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THE INFLUENCE OF TEMPERATURE, LIGHT, AND CARBON DIOXIDE ON THE GROWTH RATE AND BUOYANCY OF BLUE-GREEN ALGAE

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LOIS GAIL WOLFSON

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THE INFLUENCE OF TEMPERATURE, LIGHT, AND CARBON DIOXIDE ON THE GROWTH RATE AND BUOYANCY OF BLUE-GREEN ALGAE

By

Lois Gail Wolfson

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ABSTRACT

THE INFLUENCE OF TEMPERATURE, LIGHT, AND CARBON DIOXIDE ON THE GROWTH RATE AND BUOYANCY OF BLUE-GREEN ALGAE

By

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Many factors have been singly or collectively implicated as the major causes of blue-green algal blooms in lakes during summer. The nuisance bloom forming blue-green alga *Anabaena flos-aquae* was grown in culture flasks with limiting carbon dioxide concentrations under various levels of light and temperature to determine its growth rate and buoyancy. Growth rates were greatest at the highest light intensity used (50 $\mu E/m^2/sec$) and were dependent on carbon dioxide concentrations and temperature conditions. Algae were nonbuoyant under optimal temperatures of 21 C but increased buoyancy as carbon levels decreased. As temperature deviated from the optimal growth level, more carbon was required to maintain nonbuoyant conditions. Under lower light intensities (15 $\mu E/m^2/sec$), cells were positively buoyant, but lost buoyancy as temperatures increased above optimal temperatures. Data indicated that at these high temperatures carbon was being fixed faster than it was being assimilated by cells, resulting in gas vacuole collapse likely due to increased turgor pressure.

A kinetic growth equation model was developed to predict the interaction of light and temperature on growth rate. Equations, generated to fit the data for the maximum specific growth rate, the half saturation constant, and the threshold concentration, took the form of second order polynomials and were incorporated into the Monod equation to allow prediction of growth rate at various temperatures and carbon concentrations at three light intensities. Variations in growth rate across a range of temperatures increased as light intensity increased and/or as carbon dioxide concentrations increased.

Factors responsible for bloom formation of blue-green algae are discussed, and evidence for interaction among light, temperature, and carbon dioxide on growth rate and buoyancy regulation is presented.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The dominance of surface blue-green algae blooms in summer is a common occurrence in nutrient enriched lakes. Unlike other algae, blue-greens are not readily consumed through the food chain. They are, however, decomposed by bacteria, often leading to declines in dissolved oxygen in the water column when their numbers are high. The ability of some blue-green algae to form vacuoles and float at the water surface results in surface scums, reduces water clarity, detracts from the aesthetic quality of the water, causes odor and toxicity problems, and impedes the photosynthetic process of other algae and aquatic plants.

Blue-green algae or the cyanobacteria are prokaryotic organisms and have evolved many adaptations that have led to their successful dominance over other algae in many aquatic environments. Many of the blue-green algae can regulate their vertical position in lakes due to their gas vacuoles, organelles made up of minute hollow cytoplasmic aggregates of proteinaceous gas vesicles which provide buoyancy (Smith and Peat 1967; Walsby 1978). Other biological adaptations include heterocystous and a few non-heterocystous species with the ability to fix atmospheric nitrogen when external concentrations of nitrate and ammonia fall to low levels (Lund 1965;

Fogg et al. 1973); possession of phycobilisome pigments which increases their ability to harvest green light (Fogg et al. 1973) and an ability to sustain net photosynthesis at low light irradiances (Tilzer, 1987; Reynolds et al. 1987); a tolerance for extremes in temperature and alkalinity due to metabolic flexibility (Brock 1973); and a capacity to store nutrients in excess of their needs.

Environmental factors which have been reported to favor the blue-green algae include high nutrient concentrations (Vollenweider 1968; Edmondson 1972); elevated water temperatures (Fogg et al. 1973); low inorganic carbon availability and high pH tolerance (King 1970; Paerl and Ustach 1982); low light availability (Van Liere and Mur 1979; Zevenboom et al. 1980); low nitrogen to phosphorus ratios (Schindler 1977; Smith 1983); increased inorganic nitrogen availability (Klemer et al. 1982; Spencer and King 1985); and reduced grazing by invertebrates (Hrbacek 1964; Schoenberg and Carlson 1984).

Because blue-green algae are recognized as major components of eutrophication, a condition which results in declining water quality, it is important to identify factors which limit or promote their growth rate and the mechanism which account for their failure or success in productive waters. A variety of hypotheses have been presented, each providing pieces of information as to why the blue-greens become dominant.

The goal of this research was to determine the interaction of temperature, light and carbon dioxide on the growth

rate and buoyancy of blue-green algae. It was hypothesized that temperature, interacting with light and carbon dioxide, affects both blue-green algal growth rate and buoyancy, either directly or indirectly. The major objectives were to determine optimal conditions for blue-green algal bloom formation, the role of temperature in buoyancy, and to develop a predictive equation for assessing growth rate of a blue-green alga at various temperatures and light intensities.

LITERATURE REVIEW

Tilman and Kiesling (1984) note that changes in the algal community cannot be explained solely in terms of a single factor or process. With a wide diversity of adaptations, blue-green algal dominance may likely result from a variety of interactions governed by environmental constraints. Factors given the most attention include light, temperature, nutrients, buoyancy regulation, carbon dioxide and pH, and zooplankton grazing.

Light has been considered as one of the major factors controlling the growth of blue-green algae in several studies, and Mur et al. (1978) concluded that light intensity plays the most significant role in regulating blue-green algal dominance with low light availability in the water column favoring growth. Under limiting light, Van Liere and Mur (1979) showed that maintenance requirements in blue-greens were smaller than for eukaryotic algae, and concluded that this difference created a distinct selective advantage for many of the

bluegreens by enabling them to achieve higher growth rates. Foy et al. (1976) indicated that not only light quantity but periodicity also plays an important role in algal growth and succession, however, Van Liere and Mur (1979) found no significant influence on the specific growth rate or growth efficiency of *Oscillatoria* on a diurnal light and dark cycle compared to continuous illumination.

Various studies have noted that other factors interact with light, particularly nutrients and buoyancy. The degree to which growth is affected by these interactions has only recently received attention. Although Rhee (1978) presented data showing that inorganic nitrogen and phosphorus do not interact in an additive or multiplicative fashion for enhancing growth, Healey (1985) suggests that growth may be controlled through the interaction of a physical factor and a nutrient.

Since nutrients, particularly phosphorus and nitrogen, are the factors mainly responsible for the onset of eutrophication, many studies have examined these factors individually or in combination with an environmental parameter, such as light or temperature, in assessing the metabolism or growth of blue-green algae.

At low irradiances, biomass of the blue-green alga *Synechococcus* increased only in response to increased light, indicating that growth was light limited. At high light levels, growth response was mainly due to changes in limiting concentrations of either phosphorus or nitrogen. However, at

irradiances between the high and low levels, biomass responded to changes in both irradiance and limiting nutrient concentration (Healey 1985). This response indicated simultaneous light and nutrient limitation.

In a study of data from 22 lakes, Smith (1986) reported that an optimal total nitrogen to total phosphorus (TN:TP) ratio for blue-green algae is light dependent, and at a fixed TN:TP ratio, blue-green relative biomass increases as light availability decreases. The mechanisms by which light affected the algal biomass was not evident based on the data, since the effects of light on competition for N and P could not be distinguished from more direct effects of light on algal growth rates. Phosphorus deficiency, for example, strongly influenced nitrogen uptake and algal growth in *Oscillatoria agardhii* (Zevenboom et al. 1982) and resulted in a relatively low N content. Conversely, when nitrogen was limited, the alga had a relatively low P content.

Also, N:P ratios are not necessarily good indicators of which nutrient is limiting since the amount of external concentration is not indicative of the internal nutrient concentrations. Although Smith (1983) and others (Schindler 1977) have suggested that blue-green algal blooms occur when total N:total P ratios are low, McQueen and Lean (1987) found no correlation between blue-green algal composition and TN:TP ratios. The percent of blue-green algae was positively correlated with temperature and negatively correlated with nitrate-nitrogen, total inorganic nitrogen, and nitrate

nitrogen:total phosphorus.

With the addition of inorganic nitrogen at low light, Spencer and King (1985) found *Anabaena flos-aquae* to be positively buoyant, a priori condition for blue-green algal blooms. Reduced inorganic nitrogen levels reduced buoyancy at low light intensities.

Studies have also revealed that the responses of growth rate may be a function of the interaction of light levels and temperature. As metabolic activity increases with temperature, the amount of light needed to saturate growth rates might also be expected to increase (Reynolds 1984). The growth rate of four species of blue-green algae reached saturation at 17.5 $\mu E/m^2/sec$ at temperatures of 10 C under continuous light levels. When temperatures were raised to 20 C, the light saturation requirement was 28 $\mu E/m^2/sec$ for *Aphanizomenon flos-aquae*, and 40 $\mu E/m^2/sec$ for *Anabaena flos-aquae* (Foy et al. 1976). However, *Oscillatoria agardhii* and *O. redekei*, showed no difference in their light saturation requirements.

Photosynthetic rate decreased with increasing temperature under low light intensities for *Anabaena variabilis* (Collins and Boylen 1982). When the temperature reached 40 C, the photosynthetic rate increased as the light level increased. At mid-range temperatures and light levels, the photosynthetic response took the form of a dose response curve.

Although species response is highly variable, it is often found that as temperature increases, the highest growth rates

for broad phytoplankton groups changes from diatoms to green algae to blue-greens (Canale et al. 1974). Several studies have noted the influence of temperature in shifts of algal species. The diatom *Stephanodiscus* was favored by an increase in irradiance, but when temperature increased, the blue-green alga *Oscillatoria agardhii* was favored (Jones 1977). The author attributed the diatom decrease to the temperature's effect on its respiratory rate. Moore (1978) found temperature optima for the green algae *Cladophora* at 25 C, but suggested that even though temperature acted directly in the initiation of *Cladophora* growth in spring, its midsummer cessation of growth did not appear to be a direct effect of temperature.

Overall, a tendency exists to regard blue-green algae as organisms favored by high temperature conditions (Foy et al. 1976) mainly because they're found in hot springs and often are more abundant in tropical regions then in temperate regions, with similar conditions except for temperature (Whitton and Sinclair 1975). Also, high Q_{10} values have been measured for some blue-green algal species. Robarts and Zohary (1987) indicate that with maximum growth rates generally found above 20 C, these algae show a strong responsiveness to increasing temperature.

Cairns (1956) found in culture media with temperatures above 35 C, blue-green algae dominated and had replaced both green algae, which dominated between 30 C and 35 C, and diatoms which were dominant between 20 C and 30 C. When

temperatures were lowered, greens reappeared and dominated the cultures. The study showed that when optimal ranges were exceeded, the algae of a particular division were not killed, but were unable to successfully compete with those species more suited to a particular temperature range.

Results of experiments by Tilman et al. (1986) revealed that dominance by blue-green algae and diatoms was temperature dependent. Blue-greens dominated a broad range of N:P ratios at 24 C but were rare at these same ratios with temperatures of 17 C and 10 C.

These and other studies have shown temperature to have an effect on phytoplankton succession, but others (Robarts and Zohary 1987) points out that adaptation of species and interaction with other factors makes it unlikely that temperature alone is the major factor in determining the occurrence of algal species.

At Lake St. George, McQueen and Lean (1987) found a positive correlation between the percentage of blue-green algae and epilimnetic water temperature. When temperatures were below 21 C, the relative blue-green algal biomass never exceeded three percent of the total amount of algae present, except on one date. A blue-green algal bloom was likely if temperatures exceeded 21 C and the ratio of nitrate-nitrogen to total phosphorus was below 5:1. Blue-green algae in Lake Superior were outcompeted by diatoms and green algae when the temperature was held at 10 C and 17 C with N:P ratios ranging from 0.1 to 500. At 24 C, blue-greens dominated up to an N:P

ratio of 30:1 (Tilman and Kiesling 1984).

Working with *Oscillatoria agardhii*, Zevenboom et al. (1982) concluded that the relationship between temperature and growth rate suggests that when both nitrogen and phosphorus are in excess, which they referenced at more than 10 times the Ks value, temperature could interfere or even control the growth rate of the populations.

To determine if the absence of blue-green algae in Lake Mendota in spring was due to low temperatures, Konopka and Brock (1978) measured the photosynthetic rate of three bloom forming algae under different temperature optima. Although blue-green algal blooms were not found until early June when lake water temperature was greater than 15 C, laboratory studies indicated that temperatures below 15 C did not limit blue-green algal growth. At 4 C, photosynthesis was approximately 50 percent of what it was at optimal temperatures. The authors concluded that temperature alone was not responsible for the lack of blue-greens in spring. Evidence for interaction between temperature and low light intensities was presented, however.

Another factor often considered of primary import in blue-green algal dominance is buoyancy. Buoyancy in planktonic cyanobacteria is reported to be regulated in response to levels of light energy and inorganic nutrients (Konopka 1984). These factors may alter buoyancy as a result of their effects upon the rates of energy generation and utilization.

Within the phytoplankton, only the blue-green algae possess gas vacuoles, and most of the planktonic bloom forming species of blue-green algae have them. Evidence exists that some of these species regulate their buoyancy through vacuole formation and thus can control their vertical movements in the water column (Walsby and Booker 1980).

In their examination of buoyancy regulation, Reynolds and Walsby (1975) concluded that the physiological basis for buoyancy stems from the relationship between turgor pressure and gas vacuole formation. At high light intensity, *Anabaena flos-aquae* had a reduced gas vacuole content and reduced buoyancy (Walsby 1971). At low light intensities, buoyancy and vacuole formation increased. The species also had a higher cell turgor pressure when grown under high light then when grown under low light.

Increased cell turgor and subsequent vacuole collapse has been shown to be dependent on photosynthesis and occurs when the rate of photosynthesis exceeds the rate of nutrient limited growth (Dinsdale and Walsby 1972).

Both laboratory and field studies have shown that the formation of vacuoles and buoyancy depend on the interaction of light and growth limiting nutrients, particularly carbon, nitrogen, and phosphorus, which affect relative rates of photosynthesis, growth, and synthesis of gas vesicles (Klemer et al 1982). Klemer et al. (1988) suggested that the buoyancy response in species of *Anabaena* and *Oscillatoria* to changes in the availability of inorganic carbon was dependent on the

availability of nitrogen and phosphorus. When carbon and either nitrogen or phosphorus were present in abundance, bloom formations occurred.

Collapse of the vesicles of the gas vacuoles due to turgor occurs due to the accumulation of organic products of photosynthesis (Grant and Walsby 1977) and partly by light stimulated potassium ion uptake (Allison and Walsby 1981). But, Grant and Walsby (1977) also suggest that although photosynthesis makes a direct contribution to turgor pressure rise, another mechanism must be involved since turgor pressure changes so rapidly.

In an *Aphanizomenon* strain, turgor induced gas vesicle collapse was not important in buoyancy regulation since at high light intensities, buoyancy was lost even when no gas vesicle collapse occurred (Kromkamp et al. 1986). The authors stated that an increase in the ballast content of the cell due to polysaccharides could better explain buoyancy. In *Oscillatoria*, Utkilen et al. (1985) found that gas vacuole collapse did not occur as a results of turgor pressure rising, but rather to increased density within the cell partly caused by the accumulation of carbohydrates.

Buoyancy imparts a distinct advantage to blue-green algae by allowing them to move in and out of the euphotic zone. A vertical separation between optimal depth for light and nutrients allows the blue-greens to obtain nutrients from below the euphotic zone and utilize light within the euphotic zone (Ganf and Oliver 1982).

Light intensity at the water's surface can cause inhibition of photosynthesis and damage to the cell. Blue-green algal populations can counteract these surface light levels by either increasing synthesis of carotenoids, which protect chlorophyll a from direct photo-oxidation (Asato 1972), or through buoyancy regulation.

Although temperature may interact with light and play a role in buoyancy regulation, only one study could be found that dealt with its effects. The alga studied was *Microcystis*, a blue-green that cannot collapse its strong gas vesicles under increased turgor pressure. After exposure to light, cells were placed in the dark under high and low temperatures. Cells were able to regain buoyancy more rapidly at the higher temperature (Thomas and Walsby 1986). The authors attributed the difference to the variation in rate of carbohydrate production in the light and utilization in the dark, where those at higher temperature were able to utilize the carbohydrate that had previously accumulated, and thus regain buoyancy. These cultures also increased their gas vesicle gas space and protein concentration more so than did the cultures at low temperature.

While irradiance is likely the most important environmental factor regulating buoyancy, the interaction of light with nitrogen and phosphorus, and inorganic carbon availability also plays a role in buoyancy regulation (Walsby 1987). The mechanisms responsible for buoyancy loss, as previously discussed, likely occur when the energy generated through

photosynthesis is greater than the utilization of energy for growth (Konopka 1984). If the rate of energy utilized increased as a result of a limiting nutrient being added to a system, the amount of light necessary to cause a loss in buoyancy would also increase.

When phosphate was added to an *Aphanizomenon* population, twice as much light was needed to obtain losses in buoyancy. Also filaments that had lost buoyancy due to light irradiances regained buoyancy in the dark at a faster rate when phosphate was added (Konopka 1989).

With NH_4-N added to cultures, *Anabaena flos-aquae* became positively buoyant under reduced light conditions. In the absence of NH_4-N, the algae were buoyant when light levels were reduced, but only if carbon dioxide was also reduced (Spencer and King 1989).

Unlike the effect that phosphorus and nitrogen have on buoyancy, limiting concentrations of inorganic carbon have been found to increase buoyancy. This effect is not surprising since without sufficient carbon, photosynthesis is reduced and turgor does not increase.

In an experimental water column, where nutrients were high, *Anabaena* quickly consumed dissolved inorganic carbon and then floated to the water surface. Atmospheric diffusion of CO_2 was not sufficient to meet photosynthetic demands (Walsby and Booker 1980). The algae retained buoyant conditions even though the light intensity was ten times higher than that normally needed to cause sinking when carbon was readily

available.

The reduction in inorganic carbon, according to Reynolds and Walsby (1975) is likely one of the major factors contributing to surface algal blooms in summer. However, a weak correlation between biomass of blue-green algae and the total concentration of dissolved carbon dioxide led Canfield et al. (1989) to the conclusion that other factors in addition to CO_2 are influencing the biomass.

King (1970) states that blue-green algae can function at lower equilibrium carbon dioxide levels than most other algae and thus have lower compensation or threshold concentrations for CO_2. Thus, blue-green algae may be more efficient than other algae in obtaining CO_2 at low concentrations when pH is high, which is the condition commonly found in highly enriched lakes. King suggests that this is one reason why algal species succession in eutrophic lakes culminates in blue-green algal dominance.

The half-saturation constant (Ks) for blue-greens was generally lower than the Ks of chlorophytes between pH 8.3 and 9.3 and as pH rose so did the difference between the two groups (Shapiro 1989). Generally, the lower the Ks is for an organism, the higher its affinity is for the particular substrate in question.

Birmingham and Coleman (1979) noted that as pH increased, four species of blue-green algae decreased their compensation point to lower levels than did green algae or diatoms.

Shapiro (1973) found that the addition of CO_2 or lowering

the pH stimulated a shift from blue-green algae to green algae, particularly when nitrogen and phosphorus were both added to the enclosed containers. Nutrients added without CO_2 resulted in an increase in blue-green algae. This same response to low or high CO_2 concentration was found in sewage lagoons by Ip et al. (1982). They also suggested that low temperatures which increased CO_2 solubility favored greens and diatoms. In summer, the higher temperatures would reduce the solubility of CO_2, make it less available, and thus favor the blue-greens.

