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Title: THE EFFECT OF LIGHT INTENSITY ON THE ORGANIC COMPOSITION OF MARINE PHYTOPLANKTON DIATOMS

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Herbert C. Curl, Jr.

Investigation was made into the influence of changing light intensity on the organic and elemental composition of two species of marine diatoms, Ditylum brightwellii and Skeletonema costatum. Analysis was made of the relative changes in soluble and insoluble carbohydrate; polar, non-polar, and total lipid; amino acids; proteins; and total carbon, hydrogen, nitrogen, and oxygen. Photosynthetic quotients were calculated from both organic and elemental composition.

The response of soluble and insoluble carbohydrate was directly related to light intensity, although differences were noted between the two fractions. In general, amino acids and proteins showed little or no response to light intensity. Polar (pigment) lipids usually varied inversely with light intensity, whereas non-polar lipids varied directly with intensity. Total lipids also varied

inversely with intensity although, considering the total amount of lipid present, the changes were slight. Some differences in relative organic composition were noted between the two species. On the basis of the data, average organic composition of each species was computed and compared with similar data in the literature.

The effect of light intensity on the elemental composition of the two species yielded some differences, which were more readily apparent in the photosynthetic quotients calculated from elemental data. The photosynthetic quotient varied inversely with light intensity.

Conclusions are summarized and suggestions for future research included.

# The Effect of Light Intensity on the Organic Composition of Marine Phytoplankton Diatoms

by

Allan David Marmelstein

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Redacted for Privacy

Chairman of Department of Oceanography

Redacted for Privacy

Dean of Graduate School

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## LITERATURE REVIEW

The position of the phytoplankton as primary producers soon led to an interest in the amount of organic production they undergo. Examination of the photosynthetic equation suggests two obvious approaches to its measurement: carbon dioxide uptake and oxygen production. Historically, the first measurements of photosynthesis were made using oxygen changes. Gaarder and Gran (1927) were the first to measure phytoplankton production in this manner. Much later, Steemann Nielsen (1952) introduced the carbon-14 technique to marine primary production measurement.

Although at the time the only method available, the measurement of photosynthetic production using oxygen evolution introduced one complication. In the formation of organic matter and oxygen from carbon dioxide and water during photosynthesis, the relationship of the two gases is rarely one to one. Even though the simplified equation of photosynthesis would indicate that for every mole of oxygen produced one mole of carbon dioxide is reduced, in practice this is not the case. The relationship of carbon dioxide incorporated to oxygen released is termed the Photosynthetic Quotient (P. Q.) and is defined:

$$\text{P. Q.} = \frac{\text{moles oxygen released}}{\text{moles carbon dioxide incorporated}}$$

It is readily apparent from an examination of the basic components of a cell that, in their manufacture photosynthetically, widely varying P. Q. values would result (Davey, Marmelstein and Curl, 1969, Table 1). The P. Q. values presented assume nitrate as the nitrogen source to the autotrophic organisms. This form of nitrogen occurs most frequently and most abundantly in the marine environment (Harvey, 1963). The P. Q. can be affected by the quantity and quality of nitrogen supplied as well as metabolites produced. Nitrogen in the ammonium form results in much lower P. Q.'s of formation for those metabolites containing nitrogen. The higher P. Q.'s result from oxygen evolved during nitrate reduction.

Table 1. The P. Q. of formation of various cellular metabolites, based on nitrate nutrition[1]

| Metabolite | P. Q. |
|---|---|
| Carbohydrate | 1.00 |
| Amino Acids | 1.64 |
| Protein | 1.64 |
| Lipid | 1.40 |
| Nucleic Acids (Average of RNA & DNA) | 1.78 |

[1]After Davey, Marmelstein and Curl (1969)

When converting data on oxygen production to carbon dioxide incorporation, a P. Q. value must be chosen. Ryther (1956), noted that values of P. Q. as measured by gas exchange seldom agreed with those calculated from the elemental composition of cells. Myers and Cramer (1948) suggested that the differences found may have been due to growing the organisms at low light levels but, due to the requirements of manometric techniques, measuring gas exchange at higher light levels. Ryther, on the basis of available data, suggested a P. Q. of 1.25. More recently, Davey, Marmelstein and Curl (1969) have suggested a value of 1.45, based on nitrate metabolism, and the average composition of marine diatoms.

Davey (1970) and others have shown that a shift from nitrate to ammonium nutrition can have a marked effect on P. Q. Equally as striking, however, is the effect due to the amounts of the various metabolites produced by photosynthetic cells (Spoehr and Milner, 1949).

The research to be herein described will attempt to demonstrate how changing environmental conditions, specifically light, affects the metabolism of marine diatoms, as reflected in the concentration of various metabolites. Diatoms were chosen as the test organism because they are by far the most abundant component (in biomass if not in numbers) of the marine phytoplankton

(Raymont, 1963).

Data on the organic composition of marine diatoms are scarce. Slightly more is known of the composition of various other species of single-celled algae. Data are also available on the composition of macroscopic, benthic algae. In general, however, the latter are much different even than the unicellular members of their own taxa and will be ignored for that reason. Concentrations of the metabolites of algal species are reported in a variety of ways, the most common being carbohydrate, protein, and fat. Listed less frequently are free amino acids, nucleic acids, and other organic acids, as well as specific components of the three major groups listed first. Some data are also available on the effect of environmental influences on the concentrations of the various metabolites listed.

Lewin and Guillard (1963) present data accumulated from many sources on the percentage of fat, carbohydrate, and protein in several species of marine diatoms. Included in these data are those of Parsons, Stephens and Strickland (1961) showing the concentration of the listed compounds in Skeletonema costatum. Ketchum and Redfield (1948) give values for these same categories in Nitzchia closterium (now taken to be Phaeodactylum tricornutum; see Lewin, 1958). Collyer and Fogg (1955) have presented data showing the changes with time of fat, protein, and carbohydrate in a

fresh water species of Navicula. Spoehr and Milner (1949) also present data on the concentration of these three cellular components in the freshwater chlorophyte Chlorella pyrenoidosa. Bidwell (1957), working with two marine flagellates, reported, among other things, the presence of several different sugars.

Initially, it was reported that no free amino acids were present in marine diatoms (Low, 1955). Subsequently, the presence of free amino acids in this group was demonstrated by Rho (1958). Further, Bidwell (1957) has shown free amino acids to occur in marine flagellates as well. Chau, Chuecas and Riley (1968) have determined the amino acid composition of protein hydrolyzates of various marine phytoplankton.

By far the most abundant data available on diatom composition concerns lipid concentrations. This fact may stem in part from the belief, first stated by Beijerinck (as reported by Barker, 1935), that fats were the major metabolite found in diatoms. Subsequent authors have shown that, at times, either protein or carbohydrate or both predominate over lipid in marine diatoms (Parsons, Stephens and Strickland, 1961; Lewin and Guillard, 1962; and Strickland, 1965). In short, considerable discrepency exists, a matter to be commented on in more detail in a later section. Nevertheless, analysis of the lipid components of diatoms is prevalent in the literature. In addition to those authors listed in

the discussion of protein, carbohydrate, and lipid taken together, analysis of the fat or lipid content of diatoms was undertaken by Clark and Mazur (1941), Fogg (1956), Collyer (1962), and Kates (1966). More recently, Ackman and his co-workers have turned their attention to the fatty acid distribution in the lipids of marine plankton, including diatoms (Ackman, et al., 1964 and Brockerhoff, et al., 1964). Patton, et al. (1966) have analyzed the fatty acids of a red tide organism.

Sutcliffe and Sharp (1968) and Baraskov (as reported by Strickland, 1965) have determined the concentration of nucleic acids in marine diatoms. Rho (1958) commented on the occurrence of free organic acids in a marine diatom.

