AN ABSTRACT OF THE THESIS OF

Earl Walter Davey (Name) for the Ph. D. (Degree)
in Oceanography (Major) presented on 18 July 1969 (Date)

Title: THE PHOTOSYNTHETIC AND RESPIRATORY PHYSIOLOGY OF SKELETONEMA COSTATUM (GREVILLE) CLEVE GROWN UNDER SIMULATED ENVIRONMENTAL CONDITIONS

Abstract approved: Redacted for Privacy
Herbert C. Curl, Jr.

Effects of varying light and nutrient conditions upon the metabolism of the marine diatom Skeletonema costatum (Greville) Cleve were experimentally determined. Photosynthetic (PQ) and respiratory (RQ) quotients were used as indices of metabolism and were determined in two manners: (1) gaseous exchange monitored by gas chromatography and (2) calculation from elemental analysis. The results obtained from gaseous exchange were compared with the carbon-14 method. The difference between these results was attributed to dissolved organic excretion which was measured as dissolved organic carbon (DOC).

The quality and quantity of the nutrient nitrogen source produced the most pronounced influence on the PQ of S. costatum. An increase in PQ with the increasing oxidation state of the nitrogen source was probably due to a competition among CO_2, nitrate and/or

nitrite for the reducing potential of photosynthesis. A deficiency in nitrogen source produced an increased or decreased PQ, depending upon the original N source. No change in PQ was found for S. costatum grown on sulfur of various oxidation states.

The PQ and DOC production was found to be relatively constant with continuous light of different qualities and intensities. However, a rapid change from low light to high light decreased the PQ; whereas, a rapid change from high light to low light increased the PQ.

Suggested PQ values for a population predominantly composed of S. costatum, at relatively constant light conditions and known nutrient conditions, are 1.00 ± 0.10 for ammonia grown cells and 1.35 ± 0.15 for nitrate. A value of 0.75 was suggested for RQ.

The gas chromatographic method used in this study approaches the sensitivity of the Winkler method for analyzing oxygen. It also has the added capability of analyzing carbon dioxide, nitrogen and dissolved organic carbon. It overcomes the "iodine error" of the Winkler method and some objections to manometric measurements. It can yield a net production value for either oxygen or carbon dioxide, give an estimation of the predominant metabolic product, and possibly indicate the type of nutrient nitrogen source being utilized. It also has potential for monitoring productivity studies.

The Photosynthetic and Respiratory Physiology of *Skeletonema costatum* (Greville) Cleve Grown under Simulated Environmental Conditions

by

Earl Walter Davey

A THESIS

presented to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1970

APPROVED:

Redacted for Privacy

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Date thesis is presented 18 July 1969

Typed by Barbara Glenn for Earl Walter Davey

ACKNOWLEDGMENT

I would like to acknowledge my major professor, Dr. Herbert Curl, for his assistance both in the laboratory and in the preparation of this thesis.

I would like to thank the people in the National Marine Water Quality Laboratory who read this work.

Thanks to my family for their patience and to my wife for her words of encouragement and help in the preparation of this thesis.

Financial aid from the United States Department of Interior, FWPCA Training Grant 5TJ-WP-111 is gratefully acknowledged.

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THE PHOTOSYNTHETIC AND RESPIRATORY PHYSIOLOGY OF SKELETONEMA COSTATUM (GREVILLE) CLEVE GROWN UNDER SIMULATED ENVIRONMENTAL CONDITIONS

INTRODUCTION

The purpose of this investigation was to experimentally determine the effects of various environmental conditions upon the metabolism of the marine diatom Skeletonema costatum (Greville) Cleve.

Photosynthetic and respiratory quotients were determined and used as indices of metabolism since these coefficients are functions of (1) overall oxidation-reduction of carbon in cells, (2) the ratios of carbohydrates, lipids and proteins being metabolized and (3) nitrogen metabolism (Spoehr, 1926; Rabinowitch, 1945; James, 1953). Moreover, values of the photosynthetic quotient are used in computing carbon fixation in photosynthetic experiments in which oxygen production is measured (Strickland, 1965). Although the photosynthetic quotient (PQ) and respiratory quotient (RQ) have several definitions, here they will be defined as:

$$PQ = \frac{+\ O_2}{-\ CO_2} = \frac{\text{molecules of } O_2 \text{ liberated during photosynthesis}}{\text{molecules of } CO_2 \text{ assimilated}}$$

$$RQ = \frac{+\ CO_2}{-\ O_2} = \frac{\text{molecules of } CO_2 \text{ liberated during respiration}}{\text{molecules of } O_2 \text{ consumed}}$$

after Rabinowitch (1945). These are dimensionless numbers.

Spoehr (1926), Baly (1940), Rabinowitch (1945) and Frank and Loomis (1949) credit de Saussure for the first determination of the relative amounts of photosynthetic oxygen and carbon dioxide exchange. These authors summarized the results of PQ measurements and noted that most of them approach unity. Rabinowitch (1945) states that this remarkable constancy was independent of intensity and duration of illumination, temperature, and oxygen and carbon dioxide concentration. Photosynthetic quotients which approach unity are in agreement with the concept that carbohydrates are the principal organic products of photsynthesis. However, Spoehr (1926) suggested that even though the quotients were exactly one, this would not prove that just carbohydrates were synthesized. High values for proteins and fats and low values for acids might compensate each other and yield an average of one. Frank and Loomis (1949) mentioned that correlations between the gaseous exchange and organic production were still incomplete.

Ryther (1956) summarized some of the direct PQ measurements performed on various fresh water and marine algae; they all approached one. However, the calculation of the PQ from elemental composition of some of these algae ranged from 1.09 to 1.48 and averaged about 1.20. Myers and Cramer (1948) attributed the discrepancy between calculated and direct PQ measurements to changes in metabolism of the algae. When Chlorella was cultured at low light

intensities but measured at light saturation, a carbohydrate type metabolism was observed. Thus, Myers (1951) vigorously contends that PQ values of one have resulted from experiments carried out at low light intensities and measured at high light intensities due to manometric requirements.

Perhaps the factor which influences the PQ of an individual species of algae the most is the quantity and/or quality of the nutrient nitrogen source. Spoehr and Milner (1949) and Fogg (1956) showed that synthesis of fats increased with increasing nitrogen deficiency. Ryther (1956) used the data of Spoehr and Milner (1949) to show that the PQ for Chlorella increased from 1.11 to 1.35 as the nutrient nitrogen decreased. Cramer and Myers (1948) found that the PQ would also vary according to the source of nutrient nitrogen. A PQ of 1.06 was obtained for Chlorella pyrenoidosa when grown on ammonia and a PQ of 1.47 when grown on nitrate.

Although not reported, it is possible that the sulfur source could also influence the PQ results; sulfate, like nitrate, must be reduced before it can be incorporated metabolically (Bandurski, 1965).

James (1953) states that in air at moderate temperatures, many adult tissues exhibit an RQ close to one. A ratio of one is in accord with the assumption that a carbohydrate is being completely respired to carbon dioxide and water. The complete oxidation of proteins to carbon dioxide, water and ammonia also yields an RQ

close to one. However, respiration of fats generally gives rise to an RQ around 0.7 whereas, organic acids yield an RQ above one. It is therefore possible for a value approximating 1.0 to arise by a combination of oxidation reactions.

Apparently only Barker (1935b) has reported values for the respiratory quotients of marine phytoplankton. He found an RQ of 0.93 for unstarved Phaeodactylum tricornutum (reported as Nitzchia closterium) and an RQ of 1.00 for cells that were starved four days. Barker (1935a) also obtained RQ's of 0.91 to 1.00 for a marine Peridinium species.

One of the most common methods for measuring primary production is the determination of oxygen change in a closed system, as first reported by Gaarder and Gran (1927). The method requires that the PQ either be known or estimated if carbon assimilation is to be equated with oxygen production. Ryther (1956) concluded that PQ values of 1.0 are experimental artifacts and values of 1.1 to 1.3 and above are normal under natural conditions. Although he thought it was impossible always to know the correct value for routine measurement, he suggested that a value of 1.25 was perhaps more realistic than one. Strickland and Parsons (1960) state that the choice of the PQ and RQ value should be left to the discretion of the analyst. However, they suggest that when "normal" marine populations are exposed to moderate light intensities a PQ of 1.20 and an RQ of 1.00

can be used.

Since doubt still remains concerning which PQ and RQ values should be applied to marine primary production studies, the effects of the main environmental parameters on the PQ and RQ of marine phytoplankton were investigated. However, this study also required the development of several new analytical methods. Therefore the investigation was limited to the effects of varying light and nutrient conditions on the PQ and RQ of Skeletonema costatum.

The measurement of the photosynthetic and respiratory quotients was facilitated by gas chromatography. Gas chromatography was chosen because it has several advantages over other techniques. A one milliliter sample can yield a rapid, direct and simultaneous analysis of carbon dioxide, oxygen and nitrogen. Discrete, not continuous, samples are used. Gas chromatography does not require that cells be measured under different environmental conditions from those under which they were grown; thus, Myers' (1951) objection is overcome.

An independent estimate of PQ and RQ values was obtained by calculation from the analysis of the elemental composition of S. costatum grown under similar conditions. Cramer and Myers (1948) found that the calculation of PQ from the elemental composition of Chlorella pyrenoidosa agreed well with the PQ obtained from direct gaseous exchange measurement. This agreement depended on

minimal loss by excretion of dissolved organic compounds (DOC). Consequently, the DOC content of the medium was checked in order to obtain a complete carbon balance. The results obtained from gaseous exchange rates of total carbon dioxide were also compared with the carbon-14 method of Steemann Nielson (1952).