Elevated buoyancy in blue-greens occurred when pH levels were high, and the response could be reversed by lowering the pH. Changes in pH due to additions of CO_2 gave similar results. As the pH was lowered, the tendency to be buoyant also decreased (Paerl and Ustach 1982). The authors surmised that formation of blue-green algal "scums" at high pH levels was not a direct effect of pH but rather due to a depletion of CO_2.

Conversely, Coleman and Colman (1981) suggest that bicarbonate transport occurs in the blue-green alga, *Coccochloris*, and its inability to photosynthesize below pH 7 is not due to carbon limitation but rather by the inactivation of the enzyme ribulose biphosphate carboxylate at lowered internal pH values.

Although many hypotheses have been put forward to explain why blue-green algae become dominant in lakes, only a few studies have looked at the relative importance of several

processes simultaneously to study their interactive effects. Nutrient competition; sinking/buoyancy; physical factors, such as temperature, pH, and light; carbon dioxide; and grazing/herbivory, all may influence blue-green algal composition, both spatially and temporally.

A study done by Spencer and King (1989) combined carbon dioxide, light, and nitrogen to observe growth rates and buoyancy of blue-green algae. The rate of surface bloom formation was dependent on a three way interaction between light, nitrogen, and CO_2 availability. Depending on the experimental condition, all of the factors could be important. Other factors that may play a determining role were suggested, including phosphorus availability, water temperature, and lake stratification.

Many cellular processes are temperature dependent, and variations in temperature have marked effects on algal structure and function, including a change in the rate of chemical reactions (Fogg et al. 1973). However, the interaction of temperature, light, and CO_2 on growth rate and buoyancy has not been determined. It is important to keep in mind that chemical and physical factors show complex interactions, and an optimum level of one factor under one condition may be suboptimum under another condition.

MATERIALS AND METHODS

The objective of this research was to determine to what extent temperature, interacting with CO_2 and light, plays in regulating the growth rate and buoyancy of bloom forming blue-green algae under nonlimiting conditions of phosphorus and nitrogen. The algae used for the majority of experiments was *Anabaena flos-aquae*. *Oscillatoria sp.* was also used for one experiment. *Anabaena* was chosen because it is one of the dominant gas vacuolate, nitrogen fixing species found in inland lakes undergoing eutrophication, and prior research has given much insight into its response to individual physical and chemical conditions.

CULTURE AND NUTRIENT MEDIA

The algae, *Anabaena flos-aquae*, strain 1403/13f was purchased from the Culture Collection of Algae and Protozoa, in Cumbria, England. The culture was unialgal but not axenic. The species of *Oscillatoria* used was isolated from a sample taken under the ice in winter from a manmade pond at the Inland Lakes Research and Study Center at Michigan State University. Suspensions of *A. flos-aquae* and *Oscillatoria* were cultivated in two liter Erlenmeyer flasks, aerated by bubbling normal air (.03% CO_2) through the culture suspension

at room temperature with 25 $\mu E/m^2/sec$ of light. Algae were routinely transferred to newly autoclaved media devoid of inorganic nitrogen to keep bacterial numbers low.

The nutrient media used for both seed culture and experimental cultures was modified from Kevern and Ball (1965) and is listed in Appendix 1. Inorganic nitrogen in the form of sodium nitrate and ammonium chloride was added during growth experiments. Phosphorus concentration in both seed and experimental cultures was increased to avoid phosphorus limitation during growth. Two vitamins, B_{12} and biotin, were added. Pintner and Provasoli (1958) reported that the blue-green marine species *Phormidium persicium* required vitamin B_{12} for growth. Other blue-green species have also been found to need external vitamin B_{12} (Fogg et al. 1973). Silica was not added to the seed culture for *Oscillatoria*. This species grew as periphyton, attached to the glass flask, and formed a mat. Diatoms were conspicuous within the mat, and finally were eliminated from the seed culture through the silica depletion.

GROWTH EXPERIMENTS

Growth experiments were carried out in batch culture at various times using three light intensities at a given temperature for five temperatures. Between three and ten ml of algae, which was quantified in terms of numbers of cells per ml and calculated for total organic carbon content, were added to 600 ml Corning polystyrene tissue culture flasks containing 350 ml of nutrient media bubbled with air. The

culture flasks were sealed with a rubber stopper, which had two hole drilled through it. Two sterile pasteur pipettes were tightly fitted into each hole. One pipette was closed with a serum cap to allow withdrawal of media but not allow gaseous exchange. A plastic tube with a 0.22 μm Millipore filter disc attached to it was placed over the other pipette and served as a release for pressure from the flask but minimized atmospheric recarbonation.

Flasks of cultures grown at room temperature (19 C and 21 C) were placed directly on wooden blocks in front of two horizontal 48 inch, 40 watt Sylvania Gro-Lux fluorescent lights (Figure 1). Light intensities of 5, 15 and 50 micro-einsteins per square meter per second ($\mu E/m^2/sec$) were determined using a Li-Cor LI-188B quantum light meter with a L190SB quantum sensor, which was sensitive to wavelengths between 400 and 700 nm and provided instantaneous readings. All light intensities were continuous and were modified by placing flat black nylon cloth or fine wire mesh over the bulbs. Intermittent illumination has not been found to give better yields than continuous illumination, and blue-greens do not appear to require a diurnal alternation of light and dark periods (Fogg et al. 1973).

Cultures grown at the higher temperatures (26 C and 29 C) were placed in a water bath and wedged against the outer glass. Light readings were taken in front, beside, and behind the glass, and averaged. Cultures were placed the appropriate distance from the light to maintain readings at the chosen

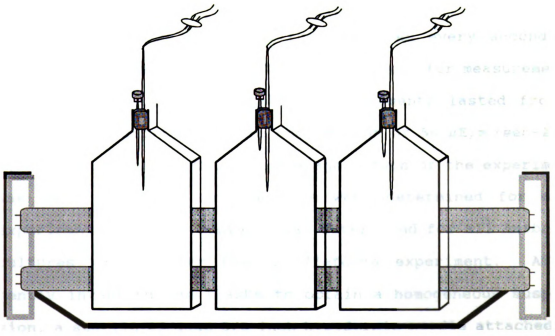

Figure 1. Apparatus used for experimental cultures at one light level and temperature.

light intensities. Cultures grown at low temperature (15 C) were placed in a refrigerated unit with a glass door and a vertical three bulb light rack propped up against the door. Mesh wire was again used to obtain the specified light intensities. All experiments were blocked from incidental room lighting by placing black polyethylene sheeting over the rack structure that held the lighting fixtures. Three replicates were used for each temperature/light combination.

SAMPLING PROCEDURE

Samples were withdrawn from cultures every second or third day for the duration of each experiment for measurements of pH, temperature, alkalinity. Experiments lasted from 9 days for a light/temperature combination of 50 $\mu E/m^2/sec$-21 C to 31 days (15 μE-29 C). The average length of the experiment was 15 ± 6 days. Cell numbers were determined for each experiment. Percent buoyancy was determined for all *Anabaena* cultures but not for the *Oscillatoria* experiment. After gently inverting the flasks to obtain a homogeneous suspension, a sterile 25 gage 5/8 inch hypodermic needle attached to a syringe was inserted into the serum cap septum to remove an aliquot sample for chemical measurement. The sample was immediately sealed with parafilm to avoid atmospheric equilibrium with the sample. An additional two ml of suspension were removed. One ml was used to measure algal densities and the other ml was used for buoyancy experiments (described later).

ANALYSES

Temperature was measured immediately upon the withdrawal of the sample using a standard laboratory thermometer. The pH was measured using an Orion digital ionalyser, model number 901, with an Orion Ross combination pH electrode. The instrument was calibrated prior to each use against standard buffer solutions of 4.0, 7.0, and 10.0. Total alkalinity was measured by titrating 10 ml of sample with 0.02N sulfuric acid to a pH of 4.8 to standardize titrations (APHA 1976), and multiplying the result by 100 to obtain total alkalinity in mg/L.

To insure that neither phosphorus or nitrogen had become limiting during the run of the experiment, both nitrate and phosphorus were added to the cultures after the pH began to decrease, in concentrations equivalent to initial conditions. No increase in growth was found. In a few experiments, distilled deionized water was bubbled with 0.03% CO_2 and added to the culture flasks after pH had begun to subside. In these cultures, the pH increased beyond its last reading after two days and quickly decreased thereafter.

GROWTH RATE CALCULATIONS

Concentration for free carbon dioxide (Equation 1) and total inorganic carbon (Equation 2) were calculated from measurements of pH, temperature and alkalinity and from the first and second dissociation constants of carbonic acid (K_1 and K_2) (Young and King 1973). Values of K_1 and K_2 were

corrected for various temperatures (Stumm and Morgan 1970).

$$CO_2 = a\left[\frac{H^2}{K_1(H+2K_2)}\right] \quad (1)$$

Where:

CO_2 = free carbon dioxide concentration (moles/L)

a = carbonate-bicarbonate alkalinity (total alkalinity minus the hydroxyl ion concentration (eq/L)

H = hydrogen ion concentration (eq/L) (using pH)

K_1 = first dissociation constant for carbonic acid

K_2 = second dissociation constant for carbonic acid.

The total molar sum of inorganic carbon (ΣCO_2) was determined by:

$$\Sigma CO_2 = a\left[\frac{\frac{H^2}{K_1}+H+K_2}{H+2K_2}\right] \quad (2)$$

Initial organic carbon measurements of seed stock were determined using a Leco Carbon Analyzer. Eighty ml of stock solution were removed and centrifuged to remove the majority of nutrient media. The concentrated algae was transferred to a crucible that had previously been dried and weighed. The crucible was placed in a drying oven at 80 C to evaporate the remaining media. Dry weight measurements for the algae were taken, and samples were sent to the Soil Science Laboratory at Michigan State University, East Lansing, to obtain total percent of organic carbon. Within the same seed culture, 0.01, 0.1, and 1 ml samples were withdrawn, filtered, and

mounted on slides for counting. The total number of cells per ml was determined and the total organic carbon per ml algae was calculated.

The amount of carbon fixed by algae could then be calculated by Equation (3) after adding the initial organic carbon concentration.

$$M = \Delta\Sigma CO_2 = \Sigma CO_{2_{t_o}} - \Sigma CO_{2_{t_1}} \qquad (3)$$

where:

M = biomass of algae in terms of organic carbon fixed

t_o & t_1 = time of initial and final total CO_2 over any two time intervals.

Following calculation of change in ΣCO_2, estimates of the specific growth rate were obtained. In addition to changes in ΣCO_2, changes in cell densities were also used and best estimates of growth rates using these two methods were compared. The specific growth rates could be calculated using Equation (4), a first order growth equation.

$$M_t = M_o e^{\mu t} \qquad (4)$$

where:

M_t = biomass at time t

M_o = biomass at time 0

μ = specific growth rate during the time increment

t = time

The specific growth rate equation measured at any two time intervals was converted to equation (5) where:

$$\mu = \frac{\ln M_t - \ln M_o}{t_1 - t_o} \quad (5)$$

The Monod (1949) equation, an adaptation of the Michaelis-Menten equation has been used to represent the saturation kinetics typical of nutrient limited algal growth and is shown in equation (6).

$$\mu = \mu_{max}\left[\frac{S}{K_s + S}\right] \quad (6)$$

where:

μ = specific growth rate (time^{-1})

μmax = maximum specific growth rate (time^{-1})

S = limiting substrate concentration (moles/L)

Ks = substrate concentration at which the growth rate μ is equal to 1/2 μmax.

This equation has been modified by Droop (1973) and further by King (1980) to account for the threshold concentration or compensation point where no net growth occurs. The term, Squit or Sq (moles/L), represents the substrate level below which no net growth occurs and is shown in Equation (7).

$$\mu = \mu_{max}\left[\frac{S-S_q}{(K_s-S_q)+(S-S_q)}\right]$$

which equals (7)

$$\mu = \mu_{max}\left[\frac{S-S_q}{(K_s+S-(2S_q))}\right]$$

The equation, which exhibits a threshold corrected hyperbolic curve, was initially used to determine the growth

rate of the algae in response to carbon dioxide at various temperatures and irradiances. Values for the kinetic constants of μmax and Ks were obtained by linear transformation of data (Lehninger 1975) followed by regression analysis of S-Sq/μ (y) against S-Sq (x). The μmax is then equivalent to 1/slope and Ks equals the intercept/slope plus Squit. Squit was derived by regressing μ against the natural log of the substrate concentration. Squit is then equal to the natural antilog of the negative constant/slope.

To account for the interdependency of light and temperature affecting algal growth, equations were developed for each kinetic constant at each of the three light irradiances across temperatures. For each kinetic constant (μmax, Ks, and Squit), an equation that best fit the data was derived. At a given light intensity, the μmax was determined for each of the five temperatures. Plotting μmax against temperature at low, medium, or high light yielded a second order polynomial equation for each light intensity. This same procedure was followed for obtaining equations for the Ks and Squit. The equations derived for these two constants were also second order polynomial equations.

By substituting the new polynomial equations into the modified Monod equation (7) a new equation (8) could be derived which accounted for the interaction between CO_2 and temperature at a given light intensity.

$$\mu = a_0+a_1T+a_2T^2\left[\frac{(CO_2)-(CO_2)_{(a_0'+a_1'T+a_2'T_Q^2)}}{(CO_2)+K_{(CO2)(a_0''+a_1''T+a_2''T^3)}-2(CO_2)_{(a_0'+a_1'T+a_2'T_Q^2)}}\right] \quad (8)$$

where:

a_o, a_1, and a_2 = constants for μmax

a_o', a_1', and a_2' = constants for Squit

a_o'', a_1'', and a_2'' = constants for Ks

T = Temperature (C)

In order to predict the interacting effects of any light and temperature combination with limiting substrate concentration, a new equation was derived. Using constant a_o from μmax at each of the three light intensities and plotting these as a function of light yielded a new equation taking the form of a power function curve. Constant a_1 graphed at each light intensity against light yielded a logarithmic curve, and a power function curve was derived for constant a_2. These three new derived equations were substituted for each of the three constants a_o, a_1, and a_2, respectively, in equation 8 to obtain the interacting effect of light and temperature on the maximum growth rate. This same procedure was followed for the Squit and Ks. Hyperbolic curves, which were found to be the best fit for both the Squit constants and Ks constants were substituted for a_o', a_1', and a_2' for Squit and a_o'', a_1'', and a_2'' for Ks, as was done with the μmax, to account for the interacting effect of light and temperature on the Sq and on the Ks. The new equation (9) was derived to predict the growth rate of *Anabaena* under any light and temperature

combination where carbon dioxide was the limiting substrate.

$$\mu = a_0 L^{-b_0} + (-a_1 + b_1 \ln(L))\, T + a_2 L^{-b_2} T^2) \left[\frac{CO_2 - CO_{2_{f_{1quit}}}}{CO_2 + K_{f_2} - 2CO_{2_{f_{1quit}}}} \right]$$

$$where\ f_{1quit} = \frac{b_0' L_0}{-a_0' + L_0} + \frac{-b_1' L_1}{-a_1' + L_1} T + \frac{b_2' L_2}{-a_2' + L_2} T_{quit}^2 \qquad (9)$$

$$f_2 = \frac{b_0'' L1_0}{-a_0'' + L1_0} + \frac{-b_1'' L1_1}{-a_1'' + L1_1} T + \frac{b_2'' L1_2}{-a_2'' + L1_2} T^2$$

where:

L = Light ($\mu E/m^2/sec$) a = intercept of the curve

T = Temperature (C) b = slope of the curve

CELL DENSITIES

In addition to calculating growth rate by total carbon fixed, growth rate was also calculated for *Anabaena* using cell densities. Cell densities for *Oscillatoria* were not made since they grew as periphyton, except when they formed gas vacuoles and became buoyant. One ml aliquots were withdrawn from each culture flask and added to 10 ml of distilled deionized water containing three drops of Lugol's solution to serve as a preservative (Wetzel and Likens 1979). Samples were filtered onto 0.45 μm Millipore filters using a membrane filtration technique similar to McNabb (1960). Filters were air dried overnight. After drying, whole filters were placed on glass microscope slides and mounted with six to ten drops of immersion oil. The filters' transparency was greatly enhanced upon placing a cover slip over the filter.

Slides were placed on a Leitz phase contrast microscope at 210X. At least 25 random fields were selected and algal filaments and number of heterocysts were counted. When variability was greater than ± 5 filaments, additional fields were counted. Only filaments that consisted of at least 5 cells were counted, however, no attempt was made to distinguish between filaments and numbers of cells per filament. The mean of filaments per ml and average number of heterocysts per field were calculated.

BUOYANCY MEASUREMENTS

One ml samples withdrawn by syringe were carefully placed on a Sedgwick-Rafter cell, topped with a cover slip, and left for approximately one hour under the light at which it was grown to let it equilibrate. Buoyancy measurements for the above mentioned experiments and for experiments under light/dark cycles were made by counting at least twenty fields of floating and sinking filaments under the microscope at 210X according to Walsby and Booker (1980). Algal cells were positively buoyant when their cells were in the upper field of focus. Cells were considered negatively buoyant when they were in the lower field of focus. Percent buoyant and nonbuoyant cells were calculated. Cells neutrally buoyant, and in between the upper and lower field of focus were not included in the buoyancy calculations.

RESULTS

An interaction between carbon dioxide concentration, temperature, and light, was apparent in affecting the growth rate of *Anabaena flos-aquae*. The growth rate of *Anabaena* increased with irradiance when carbon dioxide concentrations were at or above atmospheric equilibrium conditions but did not reach saturating conditions at the highest light intensity of 50 $\mu E/m^2/sec$ for the three highest temperatures. Growth rate increased with increasing temperature up to 22 C and then declined at all light intensities. Buoyancy response was directly dependent on light, on CO_2 at critical concentrations, and was indirectly affected by temperature.

All cultures of *Anabaena flos-aquae* began with an average pH = 8.49 ± .07 and alkalinities ranging from 110 milligrams (mg) per liter (L) to 120 mg/L $CaCO_3$. Initial carbon dioxide concentration ranged from 0.015 millimoles (mmoles)/L to 0.021 mmoles/L. Carbon dioxide concentration in equilibrium with atmospheric conditions decreases with increasing temperature. At 15 C, equilibrium free CO_2 is 0.015 mmoles/L. At 20 C, the concentration is 0.0123 mmoles CO_2/L.

TEMPERATURES, CO_2 AND GROWTH OF *ANABAENA FLOS-AQUAE*

At all temperatures, CO_2 decreased most rapidly at 50 $\mu E/m^2/sec$, and slowest at 5 $\mu E/m^2/sec$ (hereafter light intensities of 5, 15, and 50 $\mu E/m^2/sec$ will be referred to as low, medium, and high light, respectively).

Temperature at 15 C

The growth of *Anabaena flos-aquae* at 15 C ± .69 averaged .054 per day for low light, .097 for medium light, and .144 per day for high light over a 12 day period, with average doubling times of 12.8, 7.1, and 4.8 days, respectively. Although the seed culture was kept at room temperature, it was acclimated for 2 days at 15 C prior to the beginning of the experiment. However, the culture media remained at a temperature of 21 C, and approximately three days were required prior to algal cells reaching their maximum growth rate.