Strickland (1965) has prepared an excellent review of the entire question of organic composition of marine phytoplankton, encompassing nearly all of the material discussed in the preceding paragraphs.

Several authors have reported the percent carbon (Curl, 1962 and Eppley and Sloan, 1965) or percent carbon, hydrogen, and nitrogen (Ketchum and Redfield, 1949 and Parsons, Stephens and Strickland, 1961) in unicellular algae, including marine diatoms. Indeed, some of the composition data reported above were derived from carbon, hydrogen, nitrogen and, occasionally, oxygen concentrations by simultaneous equations (cf. Spoehr and Milner, 1948 or

Ketchum and Redfield, 1949). Unfortunately, this method may lead to erroneous results as a result of invalid assumptions. The equations used for deriving metabolite concentration from carbon, hydrogen, nitrogen data begin by assuming percent protein to be 6.25 times the percent nitrogen. This multiplication is only an approximation at best, and frequently the value taken for nitrogen is total nitrogen, when, in fact, the factor 6.25 is based on percent alpha-amino nitrogen only. Clearly, some portion of the total nitrogen exists as free amino acids, porphyrin rings, and nucleic acids. This method of deriving metabolite concentrations may be responsible for the discrepancies noted in the relative abundance of protein, lipid, and carbohydrate.

The majority of the work discussed to this point reflects the results of analyses performed on laboratory cultures or collections of "in situ" material. In neither case has consideration of the past environmental history of the organisms been taken. Collyer and Fogg (1955) did follow the time course change in fat, protein, and hydrolyzable carbohydrate, as well as ash, in Navicula. In addition, Spoehr and Milner (1948) reported the effects of changing light and nutrients, among other things, on the elemental composition of Chlorella. From the elemental composition, these authors also calculated the effect of the two parameters on fat, protein, and carbohydrate concentration. The effects of light quality on the

composition of Chlorella have been reported by Hauschild, Nelson and Krotov (1962).

The effect of changing light intensity on the metabolism of marine diatoms, as reflected by organic and elemental composition, has not, however, been determined. That such changes may occur can be inferred from the work of Spoehr and Milner (1948) and Davey (1970), as well as from that of Horváth and Szász (1965). The latter two authors demonstrated that change in light intensity had a marked effect on the concentration of soluble carbohydrates, starch, and soluble nitrogen, while affecting protein nitrogen only slightly, in the leaves of sugar bean. The goal, then, of this thesis will be to demonstrate what changes occur in the organic and elemental composition of marine phytoplankton diatoms as a result of changing light intensity.

# DITYLUM BRIGHTWELLII

## INTRODUCTION

Historically, determinations of primary production in the oceans were performed first by Gaarder and Gran (1927), using the oxygen method. Much later, Steemann Nielsen (1952) introduced the carbon-14 technique to production measurement. The use of oxygen as a parameter to monitor photosynthetic production introduced the necessity of converting oxygen evolution to carbon assimilation. In his review of this problem, Ryther (1956) suggests the use of a P. Q. of 1.25 when converting oxygen values to carbon assimilation. He notes, however, the discrepancy between P. Q. values as calculated from gas exchange and those calculated from the elemental composition of organisms. Myers and Cramer (1948) had suggested that the former method might produce lower values due to the high light intensities used in manometric techniques. In a preliminary paper, Davey, Marmelstein and Curl (1969) suggest a P. Q. of 1.45 for marine phytoplankton, based on gas exchange, elemental composition, and metabolite concentration, assuming nitrate nutrition. Their findings are supported and expanded by the results to be reported herein.

A small body of data has been built up in the literature concerning the elemental and organic composition of marine

phytoplankton species and related organisms. These data have been assembled in the excellent reviews by Lewin and Guillard (1962) and by Strickland (1965). Relatively little information is available on the influence of environmental conditions on the composition of unicellular algae. Spoehr and Milner (1948) have followed the change in elemental composition of Chlorella due to changing environment. With regard to light, similar work has been done by Horváth and Szász (1965) using sugar bean, a higher plant. This paper will discuss the influence of light intensity on the elemental and metabolite composition of the marine phytoplankton diatom Ditylum brightwellii and their subsequent influence on P. Q. values under the various light intensities.

# METHODS

Cultures of D. brightwellii were grown in an enriched sea water medium, following a technique modified from Guillard and Ryther (1962). Sea water aged in the dark one month was Millipore® filtered and autoclaved for sufficient time to insure sterility as regards autotrophic organisms. In addition to those nutrients specified by Guillard and Ryther, 20 ml. of a one percent $NaHCO_3$ solution and one milligram of thiourea were added per liter of sea water upon cooling. Culture size varied between eight and nine liters. The cultures were grown in 12 liter pyrex carboys at $16^{\circ}C \pm 2^{\circ}C$ and were agitated by bubbling with atmospheric air. The clone of D. brightwellii used was originally isolated from Oregon coastal waters and was derived from maintenance cultures employing similar media. The cultures were not bacteria free, and no attempt was made to remove bacteria. However, inspection by phase microscopy revealed very low numbers of bacteria. The bacterial population did increase if the cultures were allowed to reach their peak, but, as the cultures used were in early log phase, this was not a problem. On the basis of cell volume, it was estimated that bacteria represented less than 0.1% of the material analyzed.

Light was applied to the cultures through a combination of fluorescent and incandescent lamps (Table 2). Circline® fluorescent

Table 2. Light intensities achievable with various combinations of fluorescent and incandescent lamps

| Lamps | Intensity ($mw/cm^2$) | ( Langlies/ minute x $10^3$) | (Langlies/ 24 hours) |
|---|---|---|---|
| 1 warm white | 0.40* | 5.6 | 8.0 |
| 1 warm white, 1 cool white | 0.72 | | |
| 1 warm white, 2 cool white | 1.11* | 15.5 | 22.4 |
| 1 filtered incandescent flood | 1.80 | | |
| 1 warm white, 2 cool white, 1 filtered incandescent flood | 2.80* | 39.5 | 56.8 |
| 1 unfiltered incandescent flood | 10.40 | | |

*Indicates intensities used.

lamps (one warm-white and two daylight) backed by a concentric sheet metal reflector were arranged such that the culture vessel was surrounded by the circular lamps. A Sylvania Cool-lux® incandescent flood lamp was placed above the culture vessel. Light from the incandescent lamp was filtered and reduced with a Corning infra-red absorbing filter glass number 4600. The spectral distribution did not change with various combinations of lamps and is close to that found in the ocean (Figure 1). Further, the daily irradience compares favorably with levels found in the euphotic zone in temperate latitudes during early spring blooms (Raymont, 1963).

The following sequence of treatments was used to demonstrate changes induced with changing light levels. An initial culture was