METHODS

Culture and Experimental Growth Conditions

Skeletonema costatum was chosen as an experimental organism because of its nearly world-wide neritic distribution, its primacy in plankton communities, and its importance as a food organism for filter feeders. Thus its metabolism is "typical" of marine phytoplankton organisms. Skeletonema costatum can be cultured under artificial laboratory conditions, and has been used in many other physiological studies (Curl and McLeod, 1961; Jitts et al., 1964; Eppley and Sloan, 1965).

In order to avoid metabolic contamination from bacteria, attempts were made to obtain axenic cultures. None of these attempts proved lastingly successful. However, very few bacteria were observed microscopically with phase contrast at 400x magnification during logarithmic growth phase. Only when the culture approached senescence did the bacterial population became abundant. During stationary and death phase some unidentified, small, unicellular, flagellated organisms were observed in the media; they were never abundant.

S. costatum was grown in artificial sea water (ASW) to permit manipulation of inorganic carbon concentrations and sources of

nitrogen and sulfur. An initially low amount of dissolved organic carbon could be provided in order to reduce bacterial growth (Sheril Burton, personal communication). Reagent grade compounds were added to 1,050 ml of water which was glass distilled over 1 ml 85% H_3PO_4 and two grams of $K_2S_2O_8$ per liter. Final salinity was 32.5 °/oo. The basic non-nutrient salt solution was a modified Lyman and Fleming (1940) artificial sea water (Table 1). The total possible nutrient impurities in these salts were calculated by the summation of the impurities listed on the label for each reagent multiplied by the grams of each reagent added (Table 2). The basic artificial sea water was analyzed for several nutrients before the addition of nutrient salts and organic compounds. It was found to contain 5.2 μM Si, 0.000 μM PO_4-P and 0.41 μM NO_3-N.

Table 1. Basic Non-Nutrient Salts for ASW.

Major Salt	Grams
NaCl	23.477
$MgCl_2 \cdot 6H_2O$	10.635
$Na_2SO_4 \cdot 10H_2O$	8.885
$CaCl_2$	1.102
KCl	0.664
H_3BO_3	0.026

Table 2. Possible Nutrient Impurities in Basic Non-Nutrient Salts.

Nutrient Impurity	Total possible impurity in one liter of ASW	
	mg	micro-moles
Phosphate ($PO_4^{\equiv}$)	0.258	2.0
Nitrate (NO_3^-)(could also be chlorate)	0.831	14.0
Ammonium (NH_4^+)	0.213	4.0
Nitrogen compounds	0.166	-
Heavy metals (as lead)	0.159	0.8
Iron (Fe)	0.215	4.0
Strontium (Sr^{++})	0.532	3.0
Manganese (Mn^{++})	0.052	0.4
Iodide	0.482	-
Bromide	1.207	-

The artificial sea water was enriched with nutrient salts according to a formula modified from that of Ryther and Guillard (1962) (Table 3). The vitamin mix plus 1 mM $NaHCO_3$ (84.02 mg) were added to nutrient-enriched artificial sea water after the media had been autoclaved for 15 minutes and cooled to culture condition temperature. The artificial sea water contained no detectable CO_2 after autoclaving. Therefore, an exact concentration of bicarbonate could be obtained by adding $NaHCO_3$ to autoclaved ASW which had been cooled and protected against atmospheric CO_2 contamination.

It was necessary to reduce the normal inorganic carbon content of 2.3 mM found in sea water to 1 mM in the artificial medium in order to increase the sensitivity of the CO_2 measurement. Concentrations greater than 1 mM $NaHCO_3$ drove the recorder off scale,

Table 3. Nutrient Salt Additions to the Basic Non-Nutrient ASW.

Nutrient salts added to stock solution	mg per liter	micro-moles of nutrient per liter	
$Na_2SiO_3 \cdot 9H_2O$	45.0	Si,	161.0
Nitrogen source, either:			
$NaNO_3$	8.5	N,	100.0
NH_4Cl	5.3		
$NaNO_2$	6.9		
$NaH_2PO_4 \cdot H_2O$	1.4	P,	10.0
Fe-sequestrene	1.0	Fe,	2.0
Thiourea*	1.0	S,	14.0
Trace metals			
$CuSO_4 \cdot 5H_2O$	0.196	Cu,	0.790
$ZnSO_4 \cdot 7H_2O$	0.044	Zn,	0.015
$CoCl_2 \cdot 6H_2O$	0.022	Co,	0.085
$MnCl_2 \cdot 4H_2O$	0.360	Mn,	1.820
$NaMoO_4 \cdot 2H_2O$	0.030	Mo,	0.052
Vitamin mix			
Vitamin B_{12}	0.001		
Biotin	0.001		
Thiamin HCl	0.010		

*Only added when a divalent sulfur source was required for certain experiments.

requiring signal attenuation and a decrease in sensitivity. One mM of $NaHCO_3$ supplied a non-limiting concentration of carbon for growth and maximum recorder sensitivity.

S. costatum grown for gaseous exchange and carbon-14 studies

were subcultured in plastic screw-capped bottles containing either 100 or 400 ml of media labelled with a specific activity of 10 μCi ^{14}C (mmole $NaHCO_3$)$^{-1}$. These cultures, after several transfers, were assumed to be uniformly labelled. Cells for dissolved organic carbon and elemental analysis were grown in non-labelled media in one liter plastic screw-capped bottles or four liter, polyethylene, collapsible bottles.

Nitrogen and sulfur sources were varied during various experiments. Cell counts were made with a Fuchs-Rosenthal counting chamber.

S. costatum was grown under continuous light at 18°C either in a constant temperature room or a Sherer-Gillet growth chamber (Model CEL 34-7). The constant temperature room was equipped with four F40T12/CWX fluorescent lamps which emitted radiant energy of 0.030 ly min^{-1}. The Sherer-Gillet growth chamber was illuminated by ten Sylvania "Daylight" type F48T12-D-VHO and two Sylvania "Gro-lux" type F48T12-GRO-VHO fluorescent lights, plus four 60 watt incandescent lamps. A black ten-compartment box was placed inside the growth chamber. Each compartment had approximate dimensions of 16x16x16 cm. This was large enough to accomodate experimental bottles, polyethylene bottles or syringes. The compartments were covered by molded, unpolished glass filters chosen to simulate the intensity and quality of light at a given depth

in the sea (Table 4).

Table 4. Light Intensities in the Sherer-Gillet Growth Chamber

Filter Notation	Simulated depth in meters	Corning filter number	Radiant energy milliwatts cm^{-2}	Radiant energy ly min^{-1}
none			6.30	0.090
1F	surface	2815	2.70	0.046
2F	10	2781	2.06	0.030
3F	15	2380	1.43	0.020
4F	25	2827	0.95	0.014
5F	50	2278	0.55	0.008
3F & 1F	50	2380 & 2815	0.60	0.009

For gaseous exchange experiments, cells were grown in closed systems consisting of 20, 30, or 50 ml glass B-D Yale syringes with Luer-Lok tips. The advantages of these syringes were (1) when properly sealed, there was no detectable gaseous exchange between the media within the syringe and the external atmosphere, (2) volumes were variable by advancing the plunger, and (3) syringes were small enough so that they could be placed under any desired experimental light or temperature conditions.

Syringes constructed from test tubes were more difficult to operate than the B-D Yale syringes. Polyethylene syringes were permeable to permanent gases. A mineral oil surface layer over media in flasks did not reduce gas exchange.

Gas bubbles formed in the syringes due to oxygen

supersaturation during photosynthesis or nitrogen supersaturation during warming reduced photosynthetic quotient values. Therefore, the initial gas content of the medium was reduced by placing the medium, with or without cells, under 360 mmHg vacuum for 20 minutes with periodic shaking. The degassed medium was decanted into the open end of syringes, with the plungers removed and the syringe tips plugged. Three, 6 mm, solid glass beads were added before sealing the syringe to assist in mixing the cells when the syringes were shaken. The plungers, lightly coated with silicone grease, were replaced and air bubbles were worked out through the unplugged tips. When all air was removed, the tips were capped with a serum-bottle cap.

Gas exchange experiments were conducted in two manners. Cultures were either maintained in syringes during the logarithmic growth phase and the experiment run for a day or more, or the cells were first concentrated and resuspended in the desired nutrient medium and then placed in syringes for a period of several hours. Cells were concentrated by centrifugation or by siphoning the media above the cells. S. costatum tends to settle out of suspension toward the end of logarithmic growth phase. The cells were allowed to photosynthesize under the experimental light conditions until gas bubbles were just about to form inside the syringes. Gas bubble formation occurred when the combined gas concentration of nitrogen and oxygen

was greater than 30 $\mu l\ ml^{-1}$. At this point, they were placed in the dark and allowed to respire.

Measurement of Gaseous Exchange

The photosynthetic and respiratory quotients of organisms can be estimated by monitoring their gaseous exchange. High sensitivity gas chromatography can be used to obtain simultaneous measurements of oxygen, carbon dioxide, and nitrogen from a single aqueous sample. The resolution of small changes in CO_2 due to respiration and photosynthesis imposed on the large initial concentration of inorganic carbon in sea water requires gas chromatographic columns yielding sharply defined peaks, electronic stability, high gain, and frequent recalibration.