Carbon dioxide levels declined an order of magnitude at both medium and high light compared with low light by day 6 as a result of photosynthetic uptake (Figure 2a). Algae grown were able to maintain a positive growth rate through day 8 for high light, day 12 for medium light and day 10 for low light.

Cell densities increased as light intensity increased. Numbers began to decline on days corresponding with negative growth rates, as measured by the amount of carbon fixed, for high and medium light. At low light, cell densities began to decline by day 8, and further counts were not made.

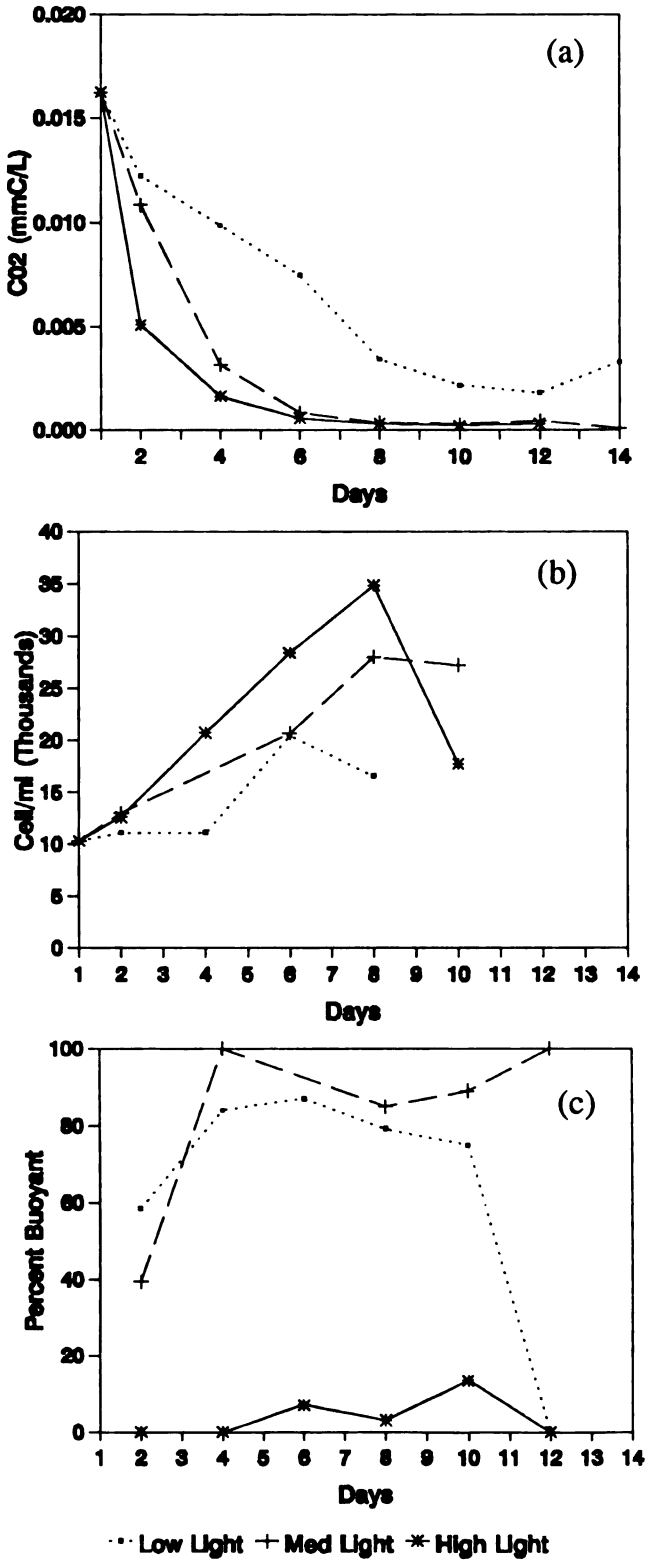

Figure 2. Time measurements at three light intensities for *Anabaena flos-aquae* at 15 C for a) carbon dioxide, b) cell densities, and c) percent buoyancy.

Whereas the other experiments used the filtration method described in the methods section for cell counting, cell densities in this experiment were determined with fresh filaments placed on a Sedgewick-Rafter cell. Filaments grown under high light were counted by both techniques. Live cell counts were approximately double the preserved counts under high light. This difference may have been due to less randomized dispersal of cells throughout the Sedgewick-Rafter cell. Cell densities for the other light intensities at this temperature were multiplied by a correction factor to more closely approximate determinations by the filtration method.

By day 10, most cells had from one to two heterocysts per filament under medium light intensities. Healthy trichomes varied in cell length, from less than 20 to more than 80 cells per filament. However, a nitrate analysis showed that 43 percent of the initial nitrate concentration remained. No determination was made as to the relative percent of heterocysts per filament; however, Spencer (1984) found that in cultures with inorganic nitrogen in the form of NH_4-N, percent heterocysts in a filament averaged two to four percent compared with 8 to 10 percent for cultures without inorganic nitrogen. Thus, a trichome of 40 cells would average four heterocysts if nitrogen were limiting.

Buoyancy at 15 C

The initial seed culture was grown with a light intensity of 25 $\mu E/m^2/sec$ at the front of the flask. As cultures

increased in numbers, the amount of light decreased significantly, and cells were buoyant throughout the media. Bubbling kept cells moving within the culture. Spencer (1984) found that the buoyancy response of algae from buoyant seed cultures was generally the same as those from nonbuoyant seed cultures except for an initial equilibration period of two to three days.

Although light intensity appeared to be the major factor in initial buoyant conditions in *Anabaena*, explanations as to why buoyant conditions occurred in several instances could not be attributed to light, but could be explained by either temperature or CO_2 concentrations.

After one day, algae growing under high light became negatively buoyant with at least 93 percent of the cells remaining nonbuoyant until day 10. At that time, carbon dioxide levels declined to an average of 1.2×10^{-4} mmoles/L, and 13.8 percent of the cells became positively buoyant (Figure 2c). By day 12, cells were senescing, disintegrating, and subsequently lost their buoyancy. At medium light, buoyancy increased from an initial 40 percent to 100 percent buoyant after two days, and then slightly decreased by day 8, likely due to deteriorating, senescing cells. Cells remained from 85 to 100 percent positively buoyant until the end of the experiment (Figure 2b).

One of the three replicates under medium light became nonbuoyant after five days. Upon examination of the culture, the nonbuoyant replicate had undifferentiated cells that were

larger in appearance, and dissimilar to the other two replicates. This same algal appearance was found after day eight in all of the cultures under low light intensity, and cells never became buoyant regardless of conditions. Agar plates were made and plated with both varieties of the *Anabaena* cells and grown at room temperature. After several days, however, both agar plates only had cells morphologically similar to the seed culture cells. Cells placed on the agar plates at low temperatures did not grow.

Blue-green algae may have considerable morphological plasticity in response to physical and chemical changes in their environment which may result in changes in cell size and trichome morphology (Wyman and Fay 1987). Walsby (1977) found filaments without gas vacuoles arose spontaneously in *Anabaena flos-aquae* and attributed it to a complete loss of the ability of the algae to make gas vacuole protein. An alternative explanation comes from the ability of filamentous gas vacuolate algae to form hormogonia, short chains of undifferentiated cells which become detached from the parent and possess motility (Fogg et al. 1983). No absolute determination was made as to whether these cells were morphological variants, had changed to non-gas vacuolate forming filaments or were hormogonia. Hormogonia, however, generally are vacuolate, and the cells in this culture possessed none. Since the algal form appeared again in a later experiment after exposure to cold conditions, it is most probable that these cells were changing morphologically in response to

adverse environmental conditions. Prior to the appearance of this morphological variant, the algae at low light were increasing their buoyancy from an initial 58 percent to 87 percent on day six. By day 12, all algae under low light conditions were 100 percent nonbuoyant but still green and healthy looking.

Temperature at 19 C

Temperature averaged 19 C ± 2.7 during this experiment, and was the most variable of the temperatures. By day 2, carbon dioxide had decreased 32 percent in low light, 70 percent under medium light, and 81 percent under high light intensity (Figure 3a). Positive growth, as measured by algal cell numbers, was sustained until day 9 for high light when CO_2 reached an average of 6.0 $x10^{-5}$ mmoles and cells numbered 71,000 cells/ml. The same trend was found for algae growing at medium light, reaching 6.7$x10^{-5}$ mmoles and 85,000 cells/ml by day 9 (Figure 3b). Increased photosynthesis as measured by decreasing CO_2 concentrations and increased pH continued until day 11 for both medium and high light intensities. Thus, maximum cell densities were reached 1 to 2 days prior to reaching minimum growth threshold concentrations. Maximum growth rates as determined by total carbon fixed averaged 0.82±.08 for high light and 0.64±.05 at medium light. Average growth rates over the 11 days were 0.384 for high light and 0.373 at medium light, corresponding to doubling times of 1.8 days and 1.85 days, respectively. A decline in algal number

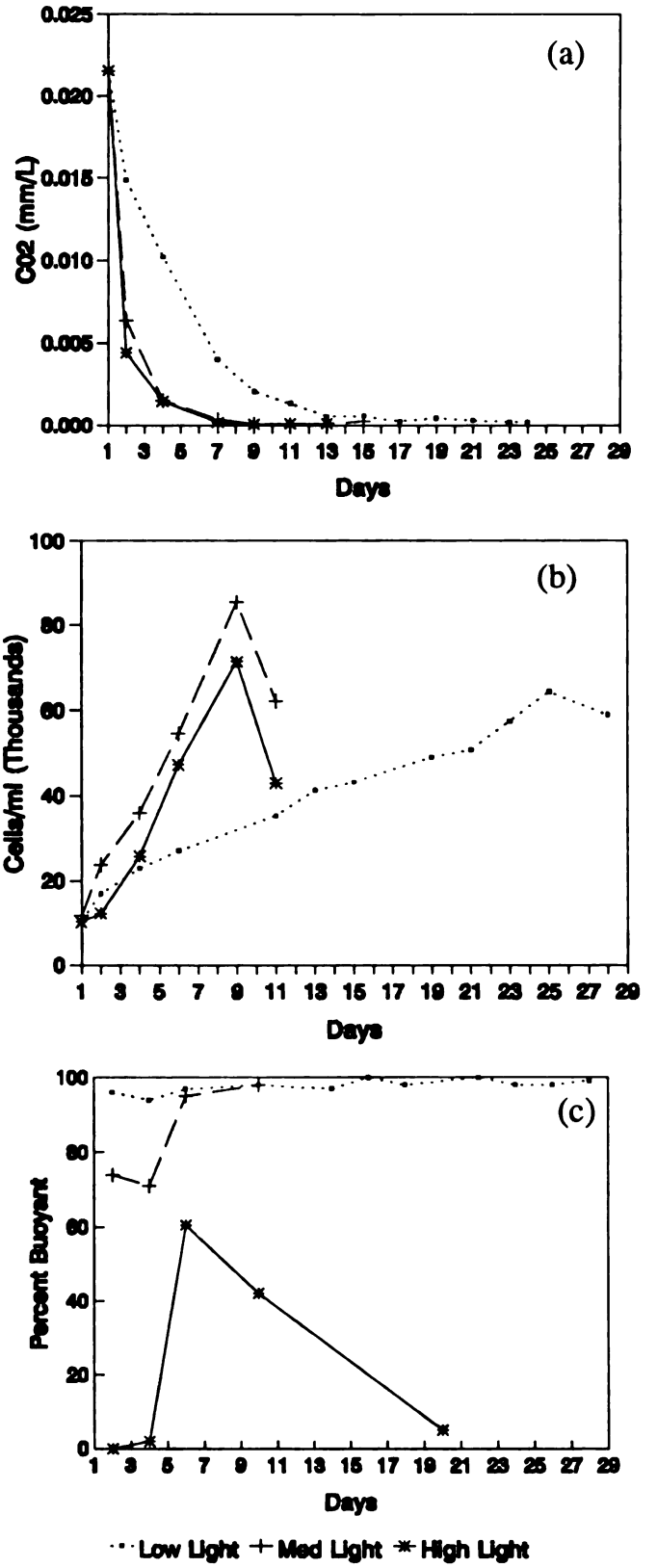

Figure 3. Time measurements at three light intensities for *Anabaena flos-aquae* at 19 C for a) carbon dioxide, b) cell densities, and c) percent buoyancy.

occurred at the termination of experiments as a result of cell disintegration.

At low light, cell numbers increased through day 28, but pH began to decline by day 23. Average growth rate per day was 0.100. The algae were able to utilize CO_2 down to an average concentration of 2.1×10^{-4} mmoles.

The total number of heterocysts increased slightly by day 4 and by day 6 were found in more filaments than on any other day under high light conditions. Under medium light, heterocyst formation was also highest by day 6, but one-third less than its high light counterpart. Under low light levels, total number of heterocysts did not vary greatly throughout the experiment.

Buoyancy at 19 C

After a short acclimation period, cultures growing under medium and low light exhibited buoyant conditions until they senesced after day 9 for medium light and day 29 for low light (Figure 3c). However, under high light, 63 percent of the cells became buoyant on day 6. By this day, CO_2 had decreased to 1.7×10^{-4}. Klemer et al. (1982) showed that a transition to carbon limitation brought about increased vacuolation in *Oscillatoria rubescens*, and Paerl and Ustach (1982) found that buoyancy of *Aphanizomenon flos-aquae* was highest under CO_2 limitation. By day 11 cells were senescing and negative growth rates were exhibited.

Temperature at 21 C

Average specific and maximum growth rates were greatest at 21 C ± 1 under high light when compared to any other experimental temperature/light combination. The average doubling time was 1.3 days for the entire run of the experiment, which lasted 8 days. Foy et al. (1976) found the maximum exponential doubling time for a blue-green alga grown at 20 C to be 0.78 days under saturating light conditions. In two days, *Anabaena* had increased from approximately 3000 to 37,000 cells/ml and decreased free CO_2 concentrations by 94 percent. Threshold CO_2 concentrations occurred slightly after maximum cell densities were reached (Figure 4a).

Algal densities at medium light intensities lagged three to four days behind densities at high light, but paralleled the growth (Figure 4b). Average growth rate over the 14 day period was 0.495 per day compared with 0.531 per day for high light. Both the threshold CO_2 concentration and maximum cell numbers occurred on day 12. Maximum growth rates were comparable with those at 19 C for this medium light intensity, but the maximum growth rate at high light and 21 C was substantially higher than that at 19 C. At 15 C, the maximum growth rate was four times lower than μmax at 21 C at medium light and five times less at high light. Foy et al. (1976) found growth rate of *Anabaena, Aphanizomenon,* and *Oscillatoria* increased between 2.4 and 3.4 fold over the range of 10 C to 20 C under continuous light.

Under low light, the threshold CO_2 concentration occurred

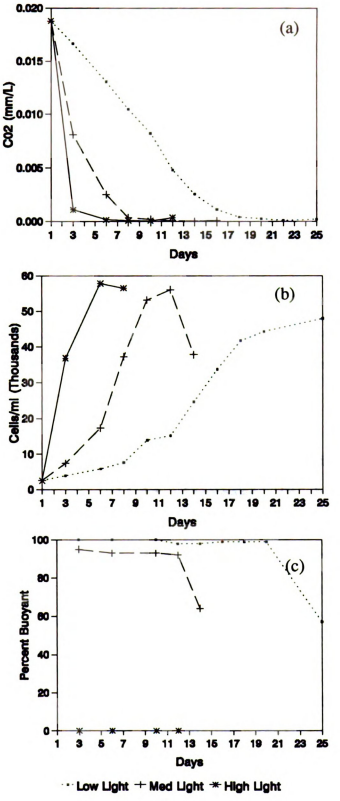

Figure 4. Time measurements at three light intensities for *Anabaena flos-aquae* at 21 C for a) carbon dioxide, b) cell densities, and c) percent buoyancy.

on day 22. CO_2 remained above atmospheric equilibrium conditions through day 6. Threshold CO_2 concentration was over an order of magnitude higher than that at medium and high light intensities. Algal densities in two of the three replicate samples continued to rise slightly until day 25, however, filament length was reduced. Cell density in cultures often appeared to increase as a result of cell breakage. Although filaments were not counted if less than five cells per filament, filament length varied, particularly as CO_2 decreased, and the algae became stressed. Further evidence of cell breakage was indicated by cells with heterocysts on the terminal end of the filaments. *Anabaena* generally has intercalary heterocysts.

Heterocysts formation did not vary greatly under low or medium light conditions, and many filaments had none present. Under high light, a slight increase in heterocysts in filaments occurred by day 5, but numbers only averaged slightly more than one heterocyst per two filaments. Heterocysts in filaments were found more often in buoyant cells than nonbuoyant ones.

Buoyancy at 21 C

At the optimum measured growth temperature of 21 C, cultures under low light were 98 percent or more buoyant through day 20, after which buoyancy was reduced. At medium light, buoyancy remained greater than 90 percent through day 12, but declined to 64 percent by day 14. This decrease

corresponded with the day on which a large decrease in cell numbers occurred and growth rates became negative. Under high light, cells remained 100 percent nonbuoyant until they senesced and disintegrated (Figure 4c). The lowest average carbon dioxide concentration recorded during these nonbuoyant conditions was $8.17 \times 10^{-5} \pm 0.000030$ mmoles/L.

Temperature at 26 C

Seed cultures, growing at room temperature (23.5 C) were not acclimated to the experimental temperature of 26 C ± 1 prior to the experiment and took two days to equilibrate at medium light in two of the three replicate samples. At high light, two of the three cultures required no acclimation time. Growth was suppressed, especially at medium and low light intensities.

Decline in the CO_2 concentration was slightly slower at high light at this temperature than the decline rate at the other temperatures. By day two, concentrations averaged 0.008 mmoles/L, but by day four concentrations were comparable with those at the lower temperatures (Figure 5a). Maximum cell densities reached approximately 31,000 by day six; the minimum CO_2 concentration occurred on day 8. Total cell densities were over two times less than those grown at 21 C, and the threshold CO_2 concentration averaged 2.7×10^{-4} mmoles/L at 26 C compared with 5.82×10^{-5} mmoles/L at 21 C. Average growth rate was 0.44 per day, with a maximum growth rate averaging 0.858 per day.