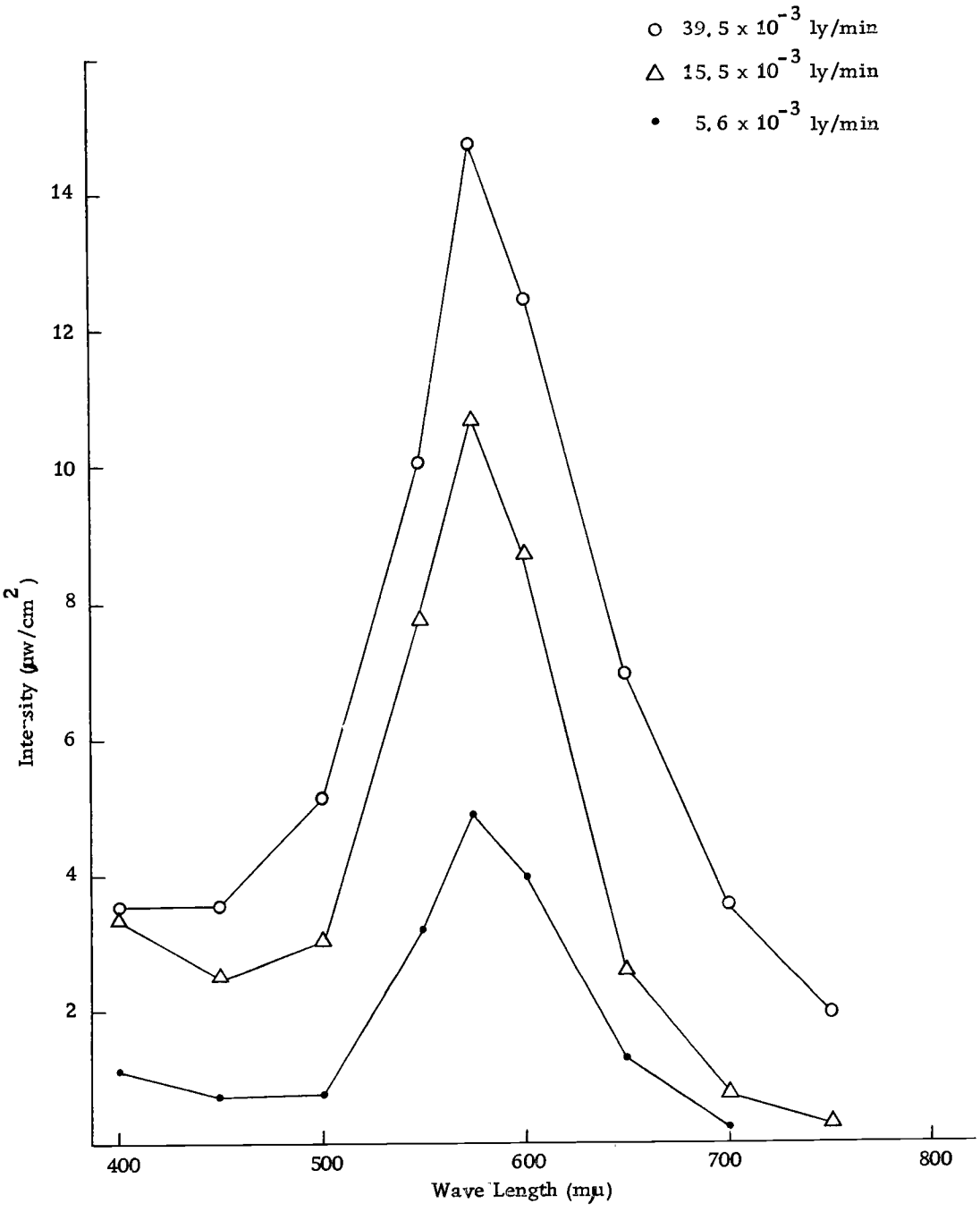


Figure 1. Spectral distribution of the three light intensities used.

grown at a light level of $5.6 \times 10^{-3}$ly/min (Langlies per minute), the inoculum having come from a culture acclimated to this light intensity. Upon harvesting, two subcultures were made from the initial culture. One of the new cultures was grown at the original light level; the other was grown at a light level increased to $15.5 \times 10^{-3}$ly/min. On harvesting the latter culture, a subculture was made from it, grown at the same light level. This culture was not harvested, but was used as a maintenance culture, allowing a somewhat longer period for acclimation to the higher light intensity. When the maintenance culture had reached the appropriate cell density, three subcultures were made from it. One of these was grown at the same intensity, $15.5 \times 10^{-3}$ly/min. The second was grown at a still higher intensity, $39.5 \times 10^{-3}$ly/min. The third culture was grown at a reduced intensity, $5.6 \times 10^{-3}$ly/min. Thus, not only were changes in metabolite concentration due to increased and decreased light intensity followed, but changes occurring with time at constant light were also followed. This sequence of treatments was repeated three times.

Cultures of D. brightwellii were harvested at a population level of $1\text{-}10 \times 10^{6}$ cells/liter. This population level was found to correspond to lower log phase under the method used. The size of the inoculum used was chosen so as to produce the above levels of growth within two to three days. Normally, this corresponded to an inoculum of 200 to 500 ml. Harvesting was accomplished by continuous

flow centrifugation. The resulting pellet was transferred to a small vial and lyophilized. The lyophilized material was stored in a vacuum desiccator for later analysis.

Analysis of the metabolic products was undertaken following fractionation of the lyophilized material. The procedure used for fractionation was modified from Kempher and Miller (1965). The dried cells were resuspended in five percent trichloroacetic acid to extract small molecules. Following centrifugation, the supernatant was analyzed for soluble carbohydrates and free amino acids. The pellet was resuspended in ten percent trichloroacetic acid and an aliquot of the suspension taken for analysis of insoluble carbohydrate and protein. The remaining suspension was again centrifuged, the supernatant discarded, and the pellet held for lipid extraction.

Carbohydrate concentrations were determined by the method of Dubois, et al. (1956) using glucose as a standard, amino acids by that of Rosen (1957) using leucine as a standard, and proteins according to Lowry, et al. (1951) using bovine albumin as a standard. Lipid extraction was done in two steps. First the pellet was extracted for at least eight hours with petroleum ether in a micro-Soxhlet apparatus, extracting non-polar lipids. Then it was extracted a second time with a mixture of methanol and chloroform, following the technique of Aaronson and Baker (1961), extracting polar lipids. Lipid content was determined by weight loss after each extraction.

Pigments were not removed in the polar fraction but did come out in the non-polar fraction.

At the time of harvest, aliquots of each culture were taken for ash-free dry weight determination and for carbon, hydrogen, nitrogen analysis. The latter were determined using an F and M Model 185 Carbon, Hydrogen, Nitrogen Analyzer, standardized with acetanalide.

Oxygen was determined either by difference from the ash-free dry weight or, more usually, by difference from the total of metabolites analyzed, as discussed in a later section.

Metabolite concentration is reported as percent of the total of fractions analyzed. P. Q. values for each treatment were calculated by weighted average from the values in Table 1 and the percent composition for each treatment. In addition, P. Q. values were computed from the elemental analysis obtained for each treatment.

The value of each organic fraction was regressed against light intensity, the three replicates being taken together. Using analysis of variance, each regression line was tested at the five percent level in order to determine if its slope varied significantly from zero. The P. Q. values computed from organic composition for each treatment were handled in a similar manner. Further, a 't' test was used to compare the P. Q. values so obtained to that of Ryther's (1956) value of 1.25. Due to the problems encountered in elemental analysis, as discussed in a later section, no statistics were attempted

on the elemental analysis or resulting P. Q. values. Only mean values of these quantities for each of the three intensities is reported.

# RESULTS

The results of the organic analysis obtained for each treatment in each of the three replicates are shown in Figure 2 (and in Tables 1A to 3A). The summarized results of the statistical computations are given in Table 3. For demonstrative purposes, the cumulative results for one fraction (free sugars) are shown graphically in Figure 3.

Free sugar concentration was found to respond in a positive manner to light intensity (Figure 3). As indicated in Table 3, the slope of the regression line was +1.84. This slope was significantly greater than zero even at the one percent level. Carbohydrate polymers behaved in a similar fashion, and the slope of the regression line for this fraction was also significantly greater than zero at the one percent level.

Both the free amino acid fraction and the protein fraction yielded slightly negative slopes when regressed against light. However, the correlation coefficients for both fractions, while negative, were extremely low, and neither slope was significantly different from zero. Therefore, both of these fractions are considered to be independent of light, and to have remained constant under the light intensities used.