An F&M Model 720 gas chromatograph equipped with a Gow-Mac Model 460 detector with four W2X filaments operated at 110 to 150 ma and 40° C was used to detect the sample gases. The more sensitive W2X filaments required the replacement of the original 12 volt power supply with a power supply capable of supplying the 40 volts at 300 ma (Lambda Model LH-124-FM). A 300 milliampere meter replaced the original 200 milliampere meter. A 250Ω, 25 watt rheostat replaced the original bridge current rheostat. This change reduced resistor heating which had caused noise in the bridge output.

Two gas chromatographic columns were necessary for the separation of carbon dioxide, oxygen and nitrogen. The first column, for the separation of CO_2 from the composite gases (O_2, Ar, and N_2), was 8' x 1/4" aluminum tubing filled with Porapak Q (150-200 mesh; Waters Associates, Inc.) coated on Chromobsorb P. NAW (3-60 mesh; F&M Scientific Corp.). This packing material was found to be far superior to silica gel (Knox, 1962) or even to silica gel coated with 1% diglyceride (Wilhite, 1966). The second column, for the separation of argon and oxygen from nitrogen, was 25' x 1/4" copper tubing with the first 20 feet filled with Chromobsorb P. NAW (30-60 mesh) and the remaining 5 feet filled with molecular sieve 5A (30-60 mesh; F&M Scientific Corp.). The length of this second column placed the CO_2 peak before the O_2-Ar peak (Figure 1). Several unsuccessful attempts were made to separate O_2 from Ar at room temperature according to the procedure of Karlsson (1966). This separation may be feasible at lower temperatures.

The two columns were connected in series by a 1' x 1/4" copper column filled with Ascarite to protect the molecular sieve column from contamination by CO_2. Both columns were "conditioned" at 200° C with He at a flow rate of 4 ml min^{-1} for 12 hours. The columns were operated at room temperature with a He flow rate of 60 ml min^{-1}.

The sample injection system for the analysis of permanent

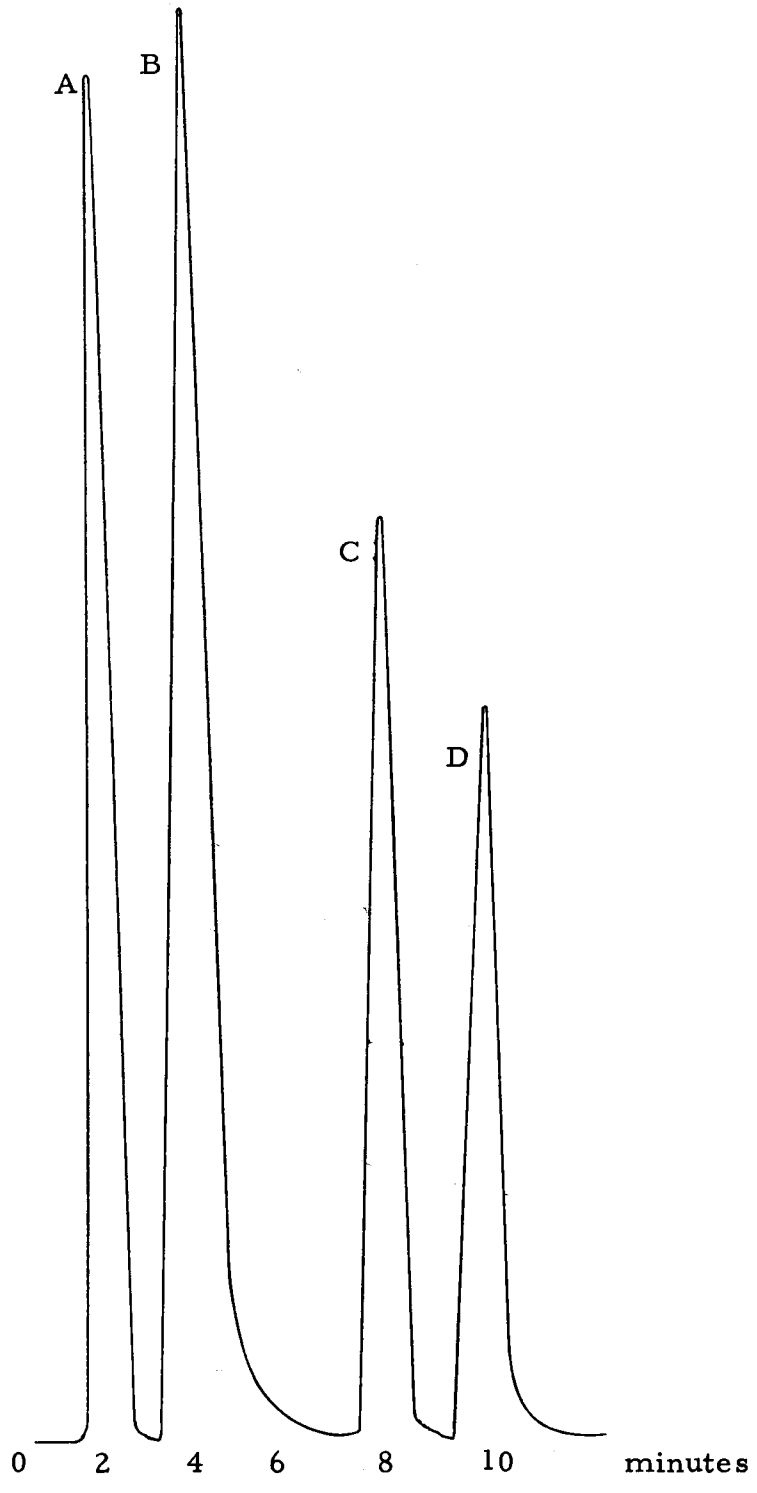

Figure 1. Time sequence of gas chromatographic separation of composite (A), carbon dioxide (B), oxygen-argon (C), and nitrogen (D).

gases and total CO_2 in sea water and the calibration technique have been described by Curl and Davey (1967). This system was modified by attaching the Swinnerton, et al. (1962) apparatus to a Perkin-Elmer valve in order to protect the Leonco gas sampling valve from corrosion and also to provide a smoother injection of calibration gas. The sample injection system was connected to the gas chromatograph by a 4' x 1/4" O. D. polypropylene tube filled with 30-60 mesh Drierite for the removal of water vapor.

One milliliter samples were taken from the culture syringes by removing the cap from the syringe tip and removing the sample with a 2. 5 ml Hamilton gas-tight syringe equipped with a Chaney adaptor. (When the rubber cap was punctured directly with a syringe needle, it did not re-seal sufficiently to prevent gas leakage back into the syringe.) The samples were injected through a rubber septum into the Swinnerton, et al. (1962) apparatus which contained 8 ml of 1N H_2SO_4 in 35 $^{o}/_{oo}$ NaCl solution. The analysis took approximately ten minutes from the time of injection to the end of the last recorded peak (N_2). Calibration gas samples were injected once an hour during sampling. The temperature was recorded at each calibration gas injection, and barometric pressure was recorded approximately every four hours to correct the volume of the gas sampling valve and loop to STP. The volume of the gas sampling valve and loop used in these studies was 208. 6 µl. The method for this volume

determination is given by Curl and Davey (1967). The areas under the sample peaks were integrated with a Disc Integrator and were corrected for base-line drift.

The total μmoles of calibration gas (CG) in the gas sampling valve (G. S. V.) was calculated as follows:

$$\mu\text{moles CG in G. S. V.} = 208.6\mu\text{l} \times \frac{1\ \mu\text{mole}}{22.4\ \mu\text{l}} \times \frac{273.16^{\circ}\text{K}}{273.16 + Z} \times \frac{y}{760\ \text{mmHg}}$$

where Z = room temperature in degrees centigrade

y = barometric pressure in mmHg.

The μmoles of the individual gases in the calibration gas mixture were determined by multiplying the percentage of the individual gas components (10.4% CO_2, 6.1% N_2, 5.6% O_2 and 0.5% Ar) times the total μmoles of calibration gas. The μmoles of CO_2, Ar-O_2, and N_2 in the samples were determined by the ratio of the integrated area of the sample peak to the calibration peak area times the μmoles of the gas desired (O_2-Ar, CO_2, or N_2) computed from the calibration gas volume. The photosynthetic and respiratory quotients were determined by the ratio of change in μmoles of CO_2 and O_2-Ar between an initial and a later measurement. No correction was made for argon in the O_2-Ar values; however, the difference between an initial and a later O_2-Ar value yields only the change in oxygen because Ar is metabolically inert and remains constant. The nitrogen peak was found to be a useful internal standard for each culture syringe.

Since N_2 is not used metabolically by these organisms, any change in N_2 concentration between samplings would reflect contamination of the sample by atmospheric air or a loss of a portion of the gases from the sample. The nitrogen peak was used to correct the oxygen peak proportionately if the nitrogen peaks between two sampling periods varied by more than 0.1 μl N_2.

Elemental Analysis of Carbon, Hydrogen, Nitrogen and Oxygen

The elemental composition of organisms can be used to compute their photosynthetic and respiratory quotients (Myers and Cramer, 1948), as well as to estimate their percentage composition of lipids, proteins and carbohydrates (Spoehr and Milner, 1948).