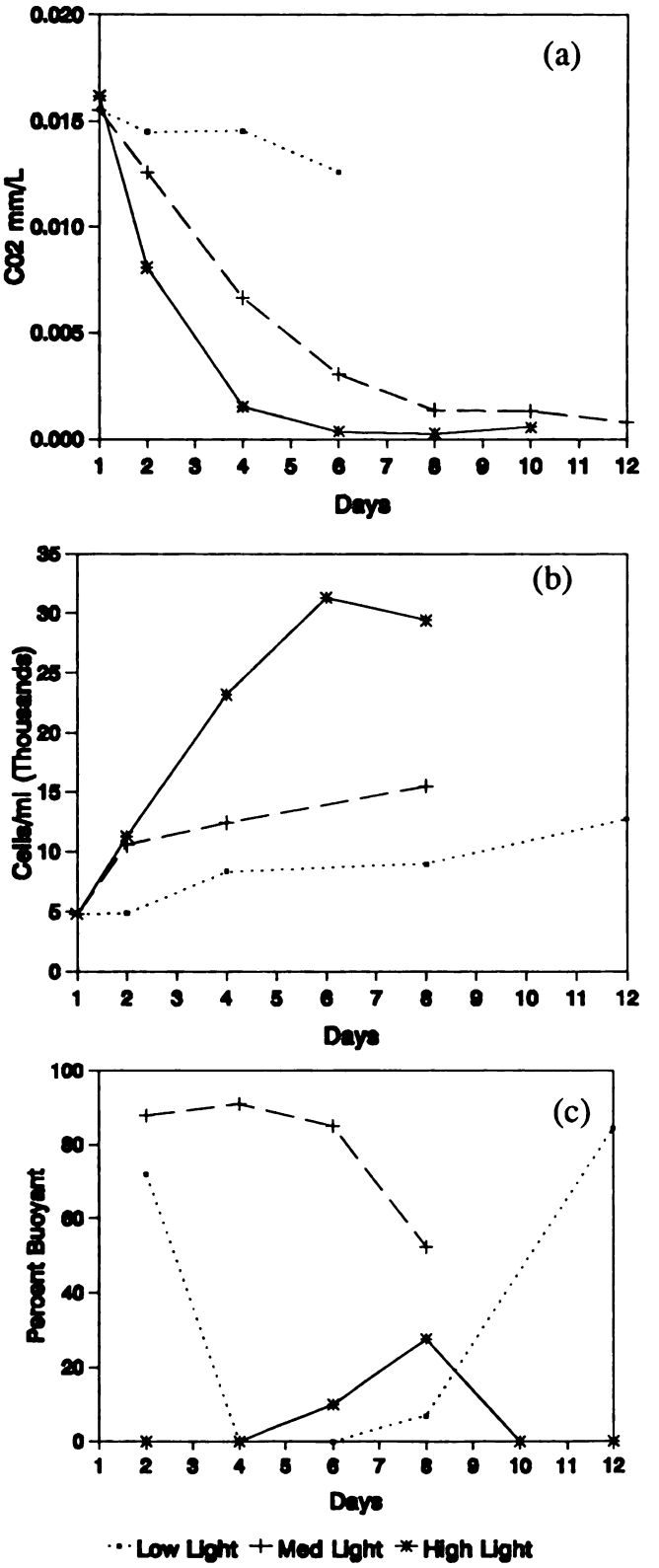

Figure 5. Time measurements at three light intensities for *Anabaena flos-aquae* at 26 C for a) carbon dioxide, b) cell densities, and c) percent buoyancy.

After two days, CO_2 concentration at medium light was still above atmospheric equilibrium conditions and declined at a slower rate than in cultures at the lower temperatures. The threshold CO_2 concentration on day 8 corresponded with the day that maximum cell densities of 15,000 were obtained. Densities were half that of high light at 26 C and almost four times less than densities growing in medium light at 21 C (Figure 5b). The average specific growth rate was 0.27, and the maximum growth rate averaged 0.32. The average threshold concentration was from one to two orders of magnitude higher than any of the cultures grown at lower temperatures under medium light intensities.

Only one of the three replicate samples showed positive growth at 26 C under low light intensity. This culture reached its threshold CO_2 concentration of 0.007 mmoles/L on day 12, corresponding with its highest cell density count of 13,000 cells/ml. The maximum growth rate of 0.19 did not vary significantly from those grown at 21 C or 19 C, but total cell numbers were two to four times less. For all three light intensities, heterocyst numbers were extremely low, averaging less than one heterocyst per filament.

Buoyancy at 26 C

Under high light, cultures were nearly 100 percent nonbuoyant until day 4, began to gain buoyancy by day 6, and were 27 percent buoyant on day 8, although growth rates were slightly negative. The CO_2 concentration was 2.7×10^{-4} mmoles/L,

slightly higher than the concentration at 19 C, when positive buoyancy first occurred. The subsequent decrease in buoyancy occurred simultaneously with cell senescence (Figure 5c).

At medium light intensity, cultures were initially between 85 and 91 percent buoyant through day 6. By day 8, buoyancy had declined to 52 percent. Growth rates were negative, and cells numbers had peaked. By day 10, cells were lying on the bottom having a greenish-yellow appearance, indicating senescing cells.

Although cells were positively buoyant at low light during the first two days, they quickly lost their buoyancy by day 4. Only one of the three replicates maintained positive growth, and decreased buoyancy was attributed to cell damage and senescing cells. After a period of acclimation to the higher temperature, cells that were able to recover, began exhibiting positive growth and buoyancy increased. Carbon dioxide concentrations were never below atmospheric equilibrium concentrations.

Temperature at 29 C

With a temperature of 29 C ± 1, light intensity at 50 $\mu E/m^2/sec$ was not high enough to meet the metabolic demands of the algae. Growth was relatively slow compared with other temperatures at high light, and took 11 days to reach a maximum cell density of 24,000 cell/ml. The threshold concentration occurred two day later when CO_2 was at $4.9x10^{-4}$ mmole/L (Figure 6a). Doubling times averaged 3.19 days for

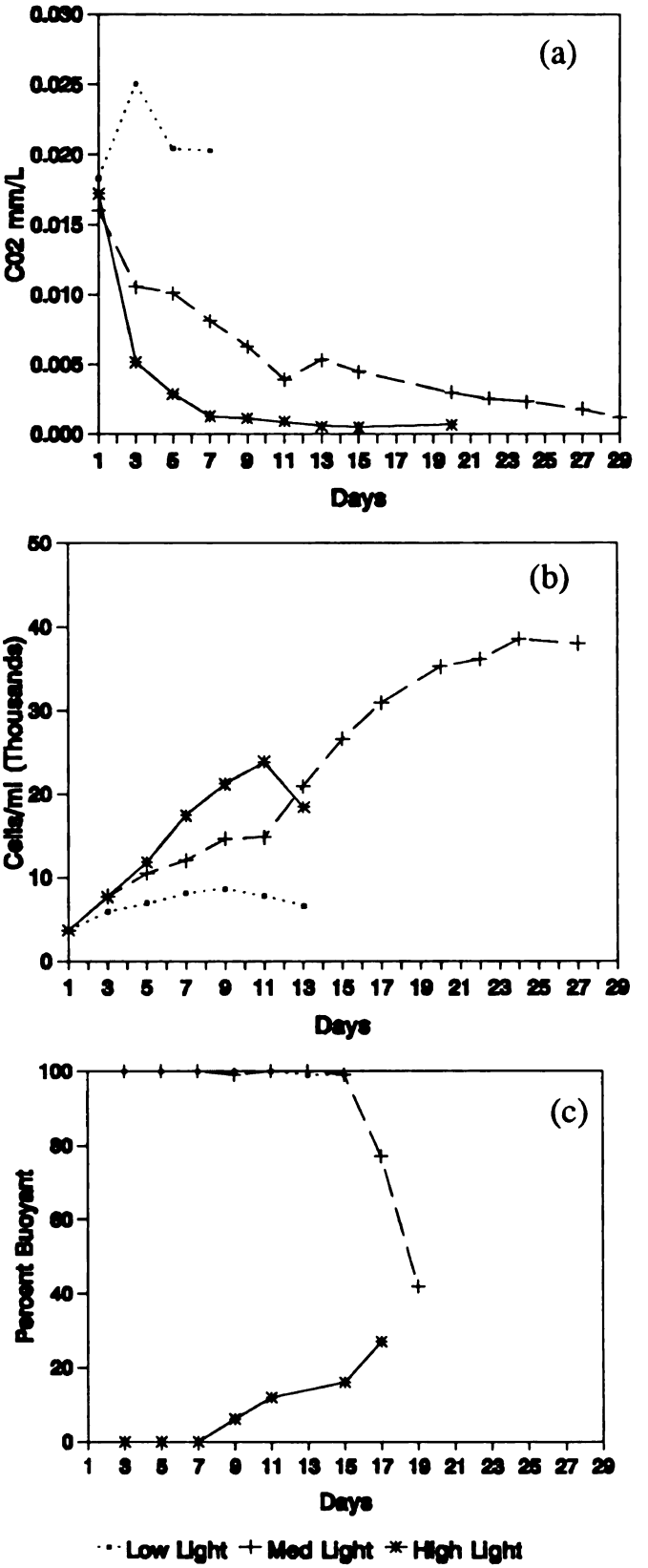

Figure 6. Time measurements at three light intensities for *Anabaena flos-aquae* at 29 C for a) carbon dioxide, b) cell densities, and c) percent buoyancy.

this light/temperature combination, and maximum growth rate was 0.69 per day.

After positive growth was terminated, temperature was decreased to 23 C. After a five day period, cells began to look much greener and healthier. Positive growth resumed, and CO_2 concentration was decreased further.

Cultures grown under medium light grew slower than those under high light but reached higher cell densities after the other cultures had terminated growth. One replicate culture increased growth until day 24 when cell densities reached 39,000/ml. Densities were higher than those at 26 C, but lower than half those grown at 19 C (Figure 6b). The second replicate terminated growth by day 22. Threshold CO_2 concentrations were reached on day 29 for replicate one and day 24 for replicate two, averaging $2.3x10^{-3}$ on day 24. Positive growth never occurred for the third replicate. Average specific growth rate was 0.099 and maximum growth rate averaged 0.15.

No positive growth occurred at low light and high temperature. On day seven, the amount of carbon fixed by algae increased very slightly, but decreased again by day nine. Cells were yellowish and breaking apart at this time, and by day 20 had senesced.

Buoyancy at 29 C

At high light intensities, algae remained nonbuoyant until day nine when six percent of the cells became buoyant.

From that day until cultures senesced, buoyancy increased to a maximum of 27 percent (Figure 6c). Carbon dioxide, at $1.1x10^{-3}$ was an order of magnitude above the concentration measured when cultures at 19 C gained buoyancy and higher than the $3.6x10^{-4}$ concentration at 26 C. However, with temperatures at 29 C, the amount of carbon fixed may not have been enough to meet the increased metabolic demands of the cell, which could then result in a turgor pressure decrease sufficient enough to maintain intact vacuoles.

At medium light, buoyancy response was initially similar to that at 26 C. Cells remained nearly 100 percent buoyant until day 15 and then slowly lost buoyancy. The decrease in buoyancy at this light intensity while cells were still fixing carbon and showing positive growth can be the result of carbon being fixed in photosynthesis faster than that assimilated by cells as a result of the growth rate being limited by other factors, such as temperature (Dinsdale and Walsby 1972). This hypothesis was supported by observing the percent of buoyant cells at medium light as a function of the ratio of the specific growth rates from carbon fixed and algal densities. As buoyancy declined, the ratio of $\mu CO_2/\mu$algae increased.

Although positive growth did not occur at low light and 29 C, those cells that were initially added to the culture remained 100 percent buoyant through day 14, although fragmentation of cells increased after seven days.

KINETIC CONSTANTS

The kinetic constants of the maximum specific growth rate (μmax), the half-saturation constant (Ks) and the threshold value (Squit) were determined for each light irradiance-temperature combination by calculating the amount of carbon fixed over time. Changes in algal densities were originally used for determining growth rate, but were not found to be as consistent as carbon fixed because of the high variability in cells per filament. However, ratios between carbon fixed and algal densities were determined to support the assumption that carbon was being utilized for growth and not just taken up and stored. Three replicates were used per treatment, however, a few combinations of light and temperature produced no positive growth. These data were eliminated in the analysis of Ks and Squit (Table 1).

The μmax as a function of temperature at the three light intensities produced a second order polynomial in the form:

$$y = \sum_{i=1}^{n} a_i X^i \quad or \quad y = a_o + a_1 x + a_2 x^2 \qquad (10)$$

where:

X = Temperature (C)

The equations for μmax as a function of temperature at 5 $\mu E/m^2/sec$ (equation 11), 15 $\mu E/m^2/sec$ (equation 12), and 50 $\mu E/m^2/sec$ (equation 13), were determined.

$$y = -1.41407 + .150134x - .00346x^2 \qquad r^2 = .950 \qquad (11)$$

$$y = -4.009 + .4297x - .0099x^2 \qquad r^2 = .974 \qquad (12)$$

$$y = -6.151 + .63267x - .01375x^2 \qquad r^2 = .985 \qquad (13)$$

The general increase in biochemical reaction rates that coincide with increases in temperature should produce an Arrhenius relationship, showing an exponential increase in growth rate with increasing temperature. However, Goldman and Carpenter (1974) point out that the relationship is applicable only in a definite temperature range. Also, a strong interaction between light intensity and temperatures can limit the general use of this exponential relationship. Four species of blue-green algae showed an exponential relationship when grown under continuous light at 1600 lux. When temperatures reached 25 C, the rate of increase in the growth rate declined for *Oscillatoria*. For *Anabaena*, a marked decline in growth occurred between 20 C and 25 C (Foy et al. 1976).

As light increased, μmax increased at each measured temperature. However, this same relationship did not prevail for μmax with increasing temperature at a fixed light intensity (Figure 7 a,b). The highest measured maximum specific growth rate of 1.233 per day occurred at high light and 21 C and decreased as temperature increased or decreased. The lowest μmax concentrations generally occurred at low light

Table 1. Average maximum specific growth rates, half saturation constants, and threshold concentrations at five temperatures and three light intensities.

	μMAX	Ks	Squit
Temperature 29 C			
High Light Ave.	0.69395	0.00375	0.00075
Std.Dev.	0.01905	0.00025	0.00024
Medium Light Ave.	0.15065	0.0045	0.001335
Std.Dev.	0.06115	0.0002	0.000815
Low Light Ave.	no growth		
Temperature 26 C			
High Light Ave.	0.858733	0.001946	0.000308
Std Dev.	0.080063	0.000247	0.000103
Medium Light Ave.	0.3215	0.001356	0.001033
Std Dev.	0.023031	0.000253	0.000047
Low Light Ave.	0.1959	0.011	0.0072
Std. Dev.			
Temperature 21 C			
High Light Ave.	1.233133	0.0016	0.000123
Std. Dev.	0.022807	0.000711	0.000045
Medium Light Ave.	0.6471	0.001	0.000281
Std. Dev.	0.004626	0.000535	0.000296
Low Light Ave.	0.216966	0.000255	0.000134
Std. Dev.	0.022128	0.000070	0.000047
Temperature 19 C			
High Light Ave.	0.82649	0.001436	0.000088
Std. Dev.	0.078757	0.000419	0.000018
Medium Light Ave.	0.6397	0.002563	0.000078
Std. Dev.	0.048615	0.000379	0.000007
Low Light Ave.	0.141766	0.00079	0.000112
Std. Dev.	0.024952	0.000420	0.000011
Temperature 15 C			
High Light Ave.	0.237933	0.000743	0.000135
Std. Dev.	0.010661	0.000330	0.000043
Medium Light Ave.	0.1597	0.000593	0.000222
Std. Dev.	0.018535	0.000206	0.000211
Low Light Ave.	0.0782	0.0026	0.0017
Std. Dev.	0.032607	0.000941	0.000326

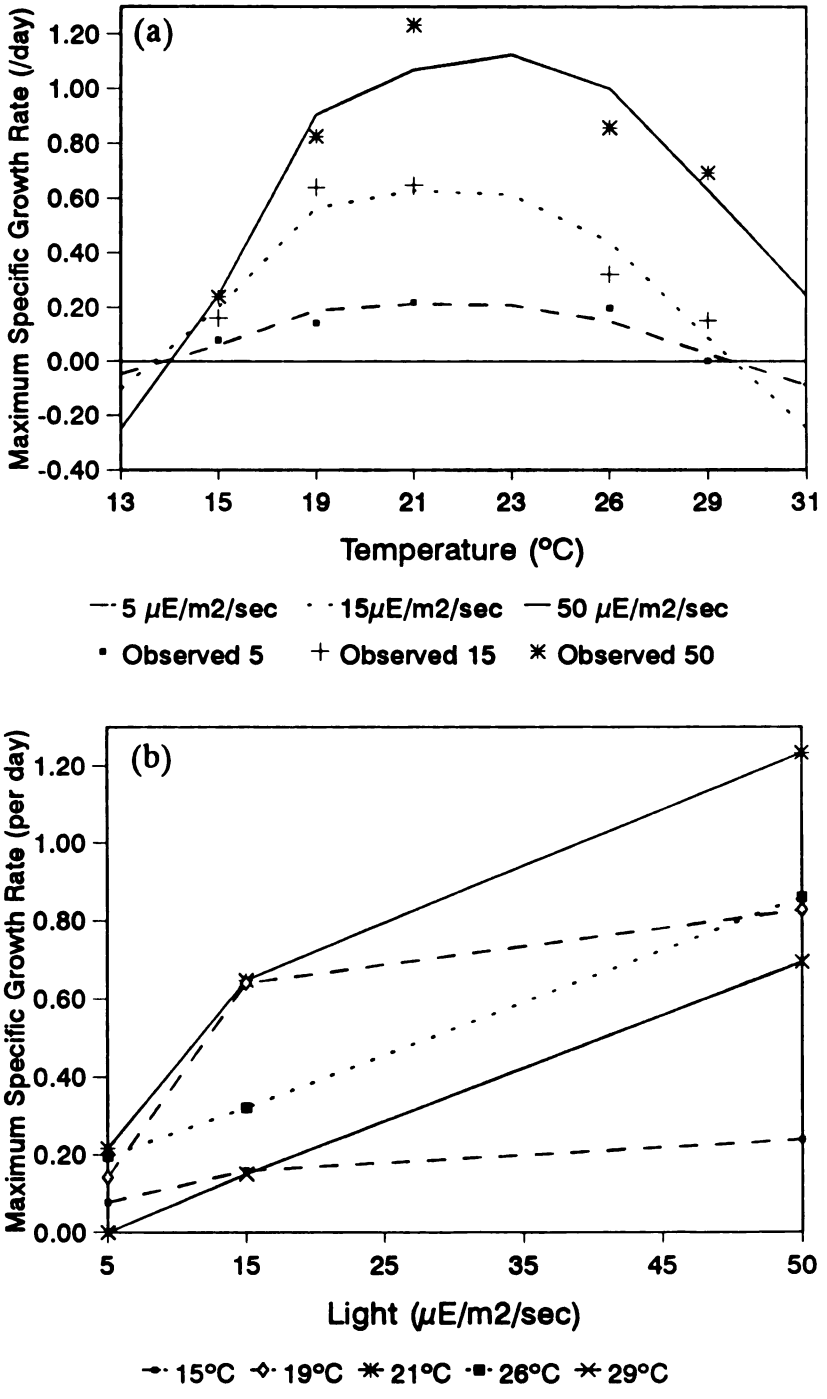

Figure 7. Maximum specific growth rate observed values and calculated equations for *Anabaena* at a) three light intensities and b) observed values for five temperatures.

intensity regardless of temperature. Maximum growth rate at low light ranged from negative growth at 29 C to .217 at 21 C. Only two cultures had a lower μmax (medium light; 15 C and 29 C) than the highest μmax at low light.

The greatest difference in μmax for a given temperature was at 21 C where the maximum growth rate at the highest light was 5.68 times the μmax at the lowest light intensity. At medium light, the μmax was 1.91 times less than high light. As temperature increased or decreased from 21 C, the difference in μmax between the highest and lowest light levels declined. A two way analysis of variance showed a significant variance in the μmax obtained due to light, temperature, and the interaction of the two ($F_{30,8}$43.6 $p<.001$) (Appendix 2).