Of the two lipid fractions analyzed, the non-polar fraction was found to have a positive correlation with light, whereas the polar

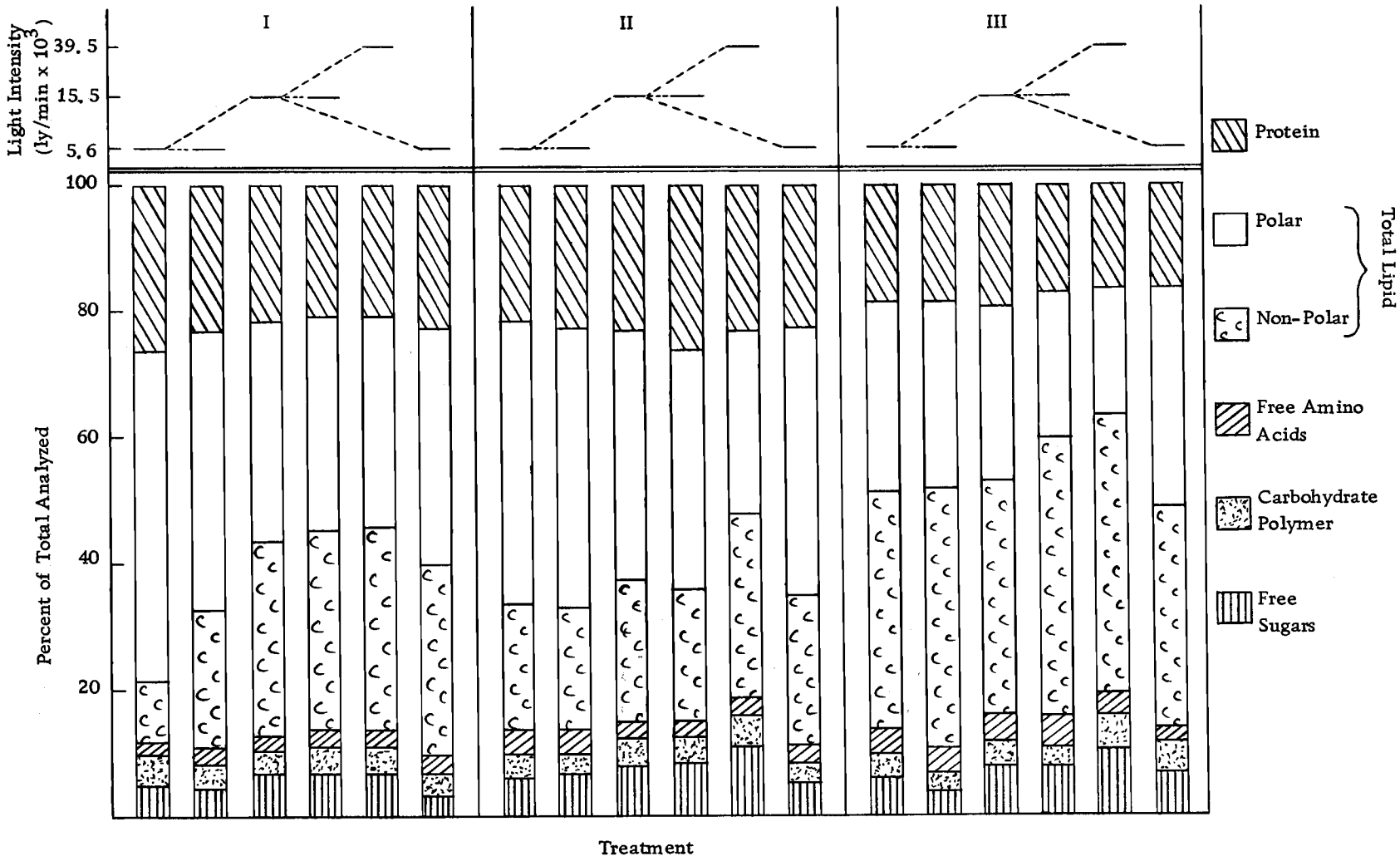


Figure 2. The influence of light intensity on the organic composition of Ditylum brightwellii, showing three replicates.

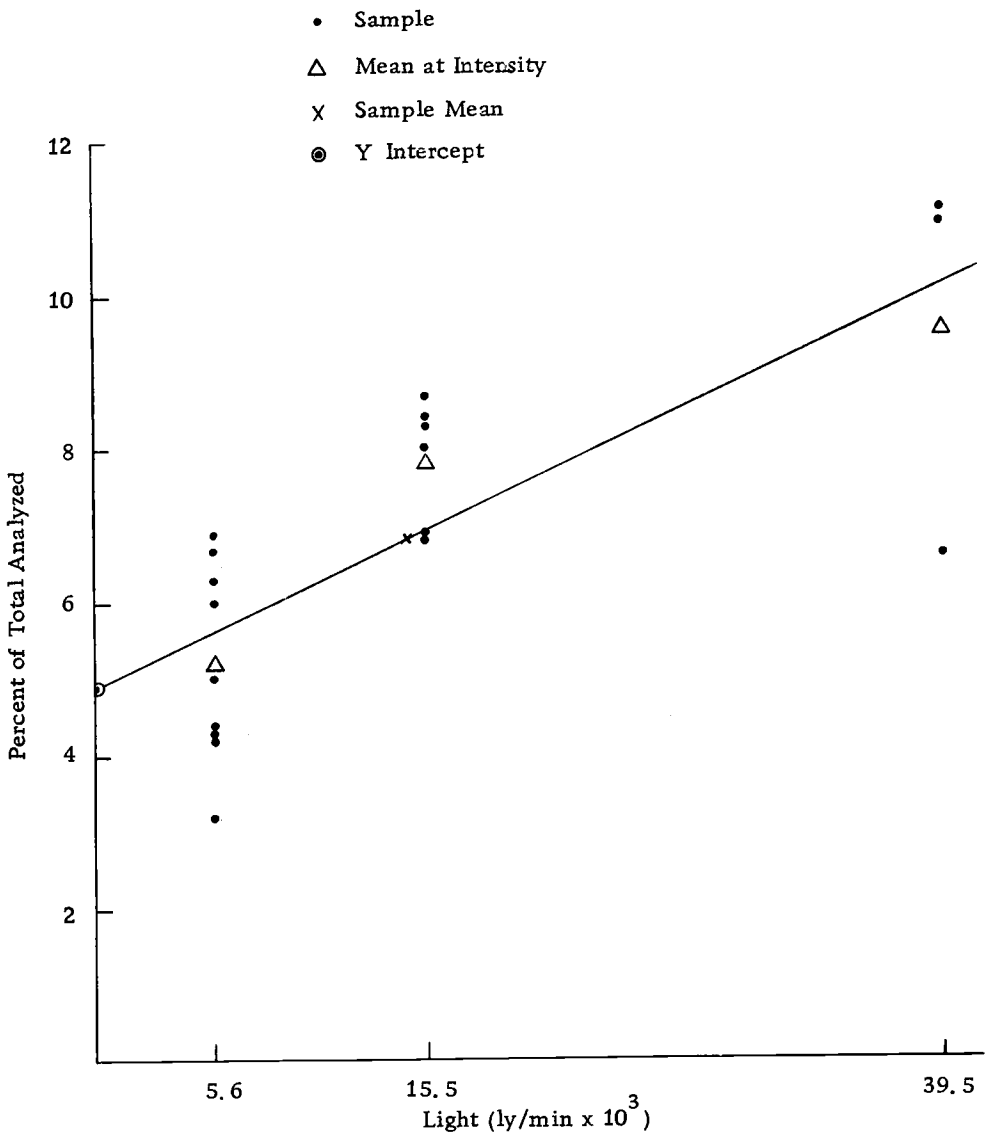


Figure 3. Cumulative data for free sugars in Ditylum brightwellii.