Samples of S. costatum under various light or nutrient conditions were taken for elemental analysis during: (1) logarithmic phase of growth at approximately 100,000 cells ml^{-1}, (2) one week later in the light, and (3) one week later in the dark. For each regime, ten samples of either 100 or 50 mls, depending upon cell number, were filtered onto 13 mm Gelman type A or E glass fiber filters in Swinney adapters, under 360 mmHg vacuum. The filters were heated at 550° C to eliminate any organic contamination from binder, dust or handling.

Blanks consisted of glass fiber filters through which 100 mls of artificial sea water had been filtered. After filtering, all filters

were dried to constant weight in a vacuum dessiccator containing silica gel.

The ash-free dry weight (AFDW) was determined on half of the prepared samples. The samples were weighed on a Cahn microbalance, heated at 550° C for at least 24 hours, cooled, dried to constant weight, and re-weighed again. The difference in weight before and after burning, corrected for the loss in weight of the blanks prepared in the same manner, was considered to be the AFDW.

The remaining samples were analyzed on an F&M Model 185 CHN analyzer. The general procedure was modified as follows: the direct recording rather than the ratio recording method was employed. The direct recording method requires the construction of standard curves for C, H, and N. Acetaniline (B. D. H. Laboratory Chemicals) was the best standard since its C, H, N composition was most similar to the S. costatum samples analyzed. The attenuator was set manually rather than automatically in order to obtain the greatest sensitivity for each element. The attentuation factor was 1 to 2 for N, 8 to 32 for C, and 4 to 16 for H.

Peak heights of all samples recorded on the CHN analyzer were measured with a 30 cm Dietzgen Excello 1574C scale. Standard curves of peak height vs. μg-atoms C, H, and N were constructed from a weight range of 0. 2 to 1. 5 mg acetaniline; the values were corrected for catalyst blanks. Peak heights of the samples were

corrected for the ASW blank.

Organic oxygen was calculated by subtraction of the total μg weight of C, H, and N from the AFDW, and converted to μg-at O. The PQ and RQ of the samples were calculated from the moles of C, H, N, and O according to the following equation:

$$w\,CO_2 + \frac{x-3z}{2}\,H_2O + z\,NH_3 \longrightarrow C_wH_xO_yN_z + \left[\frac{2w + \left(\frac{x-3z}{2}\right) - y}{2}\right]O_2$$

where w, x, y, and z are the moles of C, H, O, and N respectively. The PQ, with NH_3 as the source of nitrogen, is:

$$PQ_{NH_3} = \frac{\left[\frac{2w + \left(\frac{x-3z}{2}\right) - y}{2}\right]O_2}{wCO_2}$$

The PQ, with nitrate as the nitrogen source, is:

$$PQ_{NO_3} = \frac{\left[\frac{2w + \left(\frac{x-3z}{2}\right) - y}{2}\right]O_2 + 2z}{wCO_2}$$

since for every mole of nitrate utilized, there are two apparent moles of oxygen produced.

The accuracy of the PQ and RQ values obtained by elemental analysis was greatly reduced by the organic oxygen estimates. The carbon, hydrogen and nitrogen values, according to the F&M manufacturer, could be obtained with a 1% or better precision. However, the organic oxygen estimate relied upon placing equivalent amounts of cells on the glass fiber filters for both the C, H, N analysis and

AFDW determinations. The filters, after muffling, became brittle and were at times, as determined by examination of the filtrate, subject to some cell losses. The AFDW determination depended upon weighing the samples before and after muffling without excessive losses or gains to the filters which weighed considerably more than the filtered cells. Due to manipulative and weighing errors the organic oxygen estimate between replicates differed at times by more than 10%. Much better PQ and RQ results could be obtained if organic oxygen could be determined directly. Methods for direct organic oxygen are just now becoming available.

Analysis of Dissolved Organic Carbon

The method of Menzel and Vaccaro (1964) for dissolved organic carbon (DOC) was modified for gas chromatography by a special injection system (Curl and Davey, 1967). Duplicate 5 ml samples of the filtrate remaining from the C, H, N and O samples were filtered a second time through glass-fiber filters directly into 10 ml ampules containing 0.1 gram $K_2S_2O_8$. Blanks of artificial sea water media were prepared in the same manner. Both the ampules and glass-fiber filters were heated at 550° C before sampling. The samples in the ampules were acidified with 0.2 ml of 3% H_3PO_4 and bubbled with He or N_2 gas for five minutes before the ampules were sealed. The samples were removed from the source of bubbling He or N_2 with

dissecting forceps equipped with a cannula which force a continuous stream of N_2 or He into the ampule during the sealing process (Ronald F. Wilson, personal communication). When the ampules were sealed in this manner no trace of inorganic CO_2 could be detected. After the ampules were sealed, they were autoclaved from 1/2 to 12 hours at two atmospheres and 121° C. Ampules were sampled using the apparatus of Curl and Davey (1967). Chlorine gas expelled from the ampule was removed by an acidic KI solution (20 gm KI in 50 ml 10% H_2SO_4) placed in the Swinnerton, et al. (1962) apparatus. Carbon dioxide was separated from the composite gases on a 1/4" x 4' silica gel column at ambient temperature. The gas chromatograph detector was operated at 150 ma and 60° C.

Twelve minutes elapsed from sample introduction to the end of the CO_2 peak. Calibration gas was injected after every other sample and the barometric pressure and temperature were recorded. The μmoles of CO_2 in the samples were determined from the ratio of the integrated CO_2 peak areas recorded for the sample and the calibration gas, multiplied by the μmoles CO_2 in the calibration gas samples.

The 12 minute analysis time allowed CO_2 to separate from the large composite peak obtained in the oxidation process. Even when chlorine gas was removed with KI solution (Menzel and Vaccaro, 1964), the composite was not greatly diminished. When ampules

were sealed under a constant atmosphere of He and not autoclaved, no composite or CO_2 could be detected. Apparently the composite gas, probably O_2, is released in the oxidation process during autoclaving. It would be desirable to reduce the composite peak to speed up the time of DOC analysis as well as to protect the gas chromatograph detector from excessive exposures to an oxidizing atmosphere which limits the detector life. It is suggested that pyrogal could be used in a bubble chamber to remove O_2 expelled from the ampules.

Determination of Carbon-14 Uptake

The rates of carbon-14 uptake and loss during photosynthesis and respiration were compared with the data obtained by the gaseous exchange method. Skeletonema cells were uniformly labelled with carbon-14 by growing the cells on media containing a specific activity of 10 μCi ^{14}C (mmole $NaHCO_3$)$^{-1}$. The cells were grown through several transfers. Each time a sample was taken for gaseous analysis, 1 ml of labelled cells was also filtered onto 0.8μ type GM-4 Gelman membrane filters (2.54 cm diameters). The filters were counted for 5,000 to 10,000 counts with an end-window Geiger tube. The data were converted to disintegrations per minute (dpm) basis and corrected for background radioactivity. The rate of carbon uptake was determined by calculations based on the efficiency of the Geiger counter (25%) rather than using a $Ba^{14}CO_3$ standardization procedure.

RESULTS

Experiment I

The purpose of this experiment was to determine the photosynthetic and respiratory quotients of Skeletonema costatum grown on nitrate, nitrite, ammonia, and ammonia plus thiourea, by elemental analysis and gaseous exchange. The rates obtained for CO_2 fixation or loss were also compared with the ^{14}C method (Table 7).

S. costatum was grown in a constant temperature room at 18°C under a radiant energy of 0.030 ly min^{-1}, with 100 μM initial concentration of nitrogen from nitrate, nitrite or ammonia. One mg l^{-1} thiourea was added to supply a divalent sulfur source in the combined ammonia plus thiourea treatment.

The photosynthetic gaseous exchange and ^{14}C uptake were measured in duplicate during the log-growth phase of a uniformly ^{14}C labelled S. costatum population. When the syringes were almost saturated to the point of gas bubble formation, they were placed in the dark and the respiratory gas exchange and ^{14}C loss were measured. One ml aliquots were taken for both ^{14}C and gaseous exchange samples (Table 5).

Four 100 ml samples, two for CHN analysis and two for organic oxygen estimation, were taken for three different experimental

Table 5. The Gaseous Exchange Rate and Photosynthetic and Respiratory Quotients and Ratios of Skeletonema costatum grown on Various Nitrogen and Sulfur Sources.

Sample	Date	Time	Δ Time	$\frac{\Delta m\mu moles\ CO_2}{ml/hr}$	$\frac{\Delta m\mu moles\ O_2}{ml/hr}$	PQ	RQ	P/R
Ammonia	24 Mar	1322						
			26.4	8.70	8.53	0.98		
(NH_3)	25 Mar	1547						2.57
			47.5	3.38	5.18		0.75	
	27 Mar	1438						
Ammonia plus Thiourea	24 Mar	1336						
			26.2	8.35	8.53	1.02		
	25 Mar	1509						2.17
			46.8	3.84	4.42		0.87	
	27 Mar	1355						
Nitrite	24 Mar	1243						
			28.4	7.90	9.87	1.25		
(NO_2^-)	25 Mar	1627						2.35
			27.5	3.36	4.96		0.74	
	27 Mar	1519						
Nitrate	24 Mar	1055						
			30.9	5.13	7.32	1.43		
(NO_3^-)	25 Mar	1707						1.55
			46.9	3.30	4.55		0.73	
	27 Mar							

situations: (1) one week in the light (I), (2) one week in the light, one week in the dark (R), and (3) two weeks in the light (P). The notation P-I is used to indicate the difference in elemental composition between the initial measurement (I) and a week later in the light (P). It should represent the elemental changes under nutrient deficient conditions. The notation R-I indicates the difference in elemental composition between the initial measurement (I) and a week later in the dark. This R-I value should indicate the actual elemental loss during respiration. The PQ and RQ values were calculated from these data (Table 6) and summarized in Table 7.