Both the half-saturation constant, Ks, as defined as the substrate concentration when $\mu=1/2$ μmax, and the threshold concentration, Squit, took the form of a parabolic second order polynomial curve at all temperature/light combinations. The equations for Ks as a function of temperature at low (equation 14), medium (equation 15), and high light (equation 16) were:

$$y = .0719 - .00767x + .000205x^2 \qquad r^2 = .989 \qquad (14)$$

$$y = .0068 - .00066x + .000019x^2 \qquad r^2 = .849 \qquad (15)$$

$$y = .003308 - .00033x + .000011x^2 \qquad r^2 = .976 \qquad (16)$$

At low light, Ks was nearly a mirror image of μmax at the five

temperatures. The lowest Ks occurred at 21 C and increased above and below that temperature. The Ks was not highly correlated with temperature at medium light, and the coefficient of determination (r), was 0.55.

The Ks at the optimal growth temperature of 21 C at medium and high light was 20-fold higher than its value at low light. Little difference was found in the Ks between medium and high light at any temperature, although Ks was greatest at 29 C and least at 15 C at these two light levels (Figure 8). The fitted equation (16) for high light also showed some skewness indicating a potential error in the fit. Grover (1989) concluded from his study of 11 algal species that the half-saturation constant is not as reliable as the μmax, and most measurements of Ks have wide confidence intervals giving considerable residual variance in fitting the Monod model to algal growth kinetics.

The fitted curves for Squit are shown for low, medium, and high light (equations 17, 18, and 19, respectively).

$$y = .04966 - .0053x + .00014x^2 \qquad r^2 = .995 \tag{17}$$

$$y = .0025 - .00028x + .0000084x^2 \qquad r^2 = .984 \tag{18}$$

$$y = .00228 - .000236x + .00000063x^2 \qquad r^2 = .986 \tag{19}$$

The Squit, or the substrate concentration when no net growth occurs, was lowest at 19 C at all light intensities and did not vary much from concentrations at 21 C. Threshold

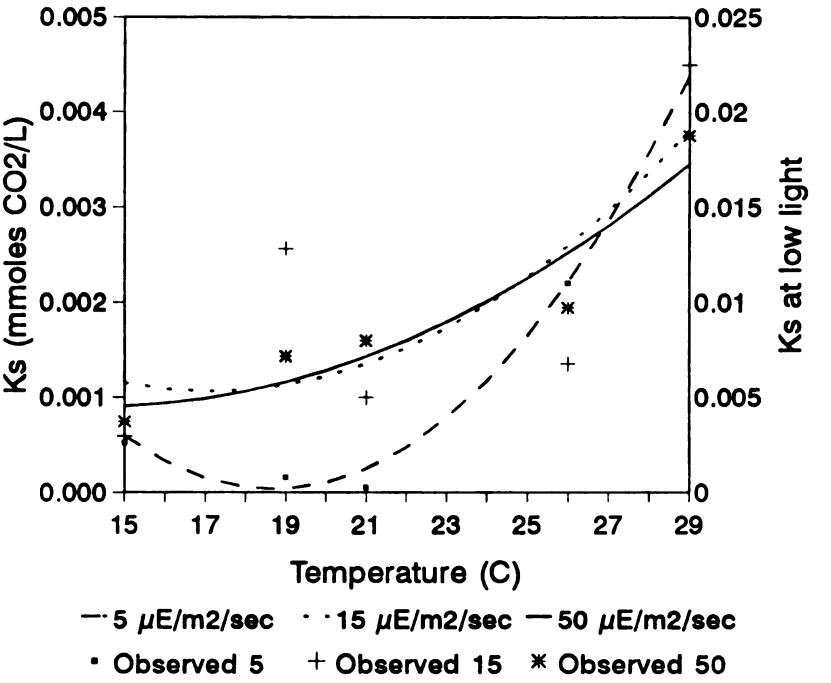

Figure 8. Observed values and calculated equations for the Ks of *Anabaena* at three light intensities.

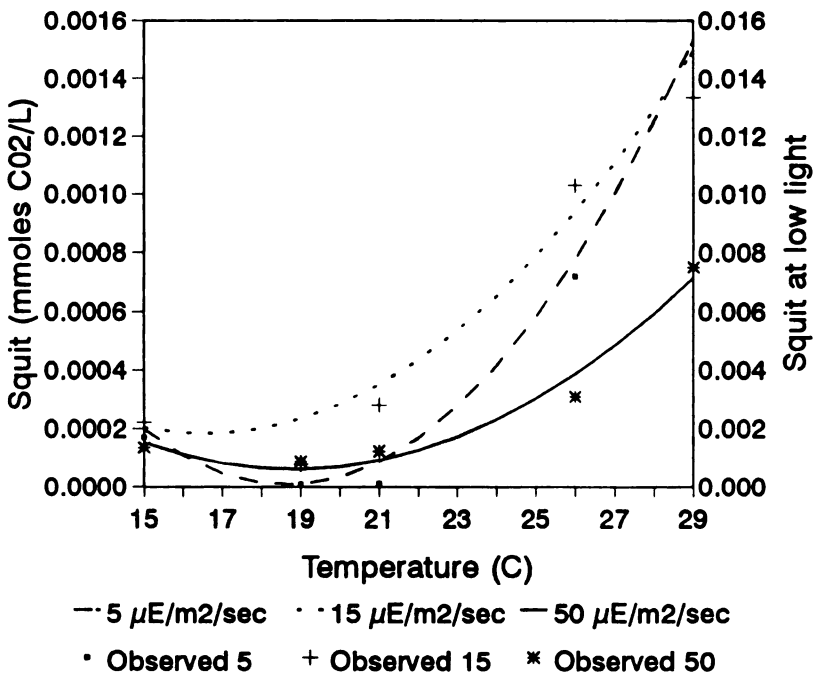

Figure 9. Observed values and calculated curves for the Squit of *Anabaena* at three light intensities.

concentrations at this temperature were as low as 7.8×10^{-5} mmoles CO_2/L. The Squit concentrations at the highest light and two highest temperatures were about an order of magnitude lower than the Squits at medium and low lights at these same temperatures. Thus, at a suboptimal temperature, light plays an important role in defining the threshold concentration of the algae. As temperature decreased, the differences in Squit at medium and high light intensities were less variable (Figure 9).

By substituting equations (11), (14), and (17) into equation (8) gives equation (20), which depicts the specific growth rate of *Anabaena* as a function of carbon dioxide concentration and temperature at low light intensity.

$$\mu = f_1 temp \left[\frac{CO_2 - f_2 temp}{f_3 temp + CO_2 - 2(f_2 temp)} \right]$$

where:

μ = *specific growth rate per day*

$f_1 temp\,(\mu max) = -1.414 + .1501\,temp - .0034\,temp^2$

$f_2 temp\,(Squit) = .0496 - .0053\,temp + .00014\,temp^2$

$f_3 temp\,(Ks) = .0719 - .00767\,temp + .000205\,temp^2$

(20)

$f_1 = time^{-1}$

f_2 & f_3 *units* = *mmoles* CO_2/L

Similar substitutions in the growth rate equation at medium light (equation 21) and high light (equation 22) can also be made.

$$
\begin{aligned}
&\textit{where:}\\
&f_1 temp\,(\mu max) = -4.0096 + .42977\,temp - .00994\,temp^2\\
&f_2 temp\,(Squit) = .0025 - .00028\,temp + .0000084\,temp^2\\
&f_3 temp\,(Ks) = .0068 - .00066\,temp + .000019\,temp^2
\end{aligned}
\qquad (21)
$$

$$
\begin{aligned}
&\textit{where:}\\
&f_1 temp\,(\mu max) = -6.1508 + .63267\,temp - .0137\,temp^2\\
&f_2 temp\,(Squit) = .00228 - .000236\,temp + .0000062\,temp^2\\
&f_3 temp\,(Ks) = .0033 - .00033\,temp + .000011\,temp^2
\end{aligned}
\qquad (22)
$$

These equations allow the prediction of the growth rate of *Anabaena* from the interaction of carbon and temperature at three different light intensities from batch cultures grown in continuous light (Figure 10 a, b, and c). A three way interaction among carbon dioxide, light, and temperature is apparent. By increasing temperature to its optimal level, and increasing available light and CO_2, the growth rate of *Anabaena* is increased.

At low light, halving the CO_2 concentration results in a decrease in the range of temperatures at which the algae can sustain positive growth. A tenfold decrease in CO_2 further decreases this growth range. Canfield et al. (1989) found a weak but significant correlation between the relative abundance or biomass of blue-green algae and the total concentration of dissolved CO_2.

As light increases, the range of temperatures at which positive growth is sustained also increases, but the lowest selected CO_2 concentration of 0.001 mmoles/L is not sufficient for growth at or above 22 C at low light intensity or at or above 27 C at medium light intensity. Positive growth is

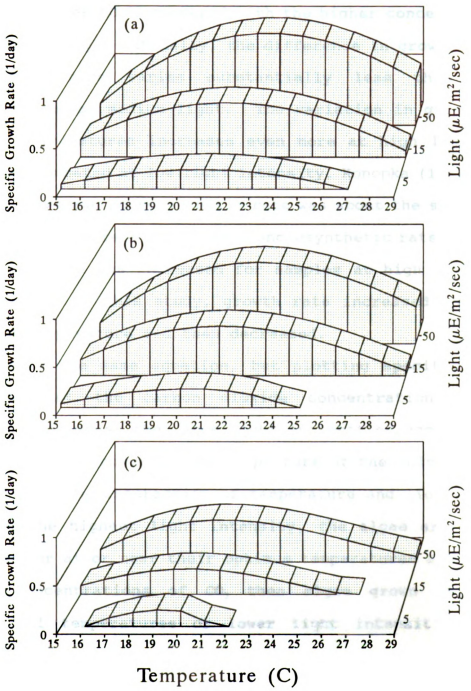

Figure 10. Specific growth rate of *Anabaena* as a function of light and temperature at carbon dioxide concentrations of a) 0.01 mmoles/L, b) 0.005 mmoles/L, and c) 0.001 mmoles/L.

sustained at all three CO_2 concentrations with high light, although considerably reduced at the substrate concentration of 0.001 mmoles CO_2/L compared with the higher concentrations.

At low light intensity, the difference in growth rate at any temperature varies substantially less than across temperatures at medium light. The variation in growth rate across temperatures increases even more at high light. In samples incubated at low light intensity, Konopka (1981) found that the average photosynthetic rate was about the same at all temperatures whereas the average photosynthetic rate increased as temperature was increased for samples at high light (180 $\mu E/m^2/sec$). In this study, growth rate increased up to the optimum temperature and then decreased.

Using the same equation, but plotting specific growth rate against the carbon dioxide concentration at five temperatures under the three light intensities used (Figures 11 a, b, and c) gives a clearer picture of the role of carbon dioxide and the interaction of temperature and light.

At the highest light intensity, the algae are able to grow faster at or near their optimum temperatures and grow at lower concentrations of CO_2 then algae grown at either suboptimal temperatures or lower light intensities. The critical CO_2 concentration needed to maintain growth is higher as light intensities decrease and as temperatures fall above and below optimal levels.

When carbon dioxide is below 0.001 mmoles at low light, growth rate is suppressed at 21 C and negative at three

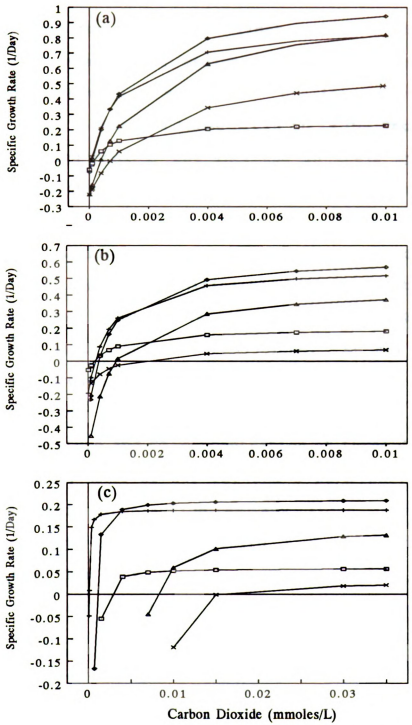

Figure 11. Calculated specific growth rates of *Anabaena* at a) high light, b) medium light, and c) low light for five temperatures.

temperatures. Growth is still positive at 19 C, the optimal temperature for algae at low light and 0.001 mmoles CO_2/L. As light increases from medium to high, and carbon dioxide increases above 0.01 mmoles, the specific growth rate of the algae at 26 C overtake those grown at 19 C. Those grown at 29 C, still lag behind, but increase in their specific growth rate of 0.068 per day at medium light to 0.46 per day at high light.

At low light intensity, the threshold carbon dioxide concentrations vary significantly with temperature. At 29 C, the cultures would have needed nearly 0.02 mmoles/L to maintain basic metabolic conditions. At all temperatures, however, growth rate was suppressed.

Equation (20) allows for the prediction of growth rate at any temperature and CO_2 concentration at three different light intensities, that of 50, 15, and 5 $\mu E/m^2/sec$. In order to predict this interaction at any light intensity, it is necessary to impose the third factor of light into the equation. For μmax, the value represented by a_o (equation 10) in equations 11 - 13 were graphed as a function of the three light levels. The best fit equation for this graph was a power function curve, which was then substituted for the a_o term. The values represented by a_1 and a_2 in equations 11, 12, and 13 were also independently plotted against light. Using least squares regression, a best fit equation was derived for these graphs also. These new equations, which produced a logarithmic curve for a_1 and a power function curve for a_2 were

then respectively substituted for a_1, and a_2 values in the parent equation (10) for μmax. The same model was also applied to Ks using the a_o, a_1, and a_2 values in equations 14, 15, and 16, and to Squit, using the constant values from equations 17, 18 and 19. The new equations formulated (23) for μmax, Ks and Squit, when substituted into the basic modified Monod equation (7), and shown in equation (9), should then account for the interaction of temperature and light at any substrate concentration.

$$\mu max = 17.43L^{-.39474} - 10 + (-.284 + .245\ln(L))T + (1.006L^{-0.0054} - 1)T^2$$

$$Ks = \frac{.0029L}{-6.451+L} + \frac{-.0003L}{-6.355+L}T + \frac{.00001L}{-5.696+L}T^2$$

$$Squit = \frac{0.002L}{-3.756+L} + \frac{-.0002L}{-4.3569+L}T + \frac{5E\text{-}6L}{-4.819+L}T^2$$

where:
$L = light\ intensity (\mu E/m^2/\text{sec})$; *and*
$T = Temperature (C)$

(23)

A significant difference was found between the observed and predicted values using this interactive equation. Since the observed kinetic constants as a function of temperature took the form of a second order polynomial, the small deviation between observed and predicted values resulted in a much larger error. Although the μmax as a function of temperature had r^2 values of 0.995, 0.984, and 0.986 for low, medium, and high light, respectively; and the resulting μmax constants a_o, a_1, and a_2 as a function of light had r^2 values of 0.999, 0.975, and 0.955, respectively, the predicted values were two times greater than those observed at the lowest

temperature. Similar error occurred for the Ks and Squit values. Due to this large error, a predictive equation for specific growth rate at any light, temperature and carbon dioxide combination could not be derived with accuracy.

BUOYANCY AND SPECIFIC GROWTH RATE

At low light intensity, the specific growth rate remained below 0.35 per day at all temperatures, and buoyancy ranged from 60 percent to 100 percent, except during the equilibration period (26 C) and for the 15 C experiment where a morphologically variant *Anabaena* arose. At the optimal temperature of 21 C, cells were 90 to 100 percent buoyant and ranged from a specific growth rate of 0.07 to 0.293. At 15 C, cells were 60 to 100 percent buoyant over the first ten days, and specific growth rates ranged from 0.028 to 0.10 per day (Figure 12).

Under high light when buoyancy measurements were taken, specific growth rate ranged from 0.007 per day at 19 C to 1.2 at 21 C per day. Except for one data point at 19 C, when growth rate was positive and buoyancy was 63 percent, cultures were 85 to 100 percent nonbuoyant. At 15 C, specific growth rate never increased above 0.20, while the highest specific growth rate at 29 C was 0.58 (Figure 13).

No immediate recognizable pattern arose for buoyancy against specific growth rate at medium light intensity for the temperature extremes of 29 C and 15 C. At 29 C, positive buoyancy ranged from 17 percent to 100 percent, and specific

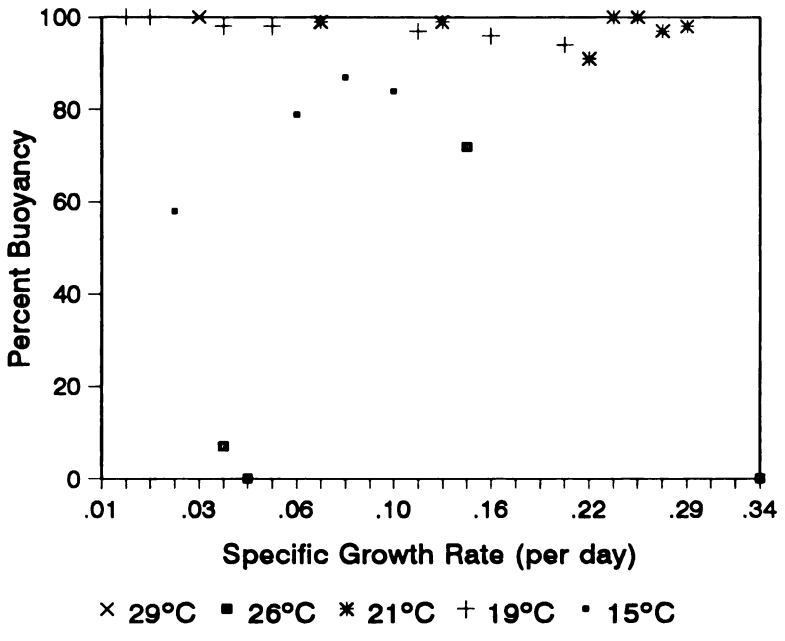

Figure 12. Buoyant and growth rate conditions of *Anabaena* at low light intensity at five temperatures

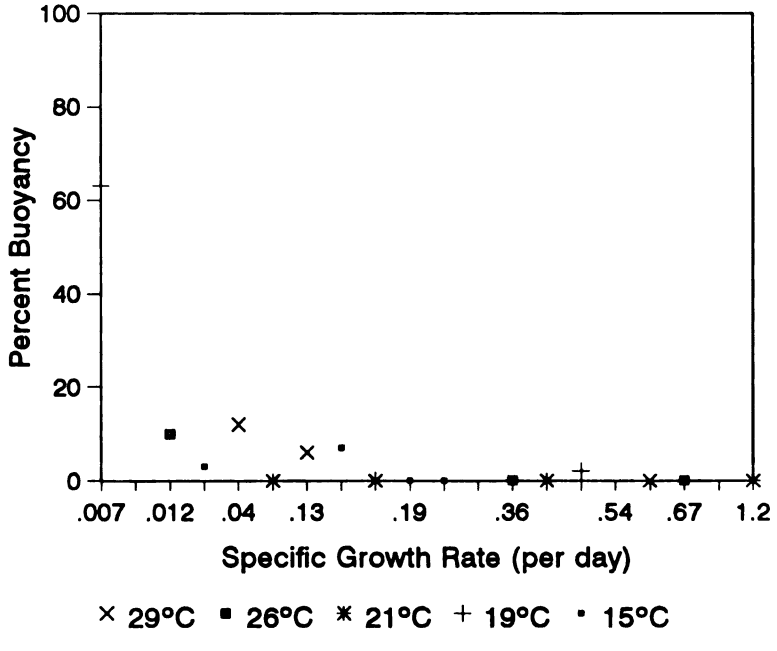

Figure 13. Buoyant and growth rate conditions of *Anabaena* at high light intensity at five temperatures

growth rates ranged from 0.016 to 0.17 per day. At 15 C, positive buoyancy ranged from 40 to 100 percent with specific growth rates ranging from 0.004 to 0.14 per day. Temperatures of 19 C, 21 C, and 26 C were greater than 75 percent buoyant with specific growth rates ranging from a low of 0.12 to a high of 0.63 (Figure 14).