Table 3. Mean values (in percent), slopes of regression lines, and correlation coefficients for the organic fractions and derived P. Q. values at three light intensities in Ditylum brightwellii.

| Fraction | Mean | | | Slope | Correlation Coefficient |
|---|---|---|---|---|---|
| | Light Intensity (ly/min x $10^3$) | | | | |
| | 5.6 | 15.5 | 39.5 | | |
| Free Sugars | 5.2 | 7.8 | 9.5 | +1.84* | +0.74 |
| Carbohydrate Polymer | 3.8 | 4.0 | 5.1 | +0.49* | +0.62 |
| Free Amino Acids | 2.9 | 2.9 | 2.8 | -0.07 | -0.10 |
| Non-Polar Lipids | 26.9 | 30.7 | 35.1 | +3.46 | +0.32 |
| Polar Lipids | 39.5 | 32.9 | 26.5 | -5.50* | -0.58 |
| Total Lipids | 66.4 | 63.5 | 61.6 | -2.03* | -0.50 |
| Protein | 21.6 | 21.6 | 21.0 | -0.23 | -0.06 |
| P. Q. | 1.42 | 1.41 | 1.40 | -0.01* | -0.51 |

* Slope significantly different from zero at the five percent level.

fraction correlated negatively with light. In addition, the total lipid fraction also correlated negatively with light. The slopes of both of these latter regression lines were significantly greater than zero at the five percent level, whereas the slope of the regression line of the non-polar lipids was not significantly different from zero.

When the P. Q. values computed from organic composition were regressed against light they, too, were shown to relate negatively to intensity, and this response was significant at the five percent level (Table 3). These P. Q. values were found to be significantly greater, at the one percent level, than Ryther's suggested value. The one percent confidence interval computed from P. Q. values obtained in this manner was 1.40 - 1.42.

The mean P. Q. values computed from elemental analysis decrease as intensity increases (Table 4). However, no significance can be attached to these results.

Table 4. Mean value of carbon, hydrogen, nitrogen, and oxygen, and P. Q. values based on these means in Ditylum brightwellii.

| Element | Mean* Light Intensity (ly/min x $10^3$) | | |
|---|---|---|---|
| | 5.6 | 15.5 | 39.5 |
| Carbon | 6.7 | 7.4 | 7.1 |
| Hydrogen | 18.8 | 17.0 | 19.1 |
| Nitrogen | 1.0 | 1.0 | 1.0 |
| Oxygen | 5.5 | 5.9 | 6.0 |
| P. Q. | 1.52 | 1.38 | 1.39 |

* Number of atoms, with nitrogen set equal to one.

# DISCUSSION

The increase seen in free sugar concentration with increasing light intensity agrees with the results obtained by Spoehr and Milner (1948) for Chlorella and by Horváth and Szász (1965) for sugar bean. Spoehr and Milner found that increasing light intensity generally resulted in a decreased R value, which would indicate, by their reasoning, an increase in carbohydrate. Horváth and Szász found, by direct analysis, that both soluble and insoluble carbohydrate increased as light intensity increased. The same was found to be true with Ditylum, although free sugars showed a greater increase than did carbohydrate polymer. As discussed by these latter authors, the increase in carbohydrate concentration is more than likely due to a shift in metabolic pathways in response to increased availability of reducing potential, in the form of NADPH+, and energy, as ATP, at higher light intensities.

Horváth and Szász (1965) found that while protein nitrogen remained constant with light, soluble nitrogen, indicating free amino acids, decreased with increasing light intensity. Ditylum analyses agree with this result as regards protein, but it was found that amino acids also remained at a constant level with increasing light. Horváth and Szász (1965) suggested that, under decreased light, amino acids may be favored over carbohydrates, as less energy is required for their formation. However, for an autotrophic organism growing with

a nitrogen source supplied as nitrate, considerable energy must be spent in reducing nitrate to amino nitrogen. For instance, reductive aminations require the expenditure of reducing potential in the form of NADPH. Therefore, for an organism receiving its nitrogen as nitrate, there may be no benefit in shifting from carbohydrate to amino acid synthesis as the energy available from photosynthesis decreases, particularly when a reshuffling of the lipid fractions toward increased pigment occurs. Furthermore, synthesis of protein from amino acids requires even greater energy expenditure for amino acid activation, condensation onto transfer RNA and polymerization. The lack of change seen in the concentrations of these two metabolites could reflect the actual energy requirements involved, resulting in maintenance amounts of amino acids rather than luxury production.

The changes seen in the two lipid fractions, as well as in total lipid, may result from the interaction of two or more processes. The lipid fraction containing pigments was seen to vary inversely with light, while the other lipid fraction varied directly with light, although not significantly. When the two fractions were summed, it was found that total lipids also varied inversely with light intensity. It might be expected that pigment content would vary inversely with the light intensity (Strickland, 1965). When available light energy decreases, an increase in the photosynthetic pigment concentration would increase the efficiency with which the available light may be

utilized for photosynthesis. Although the fraction of polar lipid decreased as light increased, the amount of non-polar lipid increased, so that the overall effect was to keep total lipid relatively constant. On a molar basis, lipids are among the more energy rich compounds found in organisms. The shifting of the two lipid functions to maintain a relatively constant amount of total lipid appears, at first glance, to be a futile expenditure of energy. However, one problem facing planktonic organisms is that of flotation. The large amount of lipid contained in marine diatoms was at first thought to reflect a flotation mechanism. Later workers demonstrated that, in general, marine diatoms control their buoyancy by adjusting their ratio of monovalent to divalent ions, relative to sea water (Strickland, 1965). However, it is possible that the latter mechanism represents a fine adjustment and that total lipid content is used as a coarse adjustment. This would explain, in part, the occurrence of nearly constant lipid content, even though the shift from the non-polar (pigment) to polar fraction requires a considerable energy expenditure.

By taking the mean value of the means under each treatment for each of the metabolites analyzed, one can arrive at an average composition for Ditylum under the experimental procedures employed (Table 5). It is clear from these averages that Ditylum, at least, shows a different composition by direct analysis than that reported by previous authors for other marine diatoms. However, as pointed

Table 5. Average organic and elemental composition of Ditylum brightwellii.

| Fraction | Percent Composition | Element | Number of Atoms |
|---|---|---|---|
| Free Sugars | 7.5 | Carbon | 7.1 |
| Carbohydrate Polymer | 4.3 | Hydrogen | 18.3 |
| Free Amino Acids | 2.9 | Nitrogen | 1.0* |
| Non-Polar Lipids | 30.9 | Oxygen | 5.8 |
| Polar Lipids | 33.0 | | |
| Total Lipid | 63.9 | | |
| Protein | 21.4 | | |

* Nitrogen set equal to one.

out earlier, some of the previous analyses were based on elemental, rather than organic, composition. Whereas Ditylum proves to be roughly 64% lipid, 21% protein and 12% carbohydrate, the majority of the previous data on composition of marine diatoms, as presented by Lewin and Guillard (1963), is in marked contrast to the above ratios. Only Serenkov and Pachoma (Lewin and Guillard, 1963), working with Rabdonema and Chaetoceros, found fat to be the predominant fraction, followed by carbohydrate in the former organism and protein in the latter organism. Collyer and Fogg found carbohydrate to be the predominant fraction in a 33-day-old culture of Navicula pelliculosa, a freshwater benthic diatom, followed by fat and protein, in decreasing order. The remaining authors quoted by Lewin and Guillard all found protein to be the predominant fraction in marine diatoms. Ketchum and Redfield (1949), working with P. tricornutum, also found protein to be the predominant fraction, followed by carbohydrate and fat, in decreasing order. However, these latter two authors' data are based on elemental composition and may be in error for that reason.

The P. Q. computed by weighted average from the data in Table 4 is 1.41. The only metabolites which may occur in significant amounts that are not reflected in this P. Q. are nucleic acids. Several authors have determined a value for nucleic acids in diatoms (Sutcliffe and Sharp, 1968 and Baraskov as reported in Strickland,

1965) and this value averages about five percent of the ash-free dry weight. The fact that large chain carbohydrate polymers varied less than short chain compounds, and that protein concentration remained constant, would imply a fairly constant level of nucleic acids. DNA concentration should remain constant, as the cells were dividing non-synchronously under constant illumination. The same should be true for RNA. Therefore, the effect of the five percent or so of nucleic acid present, with an average P. Q. of formation of 1.78, would be to increase the P. Q. determined by weighted average by a few hundredths, at most.