The notation P/R in Tables 5, 7, 9, 10, and 11 is used to indicate the ratio of photosynthesis to respiration for the rate of carbon uptake and loss. The notation C/N in Tables 7 and 12 represents the elemental ratio of carbon to nitrogen on an atom basis.

Table 6. The photosynthetic and respiratory quotients calculated from the elemental composition of Skeletonema costatum grown on various nutrient nitrogen sources.

Sample	AFEW (mgm)	µg-at N	% Change	µg-at C	% Change	µg-at H	% Change	µg-at O	% Change	PQ (NH_3)	PQ (NO_2^-)	PQ (NO_3^-)	RQ
Nitrate													
I	0.738	3.51		22.3		55.2		22.0		0.96	1.19	1.27	
R	0.615	3.34		19.7		49.3		16.6					0.93
P	1.108	4.28		37.4		83.2		32.3		1.04	1.21	1.29	
P-I		+0.77	21.9	+15.1	67.9	+28.0	50.7	+ 9.4	40.8	1.05	1.13	1.16	
R-I		-0.17	4.8	- 2.6	11.7	- 5.9	10.7	- 6.3	27.5				3.25
Nitrite													
I	0.724	3.36		24.9		57.5		21.1		0.85	1.05	1.12	
R	0.648	3.25		18.7		50.8		21.1					1.02
P	1.101	3.94		37.6		83.8		32.0		1.05	1.21	1.26	
P-I		+0.58	17.2	+12.7	51.0	+26.3	45.7	+10.9	51.7	1.05	1.12	1.15	
R-I		-0.11	3.2	- 6.2	24.9	- 6.7	11.7	- 0.0	0.0				0.80
Ammonia													
I	0.708	3.57		24.4		56.2		21.3		1.03	1.25	1.32	
R	0.649	3.00		17.3		50.0		21.0					1.02
P	0.893	3.75		32.7		75.2		20.8		1.17	1.34	1.40	
P-I		+0.18	5.0	+ 8.3	34.1	+19.0	33.8	- 0.5	2.3	1.56	1.59	1.60	
R-I		-0.57	16.0	- 7.1	29.2	- 6.2	11.0	- 0.3	1.4				0.88
Ammonia plus thiourea													
I	0.838	3.58		26.1		59.9		26.0		0.97	1.18	1.25	
R	0.712	3.54		21.0		52.8		22.4					0.99
P	0.893	3.75		32.7		75.2		32.0		1.00	1.17	1.23	
P-I		+0.17	4.7	+ 6.6	25.1	+15.3	25.5	+ 6.0	23.1	1.11	1.15	1.16	
R-I		-0.04	1.1	- 5.1	19.5	- 7.1	11.9	-3.6	13.8				1.01
Average of all N sources													
I		3.51±0.09		24.4±1.4		57.2±1.8		22.8±2.0		1.01	1.23	1.30	
R		3.28±0.19		19.2±1.4		50.7±1.1		20.2±2.2					0.99
P		3.99±0.22		35.9±2.0		80.7±4.4		32.1±5.6		1.03	1.20	1.25	
P-I		+0.48	13.7	+11.5	47.0	+23.5	41.1	+ 9.3	40.7	1.03	1.10	1.12	
R-I		-0.23	6.6	- 5.3	21.5	- 6.4	11.3	- 2.6	11.2				0.97

Table 7. Summarized Results of Experiment I.

	Nitrate	Nitrite	Ammonia	Ammonia plus Thiourea
^{14}C method (μmoles C $ml^{-1}hr^{-1} \times 10^{-3}$)				
photosynthesis	4.43	5.21	5.32	3.23
respiration	0.83	1.04	1.21	1.18
Gaseous exchange rates				
Total CO_2 (μmoles CO_2 $ml^{-1}hr^{-1} \times 10^{-3}$)				
photosynthesis	5.13	7.90	8.70	8.35
respiration	3.30	3.36	3.38	3.84
P/R	1.55	2.35	2.57	2.17
Oxygen rates (μmoles O_2 $ml^{-1}hr^{-1} \times 10^{-3}$)				
photosynthesis	7.32	9.87	8.53	8.53
respiration	4.55	4.96	5.18	4.42
PQ	1.43	1.25	0.98	1.02
RQ	0.73	0.74	0.75	0.87
Elemental analysis				
Average PQ (I)	1.30	1.23	1.01	1.01
Average PQ for senescent cells (P-I)	1.12	1.10	1.03	1.03
Average RQ	3.25	0.80	0.88	1.01

Carbon to nitrogen ratio on an atom basis for the average of all N sources of Table 6

I = 6.95
R = 5.84
P = 8.99
P-I = 23.90
R-I = 22.83

Experiment II

In this experiment the photosynthetic and respiratory quotients of S. costatum were determined when deficient and when sufficient in nutrient nitrogen. Deficient cells were concentrated by siphoning off settled cells from two-week old S. costatum cells grown under various light conditions. Nutrient sufficient cells, grown at 0.030 ly min^{-1}, in the log-growth phase, were concentrated and washed by centrifugation at 5,000 g. Measurements were made for calculations of PQ's and RQ's from deficient cells, deficient cells after enrichment, and sufficient cells (Tables 8, 9, 10).

Experiment III

The photosynthetic and respiratory quotients were determined for S. costatum grown under either high (0.030 ly min^{-1}) or low (0.008 ly min^{-1}) light and grown on either nitrate or ammonia. Cells grown under low light were measured at high and low light. Cells grown under high light were also measured under high and low light (Table 11A and B).

Table 8. Photosynthetic Quotients of Two-Week Old Nutrient Deficient and Nutrient Enriched Skeletonema costatum.

Sample	Date	Time	Δ Time	$\frac{m\mu moles\ CO_2}{ml/hr}$	$\frac{m\mu moles\ O_2}{ml/hr}$	PQ
1. Cells originally grown on nitrate at a light energy of 0.020 ly min^{-1}						
Initial	15 Jul	1740				
No added N	16 Jul	1050	17.2	18.7	18.6	1.00
Nitrate added	16 Jul	1120	17.7	14.2	18.2	1.28
Ammonia added	16 Jul	1105	17.4	27.7	18.9	0.68
2. Cells originally grown on nitrate at 0.080 ly min^{-1}						
Initial	12 Jul	1407				
No added N	13 Jul	1115	21.3	8.3	7.5	0.91
Nitrate added	13 Jul	1140	21.7	9.0	12.2	1.36

Table 9. Photosynthetic and Respiratory Quotients of S. costatum in Log-Phase, Originally Grown on Ammonia plus Thiourea at 0.030 ly min^{-1}.

Sample	Date	Time	Δ Time	$\frac{\Delta m\mu moles\ CO_2}{ml/hr}$	$\frac{\Delta m\mu moles\ O_2}{ml/hr}$	PQ	RQ	P/R
No N added	24 Aug	1100						
			12.3	28.8	36.6	1.27		
	24 Aug	2314						4.15
			40.6	6.9	8.1		0.85	
	26 Aug	1550						
Ammonia added	24 Aug	1100						
			12.5	22.9	27.9	1.22		
	24 Aug	2325						3.43
			42.2	6.7	9.7		0.69	
	26 Aug	1738						
NO_3^- added	24 Aug	1100						
			12.6	30.4	38.6	1.27		
	24 Aug	2337						4.95
			42.1	6.2	8.4		0.74	
	26 Aug	1745						

Table 10. Photosynthetic and Respiratory Quotients of S. costatum in Log-Phase, Initially Grown on Nitrate at 0.030 ly min^{-1}.

Sample	Date	Time	ΔTime	$\frac{\Delta m\mu moles\ CO_2}{ml/hr}$	$\frac{\Delta m\mu moles\ O_2}{ml/hr}$	PQ	RQ	P/R
No N added	24 Aug	0900						
			12.8	14.0	20.7	1.48		
	24 Aug	2145						5.18
			40.9	2.7	5.4		0.51	
	26 Aug	1640						
Ammonia added	24 Aug	0900						
			13.0	24.2	25.6	1.06		
	24 Aug	2254						4.81
			41.5	5.0	6.9		0.73	
	26 Aug	1626						
Nitrate added	24 Aug	0900						
			13.3	17.3	25.4	1.47		
	24 Aug	2216						4.13
			42.7	4.2	6.4		0.65	
	24 Aug	1654						

Table 11A. PQ and RQ Values of S. costatum in Log-Phase Grown under Either High or Low Light on Nitrate or Ammonia and Measured under Either High or Low Light.