At this light intensity of 15 $\mu E/m^2/sec$, temperature and nutrients appeared to influence buoyant conditions. As the intensity of light increases, more carbon dioxide can be fixed into sugars and organic compounds. If nutrients are available in adequate supply and temperature is in an acceptable range for growth, the blue-green algae can utilize the energy for growth and reproduction and maintain neutral buoyancy. However, if the amount of light available for generating energy is less than the growth rate capability, and cells continue to utilize the sugars for growth, turgor will decrease and cells will be positively buoyant. If suboptimal environmental factors such as temperature or nutrient concentration limit cell growth, then the accumulated photosynthate can increase turgor and the cells will lose buoyancy.

To determine if this scenario could provide a plausible explanation as to the lack of consistency in the buoyancy, the percent buoyancy was plotted as a function of the ratio of μ carbon/μ algae. Cultures remained 70 percent or more buoyant when the ratio was four or less. As the ratio increased, up to 22, buoyancy declined to 40 percent or less (Figure 15).

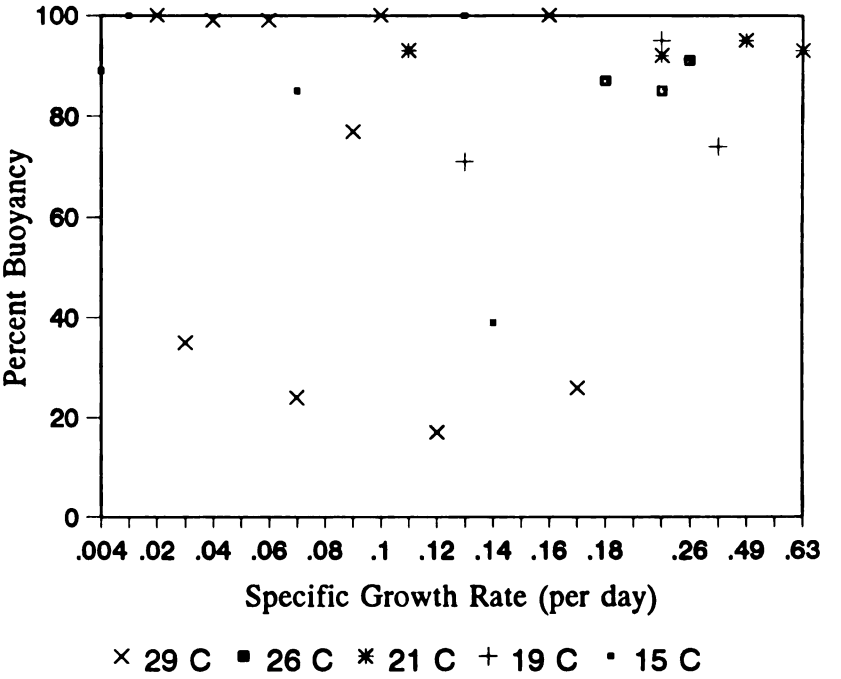

Figure 14. Buoyant and growth rate conditions of *Anabaena* at medium light intensity at five temperatures.

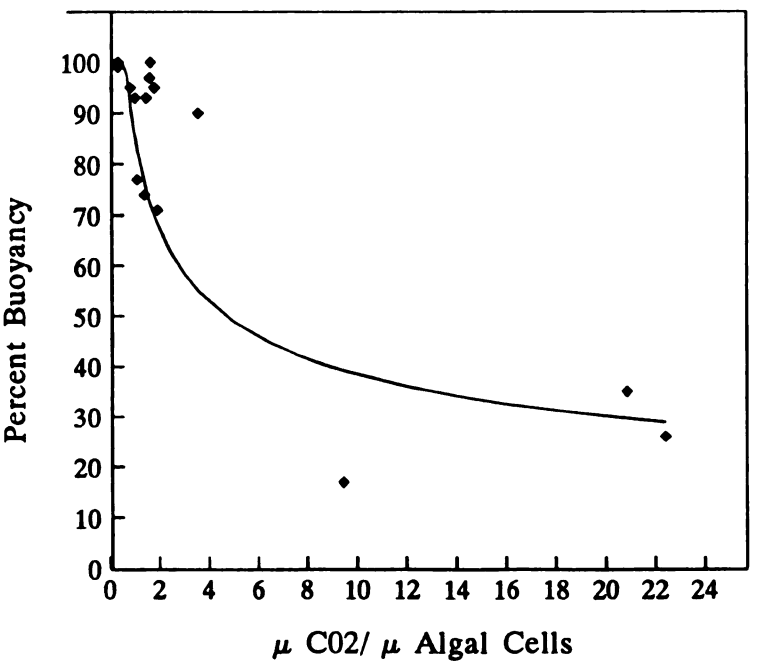

Figure 15. Buoyancy of *Anabaena* as a function of algal growth rates using total fixed carbon and cell numbers.

The carbon to algal cell ratio was highest at the temperature of 29 C, depicting more carbon relative to cell numbers. The highest ratio for the optimal temperature of 21 C was 3.35. While the amount of carbon fixed was mainly controlled by the light intensity, algal growth was also controlled by other environmental factors. Under suboptimal but high temperature, free carbon dioxide was still above threshold concentrations in the surrounding media, but the energy generated was not being utilized rapidly at this relatively low light level. Shifts in nutrient limitation can occur often and rapidly (Wetzel 1983), and a decrease in the nitrate concentration near the end of the experiment may have also been a factor in decreased cell growth and reduction in the synthesis of vacuoles.

DARK EXPERIMENTS

Cultures, grown in tissue flasks in the same manner as the other experiments, were placed in total darkness after being exposed to a two day light intensity of 25 $\mu E/m^2/sec$ at 27.5 C and 7.5 C to determine if temperature affected the resynthesis of gas vesicles. During the light phase, growth rates in the culture at the low temperature declined slightly and remained nonbuoyant. Those under the higher temperature maintained positive growth rates and were also nonbuoyant. Following 16 hours in dark conditions, both cultures exhibited negative growth rates, based on total carbon fixed. Those grown under low temperature conditions were not able to

synthesize gas vesicles after 66 hours and nonbuoyant conditions remained. However, the cells had taken the appearance of the variant *Anabaena* form, which was nonbuoyant under any light intensity in previous experiments. After 23 hours, 26 percent of the cells grown at the higher temperature were successful at regaining buoyancy. After 66 hours, no further increase in buoyancy had occurred. Thomas and Walsby (1986) found recovery of buoyancy in *Microcystis* to be 10 percent at lower temperatures (7 C) after 40 hours, while 84 percent of the colony regained buoyancy at the higher temperature of 30 C. The authors did not note if cultures continued to receive a continuous supply of carbon and other nutrients.

After 66 hours, the cultures at the higher temperature were placed under minimum light intensities (5 and 10 $\mu E/m^2/sec$). Under the lowest light levels, cells reached 100 percent buoyancy after one week. Cells appeared healthy with maximum cells per filament at approximately 165, with an average length of 45 to 60 cells per filament. Cell densities were approximately 12,000 filaments/ml. Those at the higher light intensities were 80 percent buoyant with much shorter cell lengths, averaging about 20 to 40 cells per filament. Cell density averaged 30,800/ml.

COMPARISONS WITH OSCILLATORIA

Only one experiment was performed using *Oscillatoria*. When grown in culture, the species grew attached to the flask,

entangled into one large mat, a common occurrence for many *Oscillatoria* species (Prescott 1968). The mat was aseptically cut into 10 millimeter^2 squares and added to the tissue culture flasks. Measurements for initial organic carbon concentration were not made, and results from this experiment were used for comparison purposes only. The initial experimental conditions were pH 8.42, alkalinity of 90 mg/L $CaCO_3$, and a temperature of 27 C ± 1 with the same light intensities used for the *Anabaena* experiments. Although the algae had originally been isolated from a pond during winter, cultures had been grown in the laboratory for six months at room temperature.

Using a typical organic carbon value, kinetic constants for *Oscillatoria* were determined (Table 2). Although a general trend between maximum specific growth rate and increasing light was observed, a one-way analysis of variance found no significant difference ($F_{5,2}$2.65 p >.05) (Appendix 2) between light intensities at the given temperature, likely due to the wide variability among replicate samples. Cultures at medium and high light lasted 26 days and cultures at low light showed positive growth until day 20. Although specific growth rates between *Oscillatoria* and *Anabaena* cannot be compared, the threshold concentrations can be. At high light, the Squit averaged $7x10^{-6}$ mmoles/L. This CO_2 concentration is an order of magnitude lower than that of *Anabaena* at its lowest Squit observed. At low light, the Squit averaged $1.8x10^{-4}$ mmoles/L, similar to the Squit of *Anabaena* at its optimal temperatures

Table 2. Growth rate kinetics for *Oscillatoria* at 27 C.

	μMax	Ks	S_q
High Light Ave.	0.737	0.00013	0.0000075
Std Dev	0.36	0.00005	0.000002
Medium Light Ave.	0.62	0.00076	0.0000225
Std Dev	0.12	0.00065	0.000016
Low Light Ave.	0.33	0.00066	0.00018
Std Dev	0.15	0.00064	0.00011

under low light.

Due to its adherence to the tissue culture flask and adequate carbon dioxide concentration, *Oscillatoria* did not become buoyant under low light conditions. However, under high light intensity, when CO_2 concentrations decreased to 6.14×10^{-6} mmoles/L, cells detached from the mass, became buoyant, and remained buoyant until the end of the experiment. No disappearance of gas vacuoles was found in *O. redekei* even at cultures cultivated at approximately 100 $\mu E/m^2/sec$ (Meffert 1971). Unlike *Anabaena* which usually underwent cell disintegration within two days of reaching threshold CO_2 values, *Oscillatoria* remained viable for nine days after obtaining threshold concentrations.

DISCUSSION

This investigation suggests a three way interaction with light, temperature, and CO_2 in regulating the growth rate and buoyancy of *Anabaena flos-aquae* when other inorganic nutrients are nonlimiting. At the highest light intensity of 50 $\mu E/m^2/sec$ and adequate carbon dioxide, *A. flos-aquae* was nonbuoyant regardless of temperature. Walsby and Booker (1980) found that light intensities greater than 50 to 60 $\mu E/m^2/sec$ resulted in nonbuoyant conditions in laboratory cultures. The role of carbon dioxide and temperature became more pronounced as substrate concentrations declined.

GROWTH RATE AND BUOYANCY

Under high light intensity, buoyancy loss in *Anabaena flos-aquae* has been determined to be due to the irreversible gas vacuole collapse brought about partially by increased cell turgor pressure that exceeds the critical collapse pressure of the weaker gas vesicles present (Oliver and Walsby 1984), and also by potassium ions that are transported by a light-dependent pump (Allison and Walsby 1981). Both turgor rise and subsequent buoyancy loss have been found to be dependent on photosynthesis (Dinsdale and Walsby 1972) and the accompanying rapid accumulation of low molecular weight

compounds (Grant and Walsby 1977). Turgor is eliminated in the absence of carbon dioxide or in the presence of DCMU, an inhibitor of photosynthesis.

As the carbon dioxide level decreased below a critical concentration, buoyancy increased in four of the experiments at high light. The CO_2 concentration at which this occurred varied among the different temperatures. At temperatures of 15 C and 19 C, cells began to gain buoyancy at about $1.2x10^{-4}$ and $1.7x10^{-4}$ mmoles/L, respectively. Depletion of inorganic carbon has been found to initiate buoyancy in *Anabaena* since neither carbohydrate accumulation nor turgor pressure increase can be generated (Walsby 1987). Klemer et al. (1982) found that gas vacuolation of *Oscillatoria rubescens* increased by 60 percent within three days when a shift from nitrogen limitation to carbon limitation occurred. Other studies (Paerl and Ustach 1982; Shapiro 1984) have demonstrated that the increase in buoyancy is due to CO_2 depletion and not to elevated pH levels, which would increase as CO_2 decreased.

At the optimal temperature of 21 C, cells remained nonbuoyant even when CO_2 reached $8x10^{-5}$ mmoles/L. Apparently, the filaments were producing photosynthetic products faster than they were metabolizing them (Booker and Walsby 1981, Foy and Gibson 1982) at their optimal temperature and existing light combination.

As temperature increased above 21 C, the amount of CO_2 necessary to maintain nonbuoyant conditions increased. At the highest temperature of 29 C, cells began to become buoyant at

$1.1x10^{-3}$ mmoles/L of CO_2 and increased their buoyancy as CO_2 was depleted further. Higher temperatures allow faster growth, and at this relatively high temperature, the energy utilization of the photosynthetic products was likely greater than the rate of photosynthesis, due to increased metabolic processes, decreased available CO_2 and slightly subsaturating light intensities. Even at the somewhat elevated temperature of 26 C, the cells began to become buoyant at a CO_2 concentration of $2.7x10^{-4}$ mmoles/L.

All cultures were grown in a media sufficient in all the major nutrients except for carbon dioxide. In algae with sufficient amounts of other inorganic nutrients, inorganic carbon limitation can prevent depletion of nutrient reserves and the collapse of vesicles (Klemer et al. 1982). Although nutrients may have been partially depleted during the growth phase, the decrease should not account for an increase in buoyancy. Adding a nutrient such as nitrogen would increase the assimilation of photosynthate and would reduce the turgor pressure (Klemer et al. 1988; Spencer and King 1989). Addition of phosphorus would have a similar effect (Booker and Walsby 1981; Konopka et al. 1987), and thus, cells would not lose buoyancy. In these experiments, addition of phosphorus and nitrogen in one of the replicates near the termination of a run resulted in no further increase in carbon fixation, indicating that neither phosphorus or nitrogen was limiting.

In three of the experiments, cell buoyancy increase was followed by a decline in the percentage of buoyant cells. In

each case, the decline corresponded with either a slight increase in CO_2 or senescing cells. The CO_2 increase likely resulted from increased respiration from dying cells, or photorespiration from stressed cells. While rates of respiration are affected to only a small extent due to changes in light intensity, they increase with increasing temperatures (Wetzel 1983). Whereas photorespiration may have been inhibited when carbon dioxide was more plentiful, its depletion may have promoted it. Photorespiration is very sensitive to the partial pressure of CO_2 and is completely inhibited by 0.02 atm of CO_2 (Wolk 1973).

Although several authors (Reynolds and Walsby 1975; Konopka 1984) suggest that cell senescence may promote surface blooms, the filaments in these cultures turned yellow and began disintegrating within a day of losing buoyancy. Even under medium and low light intensities, senescing cells did not promote buoyancy.

High light intensity also produced the highest growth rates at all temperatures. Van Liere et al. (1979) reported that the maximum growth rate of most temperate freshwater cyanobacteria in laboratory cultures occurs at a white light irradiance of about 50 to 60 $\mu E/m^2/sec$. Collins and Boylen (1982), however, found saturating light intensities reached nearly 300 $\mu E/m^2/sec$ between temperatures of 15 C and 30 C. As metabolic activity increases with increasing temperature, the light intensity needed to saturate growth rate should also increase, which was found to occur in this study.

As temperature rose above 21 C, growth rate decreased, likely due to light intensities not being sufficient to meet metabolic demands. Below 21 C, particularly at the lowest temperature of 15 C, growth rate was also substantially reduced. Morgan and Kalff (1979) showed in laboratory culture of *Cryptomonas* that light saturation of growth increased from 30 $\mu E/m^2/sec$ at 15 C to 138 $\mu E/m^2/sec$ at 23.5 C. Foy et al. (1976) found that the growth rate of the four common bloom forming blue-green algae were saturated at a light intensity equivalent to 1000 lux (17.5 $\mu E/m^2/sec$) at 10 C. For *Anabaena*, increasing the temperature to 20 C resulted in light saturation at 2300 lux (40 $\mu E/m^2/sec$).

Several studies (Vincent and Silvester 1979; Seki et al. 1981) have shown temperature dependence for light saturated photosynthetic rates and growth rates in various species of *Anabaena*. Other blue-green algal species including *Microcystis* (Kruger and Eloff 1978) and *Aphanizomenon* (Konopka and Brock 1978) have also been found to be temperature dependent with regard to photosynthetic rate. However, under light limited growth, photosynthetic rates of many blue-green algae, particularly *Oscillatoria* (Konopka 1981; Post et al. 1985) have been found to be temperature independent (Reynolds 1984).

In this study, where growth rate was measured as a function of total inorganic carbon fixed, temperature was a prominent factor in the growth rate of *Anabaena* at high and medium light, but was less of a factor at low light intensity. Although the light intensity at 15 $\mu E/m^2/sec$ has been

designated as medium light intensity in this study, a light intensity at this level is relatively low with respect to the optimum intensity required by algae grown in laboratory environments.

The specific growth rate at medium light intensity was less than high light intensity cultures at all corresponding temperatures. The reduced light not only resulted in decreased growth rates, but also promoted buoyant conditions. As light is reduced, the photosynthetic rate declines, and the amount of low molecular weight compounds also decreases (Grant and Walsby 1977) which causes cell turgor pressures to decline. With sufficient nutrients available, energy can be expended to produce building blocks such as amino acids and nucleotides and increase the rate of synthesis of proteinaceous gas vacuoles. At the same time, the reduced turgor pressure limits the collapse of gas vacuoles.

Although cultures were predominantly buoyant after acclimating to the environmental conditions at all temperatures, the cultures grown at the highest temperature began losing buoyancy near the termination of the experiment. This loss occurred even though growth rates, as measured by the total inorganic carbon fixed, were positive, and cell densities were still increasing.

However, as discussed in the *results* section, specific growth rates from carbon fixed were compared with specific growth rates from algal numbers. By plotting the percent buoyant cells as a function of the ratio of these growth

rates, a relationship emerged. As the ratio increased, buoyancy decreased (Figure 15). Thus, more carbon was being fixed but was not being used as rapidly for producing new cells.

Since all cultures were grown in the same nutrient media under the same light intensity, it is apparent that temperature had an effect in the physiological status of the cells, and either directly or indirectly influenced buoyancy regulation.

The three mechanisms postulated to explain buoyancy loss are collapse of the vesicles due to increased turgor, accumulation of dense molecules in the cell, and control of the rate of gas vesicle formation (Konopka 1984). The first hypothesis requires either an increase in the light intensity to increase the turgor, or a decrease in nutrients, such as phosphorus and nitrogen, sufficient to keep cell growth rate from maintaining pace with the carbon fixation rate, thereby increasing turgor and causing a decline in buoyancy. The influence of an environmental factor, such as suboptimal temperature or limiting nutrient, will affect the physiological state of the cell which can alter the cell's response to a given light intensity. Where nitrogen and phosphorus were added to cultures near the termination of an experiment, no increased growth occurred, indicating nonlimiting conditions. However, it is possible that cells were not able to recover from prior conditions, in spite of the additional nutrient input.

Heterocyst formation increased slightly at high light intensity over the course of the experiment, which could indicate a decrease in the amount of available nitrate. The presence of nitrate, ammonium and other sources of fixed nitrogen has been found to inhibit heterocyst formation in *A. cylindrica* (Wolk 1973), and high levels of ammonia inhibiting heterocysts have also been reported (Stewart et al. 1968). However, heterocysts of *Anabaena spiroides* produced from vegetative cells occurred even under conditions without nitrogen-limitation (Seki et al. 1981). A concurrent decrease in carbon dioxide concentrations may have also inhibited nitrogen fixation in this study since it is dependent on photosynthesis, and nitrate availability may have been reduced.