Considerable variation was found to occur in the elemental composition of Ditylum under the various treatments. Although the values for carbon and nitrogen are considered accurate, less confidence is placed in the hydrogen and oxygen values; therefore, only mean values have been reported. Hydrogen is measured as water, and moisture in the sample introduces an error. Determining oxygen by difference leads to further error. Due to problems with the ash-free dry weight, both percent composition of metabolites and total carbon, hydrogen, nitrogen, and oxygen, are based on the total of all metabolites analyzed, rather than ash-free dry weight. Even so, the P. Q. based on average elemental composition (Table 4) is 1.45, so that the P. Q. values based on both organic and elemental composition are higher than the P. Q. of 1.25

suggested by Ryther (1956), the former significantly so. As pointed out by Davey, Marmelstein and Curl (1969), use of a higher P. Q. value results in much closer agreement between production measurements based on oxygen and those based on Carbon-14.

# SKELETONEMA COSTATUM

## INTRODUCTION

As indicated previously, a knowledge of the influence of light on the metabolism of marine phytoplankton is, in many cases, essential to the interpretation of production data. In the preceding section, light intensity was shown to have a definite influence on the organic composition of one species of marine phytoplankton diatom, Ditylum brightwellii.

For comparison, similar manipulations were undertaken using another marine phytoplankter, Skeletonema costatum. This species is also of interest in that a considerable amount of the physiological work already accomplished with marine diatoms has employed it as the test species. Much of this physiological work is included in the review by Strickland (1965).

# METHODS

The methods employed in the culture, experimental manipulation, harvesting, preservation, fractionation, and analysis of the various organic and elemental components of Skeletonema costatum were identical to those employed previously for Ditylum brightwellii, with the following exceptions. The inorganic nutrients specified by Guillard and Ryther (1962), as well as the thiourea, were added to the sea water medium at a concentration one-half that used before. Vitamins and bicarbonate were added at the same concentration as previously used. Cultures of Skeletonema were harvested when the population reached a level of 100 to 400 x $10^6$ cells per liter. With this organism and the medium described, this population corresponded to early log phase. The inoculum was controlled so as to produce the population desired in two to three days.

The problem of bacterial contamination occurred with Skeletonema also, but was controlled in the same manner as outlined for Ditylum. Ash-free dry weights again lacked precision, so that results here are also reported as percent total metabolites analyzed, in the case of organic constituents, or as number of atoms, with oxygen calculated as the difference between total metabolites analyzed and total of carbon, hydrogen, and nitrogen analyzed, in the case of elemental constituents.

Statistical analysis was performed as before.

# RESULTS AND DISCUSSION

The concentration of the metabolites analyzed in Skeletonema varied with changing light intensity, but in an irregular manner (Figure 4 and Tables 1B and 2B). As a result, neither the organic fractions analyzed, nor the P. Q. based on organic composition, yielded a slope significantly different from zero when regressed against light (Table 6). However, some insights into the reason for this lack of significance can be gained by closer inspection of the data.

On a mean basis (Table 6), the maximum concentration of free sugars in Skeletonema occurred at the intermediate intensity of $15.5 \times 10^{-3}$ ly./min. The non-polar lipid fraction was at a minimum at this same intensity. Quite possibly, the intermediate intensity of $15.5 \times 10^{-3}$ ly/min was closest to the optimum intensity for Skeletonema under the cultural conditions employed, leading to maximum soluble carbohydrate production.

The only other fraction that one might expect to change under these conditions, to offset the sudden increase in carbohydrate, would be non-polar lipid. It is well substantiated by previous workers (cf. Strickland, 1965), and supported by the results presented herein, that pigments generally vary inversely with light. But the decrease which occurred in the mean value of polar (pigment) lipids

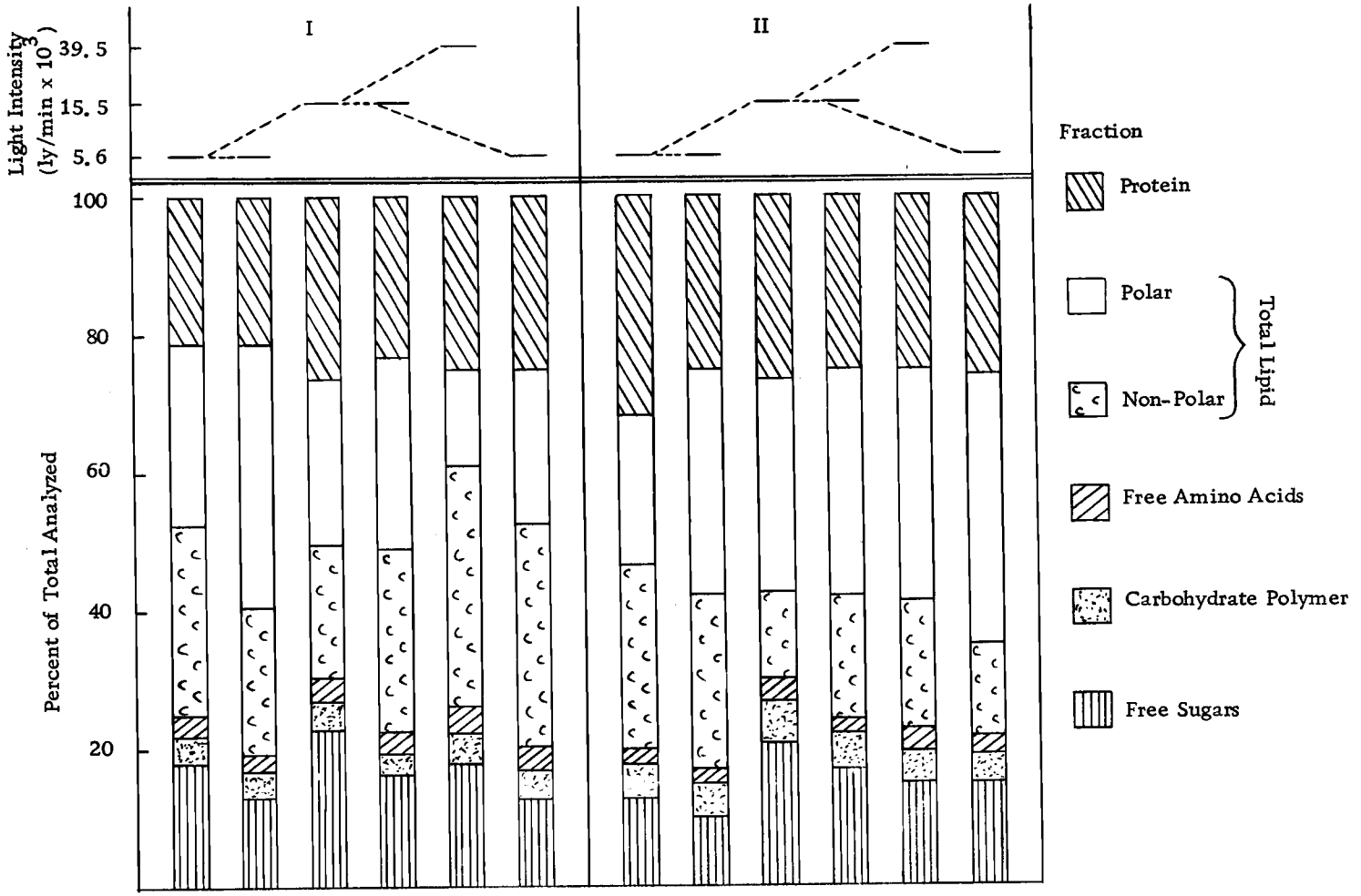


Figure 4. The influence of light intensity on the organic composition of Skeletonema costatum, showing two replicates.