Sample	Date	Time	ΔTime	$\frac{\Delta m\mu moles\ CO_2}{ml/hr}$	$\frac{\Delta m\mu moles\ O_2}{ml/hr}$	PQ	RQ	P/R
A. Cells grown in high light (0.030 ly min^{-1})								
1. Cells grown on ammonia								
a. Cells measured in high light (0.030 ly min^{-1})								
Initial	1 Aug	1530						
			22.0	4.5	2.7	0.61		
P.S.	2 Aug	1330						7.69
			46.1	0.6	0.8		0.78	
Resp.	4 Aug	1138						
b. Cells measured in low light (0.008 ly min^{-1})								
Initial	1 Aug	1530						
			22.5	5.4	5.5	1.01		
P.S.	2 Aug	1400						3.38
			45.0	1.6	2.3		0.68	
Resp.	4 Aug	1058						
2. Cells grown on nitrate								
a. Cells measured in high light (0.030 ly min^{-1})								
Initial	1 Aug	1530						
			22.0	10.2	11.2	1.10		
P.S.	2 Aug	1320						3,60
			45.9	2.8	2.5		1.13	
Resp.	4 Aug	1112						
b. Cells measured in low light (0.008 ly min^{-1})								
Initial	1 Aug	1530						
			22.0	8.1	9.8	1.19		
P.S.	2 Aug	1330						3.21
			45.9	2.5	2.5		1.03	
Resp.	4 Aug	1125						

Table 11B. PQ and RQ Values of S. costatum in Log-Phase Grown under Either High or Low Light on Nitrate or Ammonia and Measured under Either High or Low Light.

Sample	Date	Time	ΔTime	mμmoles CO_2 / ml/hr	mμmoles O_2 / ml/hr	PQ	RQ	P/R
B. Cells grown in low light (0.008 ly min^{-1})								
1. Cells grown on ammonium								
a. Cells measured in high light (0.030 ly min^{-1})								
Initial	24 Jul	1150						
			27.5	12.2	9.6	0.80		
P.S.	25 Jul	1519						5.02
			46.0	2.4	3.5		0.69	
Resp.	27 Jul	1318						
b. Cells measured in low light (0.008 ly min^{-1})								
Initial	24 Jul	1150						
			27.9	13.1	12.5	0.95		
P.S.	25 Jul	1545						4.92
Resp.	27 Jul		45.2	2.7	3.9		0.68	
2. Cells grown on nitrate								
a. Cells measured in high light (0.030 ly min^{-1})								
Initial	24 Jul	1050						
			27.9	10.9	12.7	1.13		
P.S.	25 Jul	1445						4.44
			45.5	2.5	3.9		0.62	
Resp.	27 Jul	1213						
b. Cells measured in low light (0.008 ly min^{-1})								
Initial	24 Jul	1050						
			29.8	8.2	10.6	1.29		
P.S.	25 Jul	1637						9.97
			43.2	0.8	5.0		0.17	
Resp.	27 Jul	1147						

Experiment IV

This experiment was to determine the effect of varying light quality and intensity upon the photosynthetic quotients, and upon the excretion of dissolved organic carbon by S. costatum. Cells were grown under Corning filters to simulate various depths at sea. Ten 100 ml samples (five for CHN analysis and five for organic oxygen estimation) were taken after growth for one week (I) or two weeks (P) in the light, or one week in the light and one week in the dark (R) (Table 12). The notations C/N, P-I and R-I are defined on page 27. Three replicates of each sample were also taken for dissolved organic carbon analysis (Tables 13 and 14).

The headings PQ_{-o} and RQ_{-o} in Table 12 refer to the calculation of the elemental PQ and RQ values without consideration of organic oxygen. The values for organic oxygen were so unreliable they made the PQ and RQ values meaningless. Consequently, they were deleted for the reasons mentioned on page 21 of the methods section.

Table 12. Photosynthetic and Respiratory Quotients of S. costatum Grown at Various Light Conditions Calculated from the Elemental Composition.

Sample	mg AFDW	µg-at N	µg-at C	C/N	µg-at H	PQ_{-o}	RQ_{-o}
Filter 1F (0.030 ly min^{-1})							
I	0.979	3.60	26.8	7.44	41.8	1.29	
R	0.670	3.20	22.0	6.88	31.0		0.80
P	1.691	6.40	39.6	6.19	79.0	1.38	
R - I		0.40	4.8	12.00	10.8		0.67
P - I		2.80	12.8	4.57	37.2	1.56	
Filter 2F (0.046 ly min^{-1})							
I	0.879	3.45	27.2	7.88	43.3	1.30	
R	0.404	2.65	20.2	7.62	27.4		0.83
P	1.348	5.55	34.7	6.25	72.0	1.40	
R - I		0.80	7.0	8.75	18.6		0.64
P - I		2.10	7.5	3.57	28.7	1.81	
Filter 3F (0.020 ly min^{-1})							
I	0.716	3.65	29.2	8.00	37.7	1.23	
R	0.604	3.33	22.3	6.70	27.2		0.82
P	1.564	8.20	57.8	7.05	114.0	1.39	
R - I		0.32	6.9	21.65	8.5		0.78
P - I		4.55	28.6	6.29	76.3	1.55	
Filter 4F (0.014 ly min^{-1})							
I	0.577	3.62	24.6	6.80	34.0	1.24	
R	0.483	3.42	21.3	6.23	29.4		0.82
P	1.411	7.85	46.0	5.86	88.0	1.35	
R - I		0.20	3.3	16.50	4.6		0.77
P - I		4.23	21.4	5.06	54.0	1.49	
Filter 5F (0.008 ly min^{-1})							
I	0.419	3.30	22.2	6.72	32.3	1.25	
R	0.566	2.65	19.0	7.17	28.5		0.79
P	1.588	7.28	50.0	6.87	103.6	1.41	
R - I		0.65	3.2	4.92	3.8		0.89
P - I		3.98	27.8	6.99	71.3	1.71	

Table 13. Dissolved Organic Analyses from S. costatum Cultures Grown under Various Light Conditions. (DOC = Dissolved Organic Carbon; POC = Particulate Organic Carbon)

Date	Sample	Cells mm^{-1}	$\frac{\mu moles\ POC}{cell} \times 10^{-6}$	$\mu moles\ C\ ml^{-1}$	$\frac{\mu moles\ DOC}{cell} \times 10^{-6}$
	0.030 ly min^{-1}				
6 Jul	I	69	3.88	0.095	1.39
13 Jul	P	295	1.34	0.170	0.58
14 Jul	R	63	3.49	0.061	0.97
	0.046 ly min^{-1}				
6 Jul	I	123	2.21	0.088	0.72
13 Jul	P	150	2.31	0.119	0.79
14 Jul	R	105	1.92	0.038	0.36
	0.020 ly min^{-1}				
5 Jul	I	90	3.24	0.084	0.93
11 Jul	P	186	3.11	0.127	0.68
14 Jul	R	79	2.82	0.058	0.73
	0.0140 ly min^{-1}				
5 Jul	I	130	1.89	0.093	0.72
13 Jul	P	272	1.69	0.168	0.62
14 Jul	R	79	2.70	0.029	0.37
	0.008 ly min^{-1}				
6 Jul	I	123	1.81	0.060	0.49
13 Jul	P	224	2.23	0.108	0.48
14 Jul	R	191	1.00	0.012	0.63

Table 14. Dissolved Organic Carbon Analysis from S. costatum Cultures Grown at 0.008 ly min^{-1} on Nitrate or Ammonia.

Date	Sample (corrected for blank)	μmoles C ml^{-1}
	Ammonia	
26 Jul	I	0.032
7 Aug	P	0.126
7 Aug	R	0.032
	Nitrate	
26 Jul	I	0.026
7 Aug	P	0.164
7 Aug	R	0.028

DISCUSSION

Photosynthetic and respiratory quotients are dependent on a variety of biological, biochemical and environmental factors including characteristic species of the population measured, the net value of the oxidation and reduction reactions resulting from cellular synthesis of structural compounds and substrate utilizations of storage depots, the quality and quantity of the nutrient nitrogen and sulfur sources, and possibly the quality, intensity and periodicity of light. Photosynthetic and respiratory quotients are integrated values resulting from the complex interaction of these phenomena. Since all these factors operate simultaneously, it is of interest to understand which of them exerts the most dominant influence upon the PQ and RQ values of marine phytoplankton over any period of time.

Consideration of Calculated PQ and RQ Values

Expanding and refining the approach of Rabinowitch (1945) and James (1953), the PQ and RQ values for the predominant compounds found in organisms have been calculated from their elemental composition and categorized into major groups as shown in Table 15. Each of these categories represents an average value of all the individual compounds within a major group as reported by Conn and Stumpf (1963). The group values could be somewhat higher or lower

Table 15. Calculated PQ and RQ Values and Carbon to Nitrogen Ratios for Categories of Biochemical Compounds Found in Organisms.

Biochemical Category	RQ	PQ_1	PQ_2	C/N
Organic acids	1.33	0.75	0.75	∞
Deoxyribonucleic acids (DNA)	1.29	0.85	1.62	2.3
Ribonucleic acid (RNA)	1.19	0.78	1.56	2.9
Amino acids (proteins)	1.04	0.99	1.64	4.4
Sugars (carbohydrates)	1.00	1.00	1.00	∞
Chlorophylls	0.83	1.22	1.36	13.8
Fatty acids (lipids)	0.76	1.38	1.38	∞
Carotenoids	0.74	1.35	1.35	∞

The calculations were made on the following assumptions:

1. RQ and PQ_1: Ammonia and divalent sulfur are the nitrogen and sulfur compounds utilized during respiration and/or photosynthesis.
2. PQ_2: For every mole of nitrate and sulfate utilized, there are two apparent moles of oxygen produced.

for particular organisms depending upon the actual percentage composition of individual compounds within a group. However, some generalizations can be made concerning the PQ and RQ of organisms from such a listing.