The cellular carbon to nitrogen (C:N) ratio has also been found to be a factor in algal buoyancy. Spencer (1984) found that generally, at low C:N ratios with low light, cells were buoyant. When C:N ratios were greater than 6 to 6.5, buoyancy was reduced. The latter situation generally occurred in cultures without inorganic nitrogen. Although this current study did not measure cellular C:N concentrations, it is possible that nitrate levels decreased to a level where the amount available was utilized in cell growth at the expense of gas vacuole synthesis. Loss of buoyancy in *Anabaenopsis*, for example, was suggested to be due to limited nitrogen fixation by the algae at low light intensity which limited formation of proteinaceous vacuoles (King et al. 1983).

The second hypothesis, that of increasing the density within the cell, has been found to occur in blue-greens, such as *Oscillatoria* and *Microcystis* with strong gas vacuoles that cannot be collapsed through turgor pressure alone. The third hypothesis of controlling gas vesicle formation may be important, but most experiments have been done with *Microcystis* and *Nostoc*, and overall little information is available.

At 26 C, buoyancy loss corresponded with negative growth rates. However, an instantaneous reading showed carbon was still being reduced on the day that negative buoyancy occurred. As with cultures at 29 C, the ratio of growth rates between carbon and algae revealed that carbon was being fixed faster than it was being used for cell growth. The resulting accumulation of sugars apparently caused an increase in turgor resulting in a collapse of some of the gas vesicles.

Cultures at 21 C also lost buoyancy, however, concurrent with this loss was a decrease in cell densities, occurring on day 12. By this day, cells were yellowish and beginning to disintegrate. At lower temperatures, cultures maintained nearly 100 percent buoyant conditions through the termination of the experiment, which was concluded when growth rates were negative for at least four days.

Although the temperature range of growth that could be supported under medium intensity varied with the CO_2 concentration, at 0.005 mmoles/L, growth was positive at all temperatures, and CO_2 values were well below literature

compensation values of 0.01 mmoles/L for green algae (King 1970; Birmingham and Colman 1979). As temperatures increased to optimum levels, specific growth rates increased and threshold concentrations declined, except at 15 C, where the threshold was lower than at any other temperature. Post et al. (1985) indicated that for *Oscillatoria*, temperature affected only light saturated growth and that light limited growth (< 10 W/m^2) was temperature independent. At medium light, where all cultures were light limited, temperature affected maximum specific growth rates based on carbon fixation. The ability of the algae to increase their growth rate at increasing temperatures, while decreasing their compensation point at a relatively low light intensity would impart a distinct advantage over other algal types when these conditions were present.

Although growth rates were lower at all temperatures for medium light compared with high light intensity, cell densities greatly exceeded densities at high light at 29 C. However, at day 11, when high light cultures began to decline, the cell densities were nearly two times higher than those at medium light. The high metabolic rates coupled with high light resulted in a much more rapid depletion of carbon, and cells could no longer maintain positive cell growth. At medium light, the depletion of carbon was slower, and cells could continue to divide. As Reynolds (1984) points out, the transfer of photosynthetically fixed carbon for growth is particularly efficient at low light levels since the energy

requirements for maintenance are low.

At the lowest light intensity, positive growth was partially inhibited at the two highest temperatures. Calculated equations revealed that carbon dioxide concentrations would need to exceed 0.015 mmoles/L for positive growth to occur at 29 C at this light intensity. Although maximum growth rates were substantially depressed at this light level, the combination of factors were sufficient to deal with the energy necessary for maintenance at the other temperatures between 0.001 and 0.005 mmoles CO_2/L. Below 0.001 mmoles CO_2/L, only cultures growing at 19 C and 21 C could survive.

In cultures that are nutrient limited, in this case by carbon dioxide, the maintenance concentration increases with suboptimal temperatures (Rhee and Gotham 1981), which means that the cells need more nutrients at these temperatures. With nitrogen or phosphorus, the need for additional nutrients may be the result of the cell's need for more RNA to synthesize the same amount of protein (Tempest and Hunter 1965). Healey (1985) proposed that the minimum internal nutrient concentration would be expected to increase with either decreasing light or temperature.

At this lowest light intensity of 5 $\mu E/m^2/sec$, algal filaments became buoyant within one to three days of being transferred from higher light conditions in the seed culture. The explanation as to why the culture at 21 C lost buoyancy near the end of the experiment while cell densities were increasing is similar for that at medium light intensity

previously discussed. However, the sudden loss in buoyancy at 26 C, followed by an increase, was likely due to an extended equilibration period where uptake of carbon and possibly other nutrients were restrained, and cells were not able to synthesize the gas vesicles for buoyant conditions to occur.

The synthesis of gas vesicles occurred slowly for cultures grown in the dark at high temperatures (27 C) and not at all for those grown under low temperatures (7.5 C). At higher temperatures, storage materials in the form of cytoplasmic granules are available for many metabolic and biosynthetic processes under dark conditions (Fogg et al. 1973). An initial rise in buoyancy after being placed in the dark could have occurred as a result of increased synthesis of proteinaceous material for gas vesicle formation. Synthesis of the gas vesicle may take several hours to complete, and it may take up to 40 hours before a sufficient amount of gas vesicles are synthesized to exceed neutral buoyancy (Reynolds et al. 1987). However, the available reserve was likely depleted and further synthesis of gas vesicles could not be made. Only after exposing cultures to very low light intensities (5 $\mu E/m^2/sec$) did increased buoyancy occur. Approximately, 82 percent buoyant conditions were apparent after one week.

Two major factors contributed to a lack of growth and buoyancy at the low temperature. First, lack of growth was due, in part, to the temperature being below the critical level needed for maintenance. Formation of macromolecules and

kinetics of physicochemical reactions can be profoundly affected by temperature (Rhee and Gotham 1981). Secondly, the lack of buoyancy was attributed to the rise of a morphological variant of the seed culture. Cultures of the same species often are found to show wide variations in their cell morphology and growth rates (Foy 1980). The occurrence of this form had also arisen during a previous experiment when cells were placed under cold conditions. It is possible that this species of *Anabaena* loses its buoyancy at low temperatures, which, under natural conditions, could account for the loss of *Anabaena* from the epilimnion in the fall months prior to lake turnover. Gas vacuoles were not apparent in any of the algae at this temperature even after three days in total darkness.

BUOYANCY AND GROWTH RATE INTERACTIONS

Buoyancy appears to be regulated mainly by light intensities through cell turgor pressure, but both carbon dioxide and temperature interact to affect buoyancy. Under the highest light intensity applied, cells are nonbuoyant until carbon dioxide is reduced to a particular level that is dependent on the temperature (Figures 16-17). As temperature deviates above and below the optimum level, the amount of carbon dioxide needed for buoyant conditions to occur increases, but as temperature increases, cells gain buoyancy at higher carbon dioxide concentrations. Under medium and low light, cells are mostly buoyant, but the range of carbon

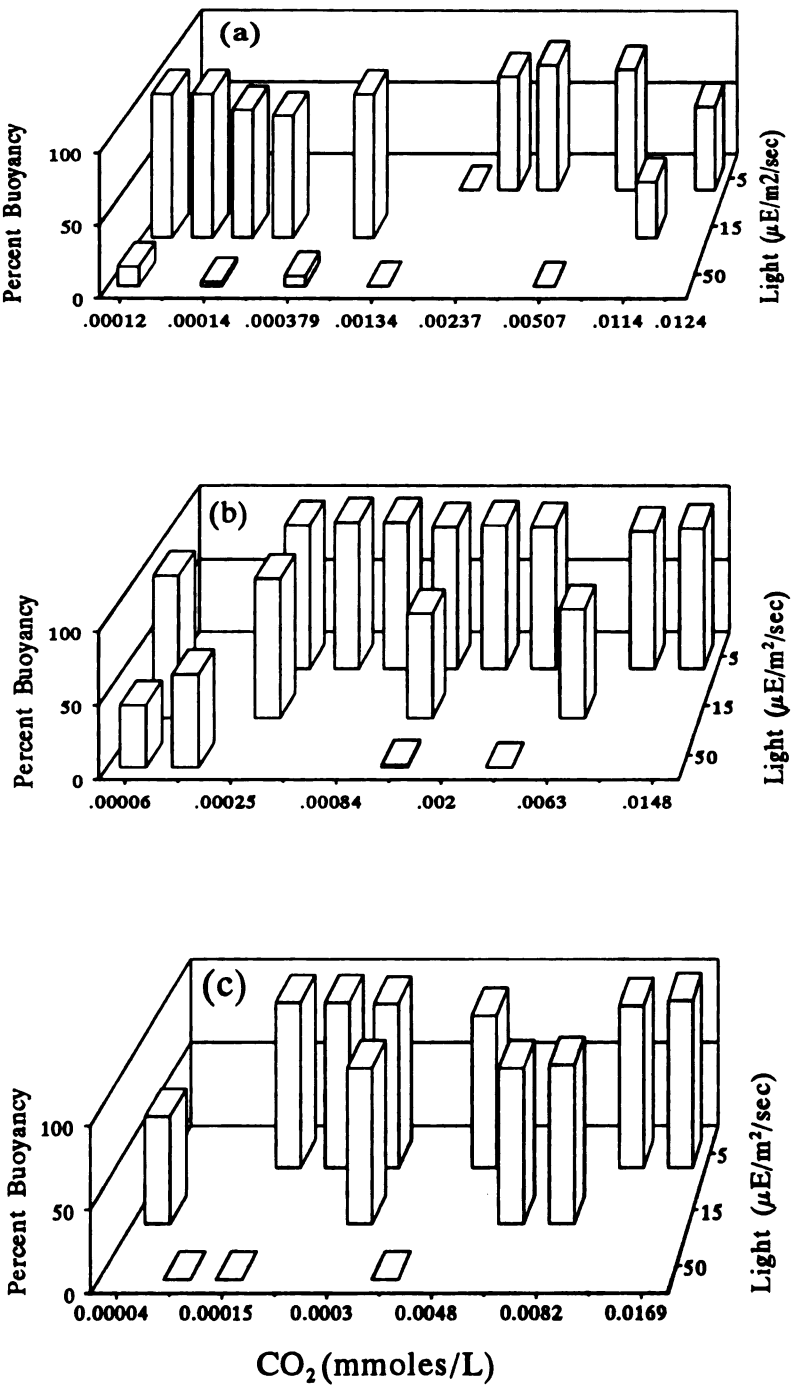

Figure 16. Relationship of buoyancy, carbon dioxide and light in *Anabaena* at temperatures of a) 15 C, b) 19 C, and c) 21 C.

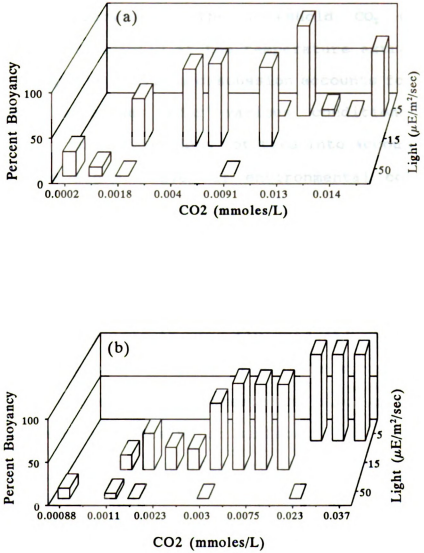

Figure 17. Relationship of buoyancy, carbon dioxide, and light in *Anabaena* at temperatures of a) 26 C and b) 29 C.

dioxide concentrations in which the cells can successfully grow decreases as light decreases and as temperatures deviate from the optimal level. At the highest temperature, cells lost buoyancy when the amount of energy generated from carbon fixation was greater than that utilized for cell growth. In low light experiments, the threshold CO_2 concentration increased significantly at the temperature extremes.

Although the previous discussion accounts for buoyancy of *Anabaena flos-aquae* under various conditions of light, temperature, and CO_2, it does not take into account the growth rate of the algae. Even if environmental conditions are optimal for buoyancy to occur, a blue-green algal bloom may not be apparent because of the low density of filaments present. Conversely, growth rates and the density of the population may be high, but environmental conditions may be such that buoyancy is greatly reduced. Although the population may be abundant in the lower strata of the water column, the algae would not create the bloom conditions known to occur near or at the water surface.

Since both the specific growth rate and buoyancy are dependent upon the interaction of carbon dioxide, nutrients, temperature, and light, the combination of these two parameters can provide a reasonable assessment of the probability of a blue-green algal surface bloom.

Specific growth rates and the percent of positively buoyant cell from algal cultures subjected to the same treatments were multiplied (referred to as μbloom) and graphed

as a function of light and carbon dioxide at the five different temperatures (Figures 18-19). Although growth rates are greatest at high light at any temperature, the probability of blooms occurring is extremely low because the overriding environmental factor of light produces nonbuoyant conditions in the algae. Even when carbon levels are reduced enough so that positive buoyancy is initiated, less than one percent per day of algal cells contribute to the bloom.

As light levels are reduced, the potential for the formation of a surface bloom increases since the percent of positively buoyant cells increases. Since growth rates are substantially reduced as both light decreases and temperatures deviate from the optimum, a moderate light intensity at optimum temperatures should produce the best conditions for bloom formation. A comparison of the highest specific growth rate of buoyant cells at medium light intensities shows that temperatures of 19 C and 21 C produce the highest growth rates at approximately 0.64 per day. At 15 C, the highest growth rate reaches 0.138 when cells are buoyant. It is seen in Figure 18c that the combination of the above mentioned factors do produce the best chance for a surface blue-green algal bloom. Nearly 60 percent of the cells per day grown under these conditions would be likely to contribute to a surface algal bloom. When light intensity is further reduced, buoyancy becomes nearly 100 percent, but growth rate declines substantially. The highest growth rate of 0.29 per day occurs at 21 C, resulting in a "μbloom" of 29 percent per day, nearly

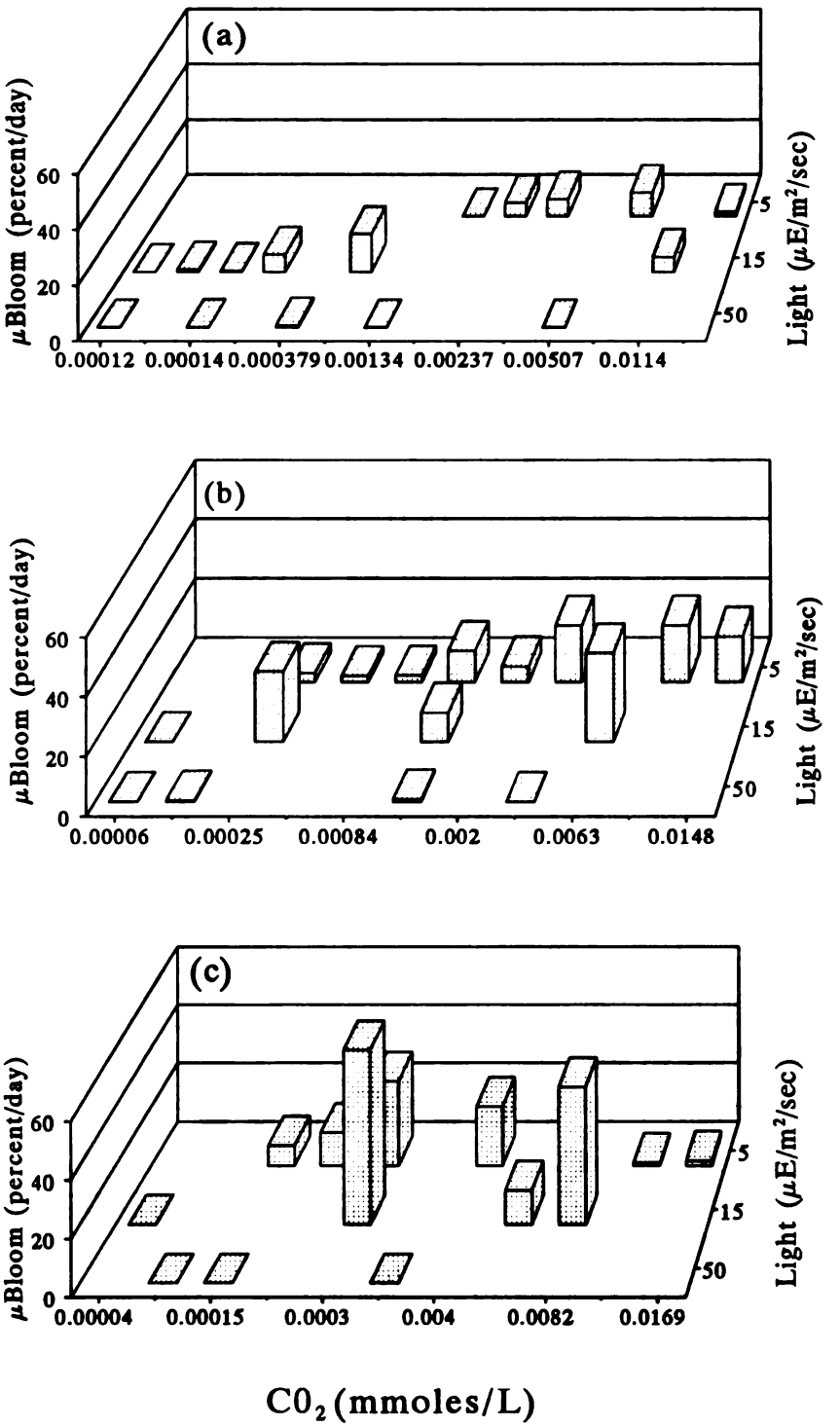

Figure 18. μBloom of *Anabaena* as a function of carbon dioxide and light at a) 15 C, b) 19 C, and c) 21 C.

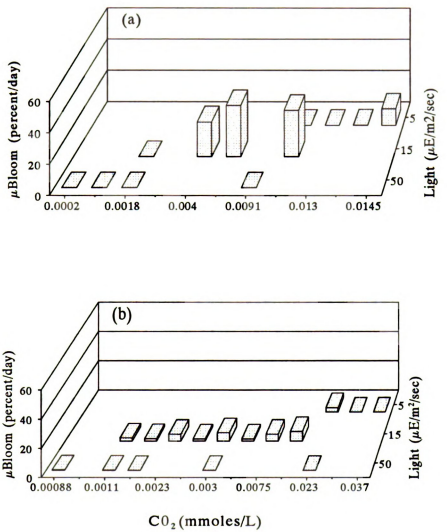

Figure 19. μBloom of *Anabaena* as a function of carbon dioxide and light at a) 26 C, and b) 29 C.

half that at the medium light intensity.

GROWTH RATE OF *OSCILLATORIA*

Although the *Oscillatoria* utilized in the experiment was isolated from a eutrophic pond during winter, its growth rate at 27 C was similar, but slightly lower, than the growth rate of *Anabaena* at high light, but was higher than *Anabaena* at medium and low light. Thus, the species utilized may not have been a cold water species as was originally desired, but was likely a dim light species. The species, however, had been isolated and grown in the laboratory at room temperature for the previous six months. Nicklisch et al. (1981) computed values for specific growth rate of *Oscillatoria redekei* at different temperatures and light intensities and concluded that this alga was a dim light species since it showed higher growth rates at lower temperatures and light intensities compared with *O. agardhii* and two other blue-green algal species, *Aphanizomenon* and *Microcystis*.