Table 6. Mean values (in percent), slopes of regression lines, and correlation coefficients for the organic fractions, and derived P. Q. values, at three light intensities in Skeletonema costatum.

| Fraction | Mean Light intensity (ly/min x $10^3$) | | | Slope | Correlation Coefficient |
|---|---|---|---|---|---|
| | 5.6 | 15.5 | 39.5 | | |
| Free Sugars | 13.4 | 19.4 | 16.5 | +1.46 | +0.34 |
| Carbohydrate Polymer | 4.4 | 4.3 | 4.8 | +0.14 | +0.17 |
| Free Amino Acids | 2.8 | 2.9 | 3.5 | +0.30 | +0.53 |
| Non-Polar Lipids | 24.4 | 19.2 | 26.4 | +0.69 | +0.09 |
| Polar Lipids | 29.8 | 28.8 | 24.0 | -2.44 | -0.28 |
| Total Lipids | 54.2 | 48.0 | 50.4 | -1.74 | -0.32 |
| Protein | 25.2 | 25.2 | 24.8 | -0.18 | -0.06 |
| P. Q. | 1.40 | 1.37 | 1.37 | -0.01 | -0.25 |

in Skeletonema at the intermediate intensity was not enough to offset the increase in free sugars; hence, non-polar lipids changed also. Protein, the only other fraction large enough to absorb the necessary change, apparently remains constant as a function of light intensity in marine diatoms. In both free sugars and non-polar lipids then, the results obtained would lead to non-significant slopes in a linear regression. The use of lower light intensities would possibly give significant results if, in fact, $15.5 \times 10^{-3}$ ly/min is the optimum intensity for Skeletonema. The mean value for carbohydrate polymer increased slightly as the light intensity increased, but the magnitude of change was much less than that of free sugars and the resulting slope of the regression line was not significantly different from zero.

Contrary to the situation in Ditylum, the mean concentration of free amino acids increases in Skeletonema as light intensity increases. However, these changes are also below the significant level.

Perhaps the most interesting results obtained from Skeletonema were those for the average composition based on all treatments, as given in Table 7. As was the case with Ditylum, the organic composition of Skeletonema costatum proves to be predominantly lipid, with protein and carbohydrate occurring in decreasing order of abundance. However, the latter organism was approximately 51% lipid, 25% protein, and 21% carbohydrate, whereas these three fractions were approximately 64%, 21%, and 12%, respectively, in Ditylum.

Aside from the differences between the two species, they were remarkably similar in two respects. Mean amino acids in both diatoms were within 0.1% of 3.0% and mean carbohydrate polymers were within 0.1% of 4.4%.

Table 7. Average organic composition of Skeletonema costatum.

| Fraction | Percent Composition |
|---|---|
| Free Sugars | 16.4 |
| Carbohydrate Polymer | 4.5 |
| Free Amino Acids | 3.1 |
| Non-Polar Lipids | 23.3 |
| Polar Lipids | 27.5 |
| Total Lipids | 50.8 |
| Protein | 25.1 |

If the amount of total lipid in diatoms does, indeed, act as the rough control over buoyancy, as postulated earlier, then the fact that Skeletonema is smaller than Ditylum (Cupp, 1942) may be responsible for the 13% difference in total lipids between the two. The smaller organism would require relatively less buoyant material. This hypothesis could be easily tested in the laboratory. In both species, the total lipid content remained relatively constant throughout, even though a great deal of shifting between the polar and non-polar lipid

pools occurred.

The similarity in amounts of carbohydrate polymer and free amino acids between the two species further strengthens the hypothesis that both of these components are present in maintenance amounts only. Carbohydrate polymer could represent some structural entity, rather than a storage product, and the amino acids are probably present as a pool, or reserve, for protein synthesis.

The reasons for a free sugar fraction in Skeletonema nearly twice that in Ditylum are not clear. It may simply relate to the relative size of the two and the fact that the presumed lower lipids requirement of S. costatum leaves more energy available for production of sugars.

It appears as if lipid is, in fact, the predominant "storage product" in marine diatoms, contrary to the findings of Parsons, Stephens and Strickland (1961), Lewin, Lewin and Philpott (1958), Collyer and Fogg (1955), and others. Further, carbohydrate appears to be the least abundant component, with protein intermediate to lipid and carbohydrate.

The P. Q. computed by weighted average from the data in Tables 1 and 7 is 1.38, somewhat less than that calculated similarly for Ditylum. This would be expected in that Skeletonema has a larger carbohydrate concentration, coupled with a lower fat concentration, than does Ditylum. The slightly higher protein concentration found

in Skeletonema helps to maintain the P. Q. at a high value. As discussed previously, inclusion of the five percent or so of nucleic acids found in diatoms would serve to increase this P. Q. by a small amount. Nonetheless, this P. Q. value (1.38) is also significantly greater than Ryther's (1956) value at the one percent level.

The elemental analyses of Skeletonema showed so much variability, as seen in the individual P. Q. values computed from this data (Tables 1B and 2B), that no further calculations were done with the elemental data.

Finally, the mean P. Q. values for both Ditylum (1.41) and Skeletonema (1.38), based on organic composition, were compared statistically and found not to differ significantly. The lack of significant difference in P. Q. values calculated in this manner for two more or less unrelated diatoms when grown under similar conditions implies that these P. Q. values may be representative of many species of diatoms grown at the light and nutrient levels specified.

The data presented from Skeletonema costatum also support the conclusion that a higher P. Q. would be more nearly representative of marine plankton diatoms than the P. Q. of 1.25 suggested by Ryther (1965). Therefore, a value of perhaps 1.40 should be used when converting oxygen data to carbon assimilated in production measurements. This figure constitutes a revised value slightly less than the value presented earlier (Davey, Marmelstein and Curl, 1969).

In conclusion, it would appear that:

1. Increasing light intensity does have an effect on marine diatoms similar to that postulated by Myers and Cramer (1948) for other photosynthetic organisms, and would therefore be expected to result in decreased P. Q. values;
2. Lipid is the predominant metabolite produced by these two species of marine diatoms, and possibly by most marine diatoms, followed by protein and then carbohydrate. The relative amounts of each of these does, however, vary from species to species;
3. A P. Q. of 1.40 more nearly reflects the metabolic activities of marine diatoms, at least under conditions of nitrate-nitrogen nutrition;
4. Lipid may, in fact, contribute to the buoyancy mechanism in marine diatoms, acting as a rough or coarse adjuster of density.

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# APPENDICES

# APPENDIX A:

# *DITYLUM BRIGHTWELLII*

Table 1A: Organic and elemental composition of Ditylum brightwellii under three light intensities: replicate one

| | Light Intensity (ly/min x $10^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 5.6 | 5.6 | 15.5 | 15.5 | 39.5 | 5.6 |
| Fraction | Percent of Total Fractions Analyzed | | | | | |
| Free Sugars | 5.0 | 4.4 | 6.9 | 6.8 | 6.6 | 3.2 |
| Carbohydrate Polymer | 5.1 | 4.2 | 3.6 | 4.3 | 4.2 | 3.3 |
| Free Amino Acids | 1.9 | 2.5 | 1.9 | 2.6 | 2.7 | 2.4 |
| Non-Polar Lipids | 11.5 | 21.1 | 30.8 | 29.7 | 32.1 | 31.0 |
| Polar Lipids | 50.7 | 44.6 | 34.1 | 35.8 | 33.1 | 37.4 |
| Total Lipid | 62.2 | 65.7 | 64.9 | 65.5 | 65.2 | 68.4 |
| Protein | 25.8 | 23.2 | 21.3 | 20.8 | 21.3 | 22.7 |
| P. Q. | 1.43 | 1.43 | 1.39 | 1.41 | 1.41 | 1.43 |
| Elements | Number of Atoms | | | | | |
| Carbon | 6.8 | 6.2 | 6.8 | 6.8 | 7.1 | 6.4 |
| Hydrogen | 18.5 | 17.4 | 18.6 | 17.6 | 16.9 | 16.9 |
| Nitrogen* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Oxygen | 3.9 | 3.1 | 5.3 | 5.4 | 4.1 | 6.3 |
| P. Q. | 1.62 | 1.69 | 1.51 | 1.47 | 1.51 | 1.41 |

* Nitrogen set equal to one.