When photosynthetic organisms are grown on ammonia and a divalent sulfur source, the photosynthetic quotient should deviate from one only if there is a predominance of fatty acids and pigment synthesis or organic and nucleic acid synthesis. The synthesis of sugars and amino acids should yield values very close to one. If, however, photosynthetic organisms are grown on nitrate and sulfate,

(column PQ_2, Table 15), the PQ could become very large, particularly if nucleic acids, chlorophyll and amino acids are the main photosynthate.

Published PQ Values of Marine Phytoplankton

The PQ values for marine phytoplankton reported in the literature (Ryther, 1956) have been revised in Table 16. These values cannot be compared, nor assessed regarding the nature of the photosynthate, because different methods of measurement were utilized, the species were not grown on the same nutrient sources, and the majority of the values were obtained manometrically under high light after being grown under low light.

The PQ Values of Skeletonema costatum

Eppley and Sloan (1965) investigated the PQ of eight marine phytoplankton species (p. 44, Table 16). They grew the organisms at 21.0° C and at a light intensity of 0.070 ly min^{-1}. They used the Winkler method for oxygen determination and the Van Slyke method for carbon dioxide determination over a five hour period. For Skeletonema costatum they obtained a PQ of 1.8 when grown on nitrate and 1.5 to 1.7 when grown on ammonia. Net oxygen production gave higher estimates of carbon assimilation (using a PQ of 1.25 for their calculation) than did their experimental carbon values

Table 16. The Reported Photosynthetic Quotients for Marine Phytoplankton Species.

Species	PQ	Author
Diatoms	1.12-1.13	Wassink and Kersten (1944)
Phaeodactylum tricornutum	1.05-1.08	Barker (1935b)
Peridinium sp.	1.03-1.11	Barker (1935a)
Raw sea water	1.09±0.23	Sargent and Hindman (1943)
Dunaliella tertiolecta	1.16-1.89	Eppley and Sloan (1965)
Skeletonema costatum		
Grown on nitrate	1.80	Eppley and Sloan (1965)
Grown on ammonia	1.49-1.70	Eppley and Sloan (1965)
Ditylum brightwellii	1.49-1.62	Eppley and Sloan (1965)
Thalassiosira rotula	1.69	Eppley and Sloan (1965)
Cyclotella nana	1.50	Eppley and Sloan (1965)
Red tide	0.89-0.98	Eppley and Sloan (1965)
Peridinium trochoideum	1.10	Eppley and Sloan (1965)
Coccolithus huxleyi	1.20	Eppley and Sloan (1965)
Syracosphaera elongata	1.60	Eppley and Sloan (1965)
*Diatoms	1.38	Brandt and Rabin (1920)
*Peridians	1.16	Brandt and Rabin (1920)
**Phaeodactylum tricornutum*		
ammonia	1.11	Ketchum and Redfield (1949)
nitrate	1.20	Ketchum and Redfield (1949)

*PQ calculated from elemental composition

obtained directly from Van Slyke or radiocarbon measurements. This difference could not be accounted for by the excretion of dissolved organic carbon nor by the excretion of ammonia after reduction from nitrate. The authors concluded they were observing an experimental artifact or that S. costatum has an unusual metabolic feature in its excess oxygen production.

Photosynthetic quotients higher than 1.40 or 1.65 for organisms grown on ammonia or nitrate respectively would seem very unlikely on the basis of known biochemical products (p. 42, Table 15). Photosynthetic quotient results obtained in this work (p. 26, Table 5) using direct gas chromatography measurements were 1.00 and 1.43 for S. costatum grown on ammonia and nitrate respectively at a light energy of 0.030 ly min^{-1}. There was substantial agreement between the PQ's obtained by gaseous exchange measurement and elemental analysis (p. 30, Table 7). Since the PQ results in this work, confirmed by two independent methods of measurement, fall within the range of calculated biochemical compounds, it would seem that the high PQ values obtained by Eppley and Sloan (1965) for S. costatum, and perhaps the other organisms, were experimental artifacts.

Strickland (1960) states that the Winkler method cannot be used with sufficient precision in waters containing very high phytoplankton populations since a significant iodine consumption can arise from an "iodine value" of phytoplankton oils. Ackman et al. (1964) has

reported that cultures of S. costatum at various stages of maturity contain long-chained highly unsaturated fatty acids. These compounds would be capable of producing high iodine values. Since Eppley and Sloan (1965) used Winkler oxygen measurements, their extremely high PQ's could be due to iodine uptake.

The Influence of the Quality of the Nitrogen and Sulfur Sources on PQ

The PQ values obtained for S. costatum grown on ammonia (0.98 to 1.01, p. 30, Table 7) would suggest a balanced metabolism between organic and nucleic acids, and pigments and fatty acids. It is not possible to draw more conclusions regarding the type of metabolism without an actual chemical analysis of cellular constituents. The PQ increases with the increasing oxidation state of the nitrogen source (p. 26, Table 5), as reported by Myers and Cramer (1948). Except in the case of S. costatum grown on nitrate (p. 30, Table 7), agreement was obtained between PQ's measured by gaseous exchange and those calculated from elemental analysis using the assumption that for every mole of nitrate or nitrite consumed, there is an apparent 2.0 and 1.5 moles of oxygen produced respectively (Davis, 1953). The word apparent has been used here and elsewhere in this thesis to emphasize that oxygen is not derived from the direct splitting of nitrate or sulfate, but is an indirect consequence of oxygen liberated during the photosynthetic production of chemical energy.

It is this energy which is used to reduce nitrate as shown by the following equations:

Light reaction of photosynthesis

$$4A + 4H_2O \xrightarrow[\text{light}]{\text{chlorophyll}} 4AH_2 + 2O_2$$

Reduction of nitrate

$$4AH_2 + NO_3^- + H^+ \longrightarrow NH_3 + 3H_2O + 4A$$

Net reaction

$$NO_3^- + H^+ + 4H_2O^* \longrightarrow NH_3 + 3H_2O + 2O_2^*$$

The symbol A represents an electron acceptor, such as pyridine nucleotide or ferrodoxin, and the asterisks (*) indicate that the oxygen liberated in nitrate reduction, analogous to carbon dioxide fixation, comes from the photosynthetic splitting of water (Bassham, 1965; Bandurski, 1965; Kok, 1965). However, Grant (1967) found no consistent relationship between the apparent oxygen evolved and the amount of nitrate or nitrite assimilated by Dunaliella tertiolecta.

The difference in photosynthetic quotients between thiourea, a divalent sulfur source used in conjunction with sulfate, and sulfate used alone as an oxidized sulfur source was slight. The difference was 0.04 PQ units (p. 26, Table 5), but it was in the opposite direction expected. If two moles of apparent oxygen are produced for each mole of sulfate utilized, then a higher PQ would be expected for the sample not containing thiourea, assuming that thiourea can be used

as a sulfur source. The significance of this difference is questionable if one considers the following calculation based on the assumption that the ratio of N:S is approximately 32:1 on an atom basis (Epstein, 1965).

$$200\ O_2 + C_{200}H_{500}O_{200}N_{32}S_1 \longrightarrow 200\ CO_2 + 200\ H_2O + H_2S + 32\ NH_3$$

$$\text{Thiourea, } PQ_{s^=} = \frac{200}{200} = 1.00$$

$$\text{Sulfate, } PQ_{SO_4^=} = \frac{202}{200} = 1.01$$

Thus, the method of analysis must be able to detect a difference of approximately 0.01 PQ units. Even though a level of 1% precision was approached for each gas analyzed in the PQ computation and triplicate samples were taken, a standard deviation of only $\pm$ 0.02 PQ units at the 95% confidence limits was achieved. Therefore, the small difference in PQ between various sulfur sources would not be detected by the methods employed in this study.

Nitrogen Deficient Conditions

Under conditions of nitrogen deficiency, with ammonia the original nitrogen source, the PQ (p. 42, Table 15) might be expected to increase with an increasing percentage of fatty acids and a decreasing percentage of nitrogen containing organic compounds; whereas, if nitrate or nitrite were the original source, the PQ might be expected to decrease since the non-nitrogen containing compounds have lower

PQ's than those compounds with nitrogen. These hypotheses are verified in the calculated PQ results (p. 28, Table 6). For example, the average of all N sources indicates the PQ change from one week in the light (I, log-growth phase) to a second week in the light (P-I, a condition of N-deficiency) for ammonia was an increase of 1.01 to 1.03; whereas, nitrite decreased from 1.23 to 1.10 and nitrate from 1.30 to 1.12.

When S. costatum was originally grown on nitrate to log-phase (p. 34, Table 10) or senescence (p. 32, Table 8) and then placed in a condition of nitrogen deficiency or ammonia or nitrate enrichment, the cells under both the ammonia and the nitrate enrichment produced approximately the same amount of oxygen but had different carbon dioxide uptakes. The nitrate repression of carbon dioxide uptake could be explained by a competition for reduced pyridine nucleotide. The latter is produced during photosynthesis and is a necessary intermediate for the reduction of both nitrate and carbon dioxide (Grant, 1967). Whether S. costatum was originally grown on nitrate (p. 34, Table 10) or ammonia (p. 33, Table 9) only an exposure to ammonia produced a reduced PQ. The apparent ammonia stimulation of carbon dioxide uptake could be explained in terms of enhanced amino acid formation from tricarboxylic acid and glycollic intermediates whose replenishment in turn would stimulate the uptake of CO_2. When S. costatum was originally grown on ammonia and

exposed to nitrate, there was no apparent response to nitrate. A response would have been indicated by an increased PQ in the nitrate sample above that of the nutrient deficient treatment. The lack of a response could be due to insufficient time or unsuitable conditions for induction of the nitrate reductase enzyme system.