Oscillatoria sp. has been found to be efficient at utilizing a wide range of the visible light spectrum and fixing carbon, particularly at low irradiance levels. The energy requirements for maintenance have been found to be about 5 $\mu E/m^2/sec$. Under continuous illumination, the growth rate of *O. agardhii* increased to 0.7 per day at 47 $\mu E/m^2/sec$, which corresponded well with these experimental data. Growth rate increased up to a saturation level of 0.85/day at 60 $\mu E/m^2/sec$ (Van Liere and Mur 1979).

Other studies have also shown algae such as *Anabaena flos-aquae* to have a higher light requirement than *O. agardhii* Under low light and low nitrate concentrations, *Oscillatoria* was able to outgrow *Anabaena* (Zavenboom et al. 1980).

In this study, the *Oscillatoria* grew as a filamentous mat, attached to the glass. At both low and medium light, buoyant conditions were not observed, and filaments remained in a mat. It is likely that buoyant conditions did not occur under the these lower light intensities because the algae adhered to the glass. Only at the higher light, when external carbon dioxide concentrations decreased to $6x10^{-6}$ mmoles/L, did the species detach and become buoyant. Van Rijn and Shilo (1983) concluded that high buoyancy in *Oscillatoria sp.* was due to both a reduction of carbon dioxide in the water and the effect of light limitation. For *O. agardhii*, Utkilen et al. (1985) found that gas vesicles could not be collapsed by a rise in cell turgor pressure, and only by accumulating carbohydrates and decreasing gas vesicle volume, could buoyancy be lost. In this study, low carbon concentrations could have prevented an increase in carbohydrates sufficient enough to increase the density of the cell. However, this *Oscillatoria* species may not possess the strong gas vesicles that *O. agardhii* does.

KINETIC GROWTH MODEL

One objective of this study was to develop an equation that could be used to predict the growth rates of *Anabaena*

flos-aquae under various conditions of light, temperature, and carbon dioxide. Assumptions made during the model's development were that the algae assimilate carbon in the form of carbon dioxide, that the constants for carbon uptake rate and growth rate are equal, and that photorespiration and excretory losses during exponential growth are negligible.

The Monod model, derived from the Michaelis-Menten equation, has been fitted to algal growth in many studies, using a wide assortment of species and a variety of limiting nutrients (Fuhs et al. 1972; Goldman et al. 1974; Gavis and Ferguson 1975; Williams and Turpin 1987; Grover 1989). In both continuous and batch culture, the rate of uptake during exponential growth depends on substrate concentration and takes the form of a hyperbolic curve in a Michaelis-Menten trend (Droop 1973).

Modification of the Monod model in this study included the addition of a threshold concentration, Squit, and taking into account the interactive effects of temperature and light in addition to the limiting substrate of carbon dioxide. Some multiplicative models often assume that temperature will affect only the maximum growth rate. However, evidence that the half-saturation constant of the Monod equation also changes with temperature has been presented (Ahlgren 1978). Also Rhee and Gotham (1981) suggest the use of the Monod equation under conditions of temperature stress may require a term for maintenance rate since the threshold (Squit) value is temperature dependent. Thus, multiplicative models in which

only μmax vary do not account for the temperature dependency of the threshold concentration and the half-saturation constant.

In the development of this model, the effects of temperature and light on the growth rate of the algae were accounted for by incorporating the fitted equations for μmax, Ks, and Squit for temperature and then light into the basic modified Monod equation. The effect of temperature was apparent on both the μmax and Squit value, but not on the Ks value.

In *Scenedesmus*, the half-saturation constant was about 0.34 μM and did not appear to vary within a temperature range of 13.5 C to 20 C in phosphorus limited cultures. At this same range of temperatures the μmax increased from 0.45 to 2.68 (Rhee and Gotham 1981). Shelef et al. (1970) found that the half-saturation constant is sensitive to temperature changes if light is saturating. In these experiments with *Anabaena*, the Ks generally increased with increasing temperatures, but no predictable pattern was observed with respect to light, except at the lowest temperature, where light was saturating. Here, Ks decreased as light increased, thus the algae had a higher affinity for the substrate at this light saturated-temperature combination.

Although Rhee and Gotham (1981) found that the maintenance value increases with decreasing temperatures, and cells require more nutrient at lower temperatures to maintain their growth rate, this study found that Squit increased both above

and below the temperature of 19 C, particularly at medium and high light intensities. The suboptimal light intensity interacting with temperature and substrate concentration may account for the increase in Squit as the temperatures increased above optimal levels, since lower light intensities resulted in increased threshold concentrations. At suboptimal light or temperature, the algal cells may be adapting to conditions by increasing enzyme concentration (Jørgensen 1968) or RNA synthesis (Tempest and Hunter 1965). Either of these processes would require a greater amount of substrate to maintain growth.

Many other models that incorporate the effects of temperature with limiting nutrient concentration are described by the Arrhenius equation ($Ae^{E/RT}$ where A = constant; E=activation energy; R=universal gas constant; T=temperature) which relates the effect of temperature on the kinetic energy of molecules in biological systems where growth rate generally approximately doubles for every 10 C rise in temperature. Where this equation has been used, it only has been found suitable for a particular temperature range, since higher temperatures veer downward from the predicted fitted exponential curve and are thus ignored. In this study, any of the temperatures measured above 21 C would have had to have been ignored if the Arrhenius equation were to be used. Since the fitted equations for all the kinetic parameters took the form of second order polynomials, the model developed allowed for the incorporation of all temperatures at given light

intensities, and took the form of a normal dose response curve to a range of temperatures and light levels.

Although the model provides for the prediction of algal specific growth rates under varying interactive carbon and temperature levels at given light intensities (Equation 20), it is derived from laboratory conditions of algae grown in batch cultures, under continuous light intensities. Light intensities have been found to exert different effects in the field compared to the laboratory, and laboratory grown algae are often unable to tolerate the intensities found in natural conditions (Fogg et al. 1973). A similar situation often occurs with temperature. *Anabaena flos-aquae*, isolated from Lake Windermere, could not tolerate temperatures above 23 C in laboratory conditions. Several other studies have found quite different temperature optima for growth in the laboratory compared with field studies (Seaburg and Parker 1983). Algae were also supplied with nutrient sufficient conditions with the exception of carbon. While it is understood that some of these conditions would not occur in natural systems, the model illustrates an interactive effect among the parameters studied and shows that growth rate is a function of all of these parameters at both optimal and suboptimal conditions. It also demonstrates that in addition to the limiting substrate concentration, a physical factor can be simultaneously limiting.

Generally, with nutrients such as nitrogen or phosphorus, the nutrient status of the natural populations cannot be

determined from external concentrations since observations are instantaneous readings (Zavenboom et al. 1982), and the internal nutrient is not a reflection of the external nutrient concentration in nature. When algae are depleted of phosphorus and then placed in fresh phosphorus rich media, the media is rapidly depleted of phosphorus and the initial rates of phosphate uptake are far in excess of the organism's specific growth rate (Droop 1973).

Although recent studies have concluded that some algae are able to concentrate carbon dioxide (Badger 1985), this study assumed that the rate of uptake was equivalent to the rate of utilization for maintenance and growth. In all experiments, cell density maxima occurred on the day when threshold concentrations were reached or occurred one to two days prior to reaching final positive carbon fixation values. This supports the assumption that algae were utilizing the carbon they were assimilating and not storing it for use when carbon depletion occurred. Further, the studies indicating differences in external and internal inorganic carbon concentrations occurred at lower pH's where the enzyme ribulose bi-phosphate carboxylase is somewhat inactive.

With respect to the assumption of assimilation of carbon in the form of carbon dioxide, some evidence has been presented for direct assimilation of bicarbonate (HCO_3) (Tabita 1987), however, proof as to whether or not blue-green algae can assimilate it seems lacking (Gavis and Ferguson 1975; King and Novak 1974). In green algae, Findenegg (1980)

suggested that the algae were able to take up bicarbonate ions provided they had been adapted to low carbon dioxide levels prior to the experiment. However, Lehman (1978) said while CO_2 is the only substrate used for carbon fixation in the green alga, *Chlamydomonas*, rates of carbon fixation could be enhanced at low carbon dioxide concentrations if bicarbonate is transported into the cell, and converted to carbon dioxide by either lower intracellular pH or by the enzyme carbonic anhydrase. Burris et al. (1980) hypothesized that although bicarbonate may be present in sufficient amounts, either the algae could not directly use it for photosynthesis, or if they could, the rate of bicarbonate dehydroxylation to carbon dioxide is slower than the utilization of carbon in photosynthesis.

The use of this model requires a growth limiting substrate concentration. In this study, carbon was chosen as the rate limiting substance. Although phosphorus or nitrogen is commonly the rate limiting nutrient in natural conditions, under highly eutrophic conditions, carbon can become limiting. Short term carbon limitation in freshwater lakes has been reported in eutrophic systems in which CO_2 influx into the lake from the atmosphere was not sufficient to support photosynthesis (Schindler and Fee 1973; Burris et al. 1981). Dense populations of blue-green algae crowded into the upper few millimeters of the water column have also been found to locally deplete dissolved nutrients so that growth is prevented. The algae may consume CO_2 at such a fast pace that

the rate of supply by diffusion from the surrounding water and overlying atmosphere becomes rate limiting to photosynthesis (Walsby 1987).

More importantly, the occurrence and eventual dominance of blue-green algae is routinely found under conditions of low carbon dioxide concentrations and high pH values, brought about by a complex interaction of physical, chemical, and biological parameters that provide advantage to the blue-greens who have a high affinity for carbon dioxide at low concentrations.

BLUE-GREEN ALGAE IN LAKES

A large number of ecological factors influences the occurrence and dominance of blue-green algae in lakes. The environmental factors of light, temperature, and nutrient status, combined with the potential for positive buoyancy can be applied toward developing a plausible explanation of bloom forming conditions in nutrient enriched lakes.

Available light intensity is important not only for determining the status of the blue-green algae, but also in determining the status of other algae. With blue-greens, a decreased light intensity will reduce growth rate, but the concomitant increase in buoyancy allows them to migrate into the upper portions of the water where light may be higher. Their vertical migration up and down in the water column as a result of buoyancy regulation allows them to exploit areas where nutrients may be more plentiful. With other algae, such

as diatoms and green algae, increased stress and decreased growth rates brought about by a decrease in light and carbon dioxide can result in increased sinking rates (Jaworski et al 1981; King et al. 1983). Even if the algae have low threshold carbon dioxide concentrations, King (1980) reported that, with *Chlorella*, its inability to maintain mass in the photic zone would limit its development at carbon dioxide concentrations three to four orders of magnitude higher than its true physiological threshold concentration.

Following spring turnover, nutrient and CO_2 concentrations would be high under eutrophic conditions. Algal populations are generally first dominated by diatoms and succeeded by small flagellates and green algae (Wetzel 1983). As spring progresses, solar radiation increases, and photosynthesis increases. Because water temperatures generally decrease with depth, blue-greens that have a higher temperature optimum for growth might be present in the lower water strata of the photic zone, but their growth rate would be retarded. Reynolds (1971) suggests that surface accumulation of blue-green algae in some of the mires he studied resulted from concentrations previously distributed in deeper water rather than from growths at the surface. Thomas and Walsby (1986) found that *Microcystis* colonies became entrapped on the sediment during autumn and were released in spring when temperatures began to warm. Thus blue-greens, such as *Anabaena,* may be present in the water column, but a low growth rate brought about by suboptimal temperatures keep them from

gaining dominance. Colder water species like *Oscillatoria rubescens* may be more successful in the early spring at forming deep water population maxima, but often tend to remain at this depth since they favor low light and nutrient rich conditions (Klemer et al. 1982).

As spring progresses and temperatures warm, growth rates of many of the algal groups increase. With a sufficient amount of phosphorus and other nutrients present, photosynthesis will increase causing a rise in pH and subsequent decline in free carbon dioxide. Concurrent with rising photosynthesis is a decrease in light intensity due to the increased density of algae.

As light intensities decline in the epilimnion, blue-greens in the deeper depths will begin to synthesize gas vacuoles. With decreased carbon dioxide concentrations and increased temperatures, blue-greens could obtain buoyant conditions even if light levels are above an intensity that normally could cause collapse of the vesicles. Growth rates would continue to increase until light levels were reduced significantly. Because of their high affinity for low carbon concentrations and their ability to remain buoyant, blue-greens would outcompete other algae that were sinking from the photic zone.

The key for all of these conditions to occur is phosphorus enrichment. An increase in phosphorus is usually the stimulus for increased plant production, since in many temperate lakes phosphorus naturally occurs in the least

amount relative to the needs of the algae. Several studies have shown a strong correlation between phosphorus and algal standing crop (Dillon and Rigler 1974; Vollenweider 1976). In the development of a model for predicting the summer peak biomass of blue-greens in Swedish lakes, Smith et al. (1987) concluded that the most effective management practice for the four major bloom forming algae involves control of phosphorus loading to the lakes.

During cultural eutrophication of lakes, phosphorus can enter the water from a variety of human controlled sources, such as wastewater and industrial discharges, agricultural and urban fertilization, septic system leachate, and stormwater runoff. As phosphorus input increases, phytoplankton productivity increases, and the dominance of algae in the open water will begin the series of events previously mentioned.

However, prior to a depletion of carbon dioxide and reduction in light intensity, inorganic nitrogen levels begin to decrease through a multitude of processes including uptake by plants, volatilization of ammonia to the atmosphere as pH increases (King and Burton 1979), and denitrification, which usually occurs in the hypolimnion of a stratified lake.

The ability of blue-green algae to fix atmospheric nitrogen may lend a physiological advantage to them over non-nitrogen fixing algae, however, several studies (Spencer and King 1989; Klemer et al. 1982) have found that buoyancy is reduced when inorganic nitrogen is in short supply, but its presence promotes bloom formation under low light conditions.

Thus, the nitrogen fixing blue-green algae could benefit from low nitrogen conditions, where they would have a growth advantage over other non-nitrogen fixing algae, since they could undergo nitrogen fixation. They would also benefit from high nitrogen conditions, which would increase the buoyancy of the algae and establish their presence in the upper waters of the lake.

In temperate dimitic lakes, a series of events that are usually subject to phosphorus enrichment must occur if blue-greens are going to experience bloom forming conditions. Since low light intensities are necessary for buoyancy to occur in the presence of adequate carbon concentrations, other non-blue-green algae must first become dominant. Only if nutrients are available in sufficient quantity will this condition be met.

For any algal species, a series of conditions must be met in order to be successful at competing with other biological components of the system. It must remain in the photic zone; it must be able to maintain access to available nutrients needed for maintenance and growth; and it must be able to utilize these nutrients for growth (Reynolds 1984). Through the evolution of morphological and physiological adaptations in response to changing environmental and biological factors, the blue-green algae have proven successful at meeting these conditions.

As a result of their nitrogen fixing ability, their high affinity for carbon dioxide, the ability to synthesize gas

vacuoles and move up and down in the water column, their relatively higher temperature optima, and their lack of grazing pressure, blue-green algae have become well adapted to eutrophic waters during summer and often dominate the phytoplankton assemblage. Their dominance and success in these waters appears to be dependent upon a number of interacting factors that affect both their growth rate and buoyancy.

CONCLUSIONS

This study demonstrated that both the growth rate and regulation of buoyancy of the blue-green alga *Anabaena flos-aquae* were dependent on an interaction of light, temperature, and carbon dioxide concentration. The growth rate was affected by light, and by temperature, even under somewhat subsaturating light conditions. When higher concentrations of external carbon dioxide were available to the algae, the temperature range for positive growth increased, and specific growth rates were higher at their respective light intensities and temperatures.

Under optimal conditions of temperature and the highest light intensity used, doubling time for the species was 1.3 days; at 15 C, the doubling time was 11 days.

Buoyancy was directly dependent on light while temperature and carbon dioxide affected buoyancy indirectly. Under high light, as temperature increased above and below optimal growth temperatures, the amount of carbon dioxide needed to maintain nonbuoyant conditions increased. The effect was most dramatic at the highest temperature of 29 C, where nearly ten times more carbon was needed than at 21 C to maintain nonbuoyant conditions. The blue-green *Oscillatoria sp.* was able to remain nonbuoyant at carbon dioxide concentrations two

orders of magnitude lower than *Anabaena flos-aquae* at similar temperatures.

Generally, under low light intensity and any temperature, cells became buoyant, but growth rates were always less than 0.35 per day. However, *Anabaena* grown in experimental conditions under cold temperature at low light intensity did not form gas vacuoles and were not buoyant. This anomaly may be a factor in the loss of buoyant conditions in fall when temperatures begin to decline.

At medium light, temperature did affect cell buoyancy. While cells were always buoyant under optimal temperatures, the combined temperature extremes and carbon levels influenced whether cells were positively or negatively buoyant.

At high light and at any temperature where positive growth occurred, growth rate increased to as high as 1.2 per day, but gas vacuoles collapsed and positive buoyancy declined.

A predictive equation for growth of *Anabaena* at any temperature under three light levels was developed using the kinetic constants of the maximum specific growth rate, the half-saturation constant, and the threshold concentration. This equation, although based on results from laboratory conditions, provides insight on both the interaction of factors affecting growth and on the algal's kinetic constants, which provides an indication as to their competitive abilities with other algae.

Since both growth rate and buoyancy are functions of

light, temperature, and nutrients, the potential for bloom formation in *Anabaena* was predicted by determining the multiplicative relationship of these two processes. In natural systems, blue-greens may be influenced by other factors such as grazing and mixing.

Although factors such as light, N:P ratios, carbon concentrations, temperature, grazing, and buoyancy have each been individually presented in the literature as the most important contributor to the rise and dominance of blue-green algae in temperate lakes during summer, this data provides evidence of multiple interaction among physical and chemical factors in affecting both the growth rate and buoyancy of blue-green algae.

APPENDICES

APPENDIX I.

Table A1. Algal nutrient media and concentrations.

Compound	Concentration (mg/L)
Sodium bicarbonate ($NaHCO_3$)	168
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	20
Ferric chloride ($FeCl_3 \cdot 6H_2O$)	6.66
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	40
Potassium phosphate (K_2HPO_4)	39
Ammonium chloride (NH_4Cl)	5
Sodium Nitrate ($NaNO_3$)	250
$EDTA \cdot 2H_2O$	11.07
Sodium meta-silicate ($Na2SiO_3 \cdot 9H_2O$)	58
Biotin	0.002
B_{12}	0.002

Micronutrient Solution	Concentration (g/L)[1]
Boric Acid (H_3BO_3)	2.86
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	1.81
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	0.08
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.22
Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	0.39
Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.049

[1] Removed one ml/L from micronutrient solution for stock solution

APPENDIX 2.

Table A2. Analysis of variance for the μmax of *Anabaena flos-aquae* and *Oscillatoria sp.*

TWO-WAY ANOVA - *Anabaena*

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F RATIO
COLS	1.643	4	.411	44.97
ROWS	2.997	2	1.498	164.093
INTERACTION	.698	8	.087	9.561
ERROR	.274	30		9.13E-03
TOTAL	5.612	44		

ONE-WAY ANOVA - *Oscillatoria*

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F RATIO
BETWEEN	.235	2	.117	14.417
WITHIN	.033	4	8.1349E-03	
TOTAL	.267	6		

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LIST OF REFERENCES

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