Table 2A: Organic and elemental composition of Ditylum brightwellii under three light intensities: replicate two

| | Light Intensity (ly/min x $10^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 5.6 | 5.6 | 15.5 | 15.5 | 39.5 | 5.6 |
| Fraction | Percent of Total Fractions Analyzed | | | | | |
| Free Sugars | 6.3 | 6.7 | 8.0 | 8.7 | 10.9 | 4.2 |
| Carbohydrate Polymer | 3.7 | 3.2 | 4.4 | 3.8 | 5.4 | 3.8 |
| Free Amino Acids | 3.3 | 3.0 | 2.4 | 3.0 | 2.6 | 3.1 |
| Non-Polar Lipids | 20.4 | 20.2 | 22.2 | 20.4 | 29.2 | 23.6 |
| Polar Lipids | 44.7 | 43.0 | 39.0 | 38.0 | 27.1 | 41.8 |
| Total Lipid | 65.1 | 63.2 | 61.2 | 58.4 | 56.3 | 65.4 |
| Protein | 21.5 | 23.9 | 24.0 | 26.7 | 24.8 | 23.6 |
| P. Q. | 1.42 | 1.42 | 1.37 | 1.43 | 1.40 | 1.43 |
| Elements | Number of Atoms | | | | | |
| Carbon | 6.5 | 7.6 | 7.2 | 7.0 | 7.2 | 7.0 |
| Hydrogen | 12.9 | 17.4 | 16.2 | 13.2 | 15.6 | 22.4 |
| Nitrogen* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Oxygen | 6.5 | 6.7 | 3.6 | 5.1 | 4.4 | 3.9 |
| P. Q. | 1.23 | 1.33 | 1.53 | 1.31 | 1.51 | 1.78 |

* Nitrogen set equal to one.

Table 3A: : Organic and elemental composition of Ditylum brightwellii under three light intensities: replicate three

| | Light Intensity (ly/min x $10^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 5.6 | 5.6 | 15.5 | 15.5 | 39.5 | 5.6 |
| Fraction | Percent of Total Fractions Analyzed | | | | | |
| Free Sugars | 6.0 | 4.3 | 8.4 | 8.3 | 11.1 | 6.9 |
| Carbohydrate Polymer | 3.6 | 3.4 | 4.0 | 3.7 | 5.6 | 4.4 |
| Free Amino Acids | 3.8 | 3.4 | 3.7 | 4.0 | 3.0 | 3.0 |
| Non-Polar Lipids | 38.5 | 40.9 | 37.3 | 43.8 | 44.0 | 34.7 |
| Polar Lipids | 29.3 | 29.6 | 27.2 | 23.1 | 19.4 | 34.6 |
| Total Lipid | 67.8 | 70.5 | 64.5 | 66.9 | 63.4 | 69.3 |
| Protein | 18.7 | 18.2 | 19.4 | 17.1 | 16.9 | 16.5 |
| P. Q. | 1.41 | 1.42 | 1.40 | 1.40 | 1.38 | 1.40 |
| Elements | Number of Atoms | | | | | |
| Carbon | 7.4 | 6.5 | 8.9 | 7.9 | 7.1 | 6.0 |
| Hydrogen | 17.3 | 21.6 | 20.7 | 16.0 | 25.0 | 24.5 |
| Nitrogen* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Oxygen | 8.2 | 4.8 | 6.8 | 9.2 | 9.6 | 6.5 |
| P. Q. | 1.20 | 1.69 | 1.37 | 1.11 | 1.41 | 1.73 |

* Nitrogen set equal to one.

# APPENDIX B:

# SKELETONEMA COSTATUM

Table 1B: Organic and elemental composition of Skeletonema costatum under three light intensities: replicate one

| | Light Intensity (ly/min x $10^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 5.6 | 5.6 | 15.5 | 15.5 | 39.5 | 5.6 |
| Fraction | Percent of Total Fractions Analyzed | | | | | |
| Free Sugars | 17.5 | 13.7 | 23.0 | 16.5 | 18.2 | 12.1 |
| Carbohydrate Polymer | 4.2 | 3.3 | 3.8 | 3.1 | 4.5 | 4.8 |
| Free Amino Acids | 3.3 | 2.4 | 3.6 | 3.1 | 4.0 | 3.2 |
| Non-Polar Lipids | 28.0 | 20.9 | 19.4 | 25.7 | 34.6 | 32.4 |
| Polar Lipids | 25.7 | 37.9 | 23.4 | 28.2 | 13.6 | 22.0 |
| Total Lipid | 53.7 | 58.8 | 42.8 | 53.9 | 48.2 | 54.4 |
| Protein | 21.4 | 21.7 | 26.8 | 23.3 | 25.0 | 25.5 |
| P. Q. | 1.37 | 1.39 | 1.36 | 1.38 | 1.38 | 1.40 |
| Elements | Number of Atoms | | | | | |
| Carbon | 5.7 | 4.0 | 4.6 | 3.6 | 2.6 | 6.0 |
| Hydrogen | 16.8 | 13.5 | 24.0 | 19.9 | 19.3 | 24.3 |
| Nitrogen* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Oxygen | 5.7 | 8.0 | 5.1 | 6.8 | 5.9 | 7.0 |
| P. Q. | 1.51 | 1.22 | 2.09 | 1.86 | 2.31 | 1.68 |

* Nitrogen set equal to one.

Table 2B: Organic and elemental composition of Skeletonema costatum under three light intensities: replicate two

| | Light Intensity (ly/min x $10^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 5.6 | 5.6 | 15.5 | 15.5 | 39.5 | 5.6 |
| Fraction | Percent of Total Fractions Analyzed | | | | | |
| Free Sugars | 12.6 | 10.1 | 21.2 | 17.2 | 14.8 | 14.3 |
| Carbohydrate Polymer | 5.3 | 4.5 | 5.3 | 5.0 | 5.0 | 4.2 |
| Free Amino Acids | 2.2 | 2.9 | 2.6 | 2.4 | 3.0 | 2.5 |
| Non-Polar Lipids | 26.6 | 24.6 | 14.4 | 17.4 | 18.3 | 13.6 |
| Polar Lipids | 21.5 | 32.9 | 30.2 | 33.5 | 34.3 | 39.2 |
| Total Lipid | 48.1 | 57.5 | 44.6 | 50.9 | 52.6 | 52.8 |
| Protein | 31.9 | 24.9 | 26.3 | 24.4 | 24.6 | 26.1 |
| P. Q. | 1.41 | 1.41 | 1.36 | 1.37 | 1.39 | 1.39 |
| Elements | Number of Atoms | | | | | |
| Carbon | 2.9 | 6.3 | 8.5 | 9.5 | 6.7 | 6.0 |
| Hydrogen | 16.7 | 16.3 | 21.2 | 18.6 | 20.2 | 14.9 |
| Nitrogen* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Oxygen | 5.5 | 8.6 | 6.9 | 5.9 | 9.4 | 8.1 |
| P. Q. | 2.07 | 1.21 | 1.39 | 1.34 | 1.28 | 1.20 |

* Nitrogen set equal to one.