Carbon to Nitrogen Ratios (C/N)

A survey of the C to N ratios (p. 42, Table 15) indicates the predominant biochemical compounds capable of contributing the majority of nitrogen to the C to N ratio are the amino acids and the ribo- and deoxyribo-nucleic acids. Sugars, organic acids, pigments and fatty acids will tend to increase the C/N ration.

Ketchum and Redfield (1949) stated that the ratio of carbon to nitrogen in culture of one marine and several freshwater phytoplankton was nearly identical for the various species (8.60:1, on an atom basis). This value was also very similar to the C/N values obtained for natural phytoplankton collections at sea (8.65:1). They also reported that the C/N ratio of Chlorella pyrenoidosa under "normal" nutrient conditions (8.4:1) was found to change under phosphorus deficiency to 7.5:1 and under nitrogen deficiency to 25.8:1. Strickland (1965) reported a much wider range (3.0:1 to 9.0:1) of C to N ratios of various marine phytoplankton species. A C to N value of 4.4:1 was indicated for S. costatum in nutrient rich medium.

The C to N ratios obtained in this study for S. costatum under nutrient sufficient conditions were 7.37:1 (average of all I, C/N values on p. 38, Table 12) and 6.95:1 (I, C/N ration, p. 30, Table 7). Under nitrogen deficient conditions the C to N ratio increased (P = 8.99:1 and P-I = 23.90:1, p. 30, Table 7). These values for nitrogen deficiency would indicate a decreased amino and nucleic acid synthesis and a predominant synthesis of non-nitrogen containing compounds.

In the dark, the cellular C/N ratio decreased (R = 5.84, p. 30, Table 7) due to the respiration of biochemical compounds of high C/N ratios (R-I = 22.83, p. 30, Table 7). Thus, there is an apparent cellular conservation of nitrogen in the probable form of proteins and nucleic acids and a respiration of carbohydrates, lipids and possibly pigments.

The Influence of Rapid Light Intensity Changes on the PQ

When S. costatum was grown in low light (0.008 ly min^{-1}) and changed to high light (0.030 ly min^{-1}), the PQ decreased whether grown on nitrate or ammonia; whereas, when grown in high light and switched to low light, the PQ increased (p. 35 and 36, Table 11A and B). The decrease in PQ when the cells were grown in low light and measured at high light was also observed by Myers and Cramer (1949). Myers (1951) stated that the change in light intensity from

low to high causes a shift in metabolism toward carbohydrate production Horvath and Szasz (1965) found that at low light intensities, photosynthesis in Phaseolys vulgaris L. (var. 'Sugar bean') results in a higher proportion of nitrogen compounds; whereas at higher light intensities carbohydrates are predominant.

The photosynthetic quotients obtained for S. costatum when grown on ammonia at both high and low light and measured at only high light (0.61 and 0.80 respectively) would indicate a predominant synthesis of organic acids and possibly nucleic acids. Compounds such as the carboxylic acids malate, citrate and glycolate, certain amino acids like glycine, cystine and aspartate and nucleic acids would yield PQ's less than 0.80. The ammonia grown cells measured at low light yielded PQ values (1.01 and 0.95) very close to that which would indicate a more balanced metabolism among the various cellular components.

Similar trends were also obtained for S. costatum grown on nitrate when grown and measured at high and low light. However, the shifts in metabolism as light intensity is varied are somewhat complicated by possible competition between nitrate and carbon dioxide for photosynthetically produced reducing power. Grant (1967) reported that Dunaliella tertiolecta reached maximum rates of nitrate and nitrite assimilation at 300 and 1000 ft. candles respectively, while both maximum oxygen evolution and carbon fixation rates were

obtained at 1800 ft-candles. Consequently, proportionately more carbon dioxide is fixed than oxygen produced as the light intensity increases. This relationship in turn could yield a decrease in PQ as the light intensity increases, as observed for nitrate (p. 35 and 36, Table 11A and B).

PQ Values After Long Period Exposure to Different Light Conditions

Skeletonema costatum grown at various intensities and qualities of continuous light were found to have relatively constant PQ_{-o} values (p. 38, Table 12). The PQ_{-o} and RQ_{-o} results in Table 12 were obtained by calculation from carbon, hydrogen and nitrogen contents of S. costatum. Organic oxygen values were not included because they were inconsistent. Although the PQ_{-o} results are not absolute, they can be used for the basis of comparison of metabolism at different light intensities and qualities. The average value for all light intensities after one week in the light was $I = 1.26 \pm 0.03$, for two weeks in the light it was $P = 1.39 \pm 0.02$ and the difference $P-I = 1.62 \pm 0.12$. These results are similar to those of Amman and Lynch (1965). The PQ changes only slightly at different light intensities and qualities for Chlorella pyrenoidosa.

RQ Measurements

The measured gaseous exchange respiratory quotients were almost the same regardless of the nitrogen source, but the RQ's calculated from elemental analysis were higher than the RQ's calculated for gaseous exchange (p. 30, Table 7). Only slight changes (R-I) in elemental composition occurred between the initial value (I) and one week later in the dark (R) (p. 28, Table 6); consequently, these values are not considered as reliable as the gaseous exchange RQ values.

Respiratory quotient values for S. costatum, obtained by gaseous exchange after two days in the dark, ranged from 0.51 to 1.03 and averaged 0.75. A value of 0.75 would indicate a predominant respiration of fatty acids and perhaps pigments (p. 42, Table 15).

The RQ is not necessarily the reciprocal of the PQ. First, the percentage respiratory loss of nitrogen is much lower than that for carbon (p. 28, Table 6); therefore, not all of the products formed during photosynthesis are respired in the same proportions. Secondly, the "extra" oxygen produced in the photosynthetic reduction of nitrate is not matched by a corresponding oxidation of ammonia during respiration.

Photosynthesis to Respiration Ratios (P/R)

Ryther (1954) reported that during exponential growth of Chlamydomonas respiration was 5-10% of photosynthesis, but in nutrient starved, non-growing cultures respiration could equal photosynthesis. A calculation of the data of Eppley and Sloan (1965) indicates an average P to R ratio for Skeletonema costatum of 3.27±0.48, or respiration was 30.6% of photosynthesis. In this work, an average of 18 P to R ratios resulted in a P/R of 4.27±1.95 or respiration was 23.4% of photosynthesis. However, the relatively low P/R ratios obtained here could have been influenced by the use of non-axenic cultures and the long period of containment, up to two days, required by the method of analysis. These conditions could promote excessive bacterial respiration and bias the results by lowering the P/R ratio.

Excretion of Dissolved Organic Carbon

Hellebust (1965) states that S. costatum excretes considerably more dissolved organic carbon (DOC) at 300 ft-candles (9%) than at 1000 ft-candles (4.6%). The higher excretion values obtained at low light rather than at light intensities approaching light saturation are thought to be due to the organic excretion of a relatively fixed proportion of the total carbon rather than the net photoassimilated

carbon. The data in Table 13 (p. 39) would tend to confirm this suggestion, since approximately the same amount of DOC is produced at various light intensities and qualities ($I = 0.084 \pm 0.013$ $\mu mC\ ml^{-1}$). The production of DOC nearly doubled as the culture aged for two weeks in the light ($P = 0.138 \pm 0.026$ $\mu MC\ ml^{-1}$). There was an indication of possible respiration of the DOC. The amount of DOC produced in the light decreased after one week in the dark ($R = 0.040 \pm 0.018$ $\mu MC\ ml^{-1}$). The source of nutrient nitrogen apparently had little or no influence on the production of DOC; approximately the same amount of DOC was produced whether S. costatum was grown on nitrate or ammonia (p. 40, Table 14).

Comparison of the Carbon-14 and Gaseous Exchange Methods

A comparison of the carbon-14 and gaseous exchange methods was made in experiment I (p. 30, Table 7). The rate of photosynthetically fixed carbon measured by the carbon-14 method was found to be consistently lower than the carbon dioxide rate monitored by gaseous exchange. The carbon-14 method measures indirectly the amount of carbon incorporated into the algae, but is not an absolute measure. Moreover, labelled DOC can be excreted without detection. Gaseous exchange measures the net exchange of inorganic carbon and also does not discriminate between particulate fixed and excreted dissolved organic carbon. Thus, the difference between the two methods

could be explained on the basis of the loss of dissolved organic compounds by S. costatum. S. costatum, in this study, was found to excrete up to 20% of the carbon fixed in photosynthesis as dissolved organic carbon.

There was even a larger discrepancy between the rates of respiration measured by the two methods. The difference could be attributed to the assimilation and respiration of dissolved organic compounds. The gaseous exchange technique would give an indication of DOC respiration; the carbon-14 method would not.

CONCLUSIONS

This study indicates that, of the factors tested, the quality and quantity of the nutrient nitrogen source exerts the most pronounced influence on the photosynthetic quotient of Skeletonema costatum. The PQ obtained for cells grown on ammonia at constant light indicated a balanced metabolism among the various cellular constituents. The increase in PQ with an increasing oxidation state of the nitrogen source is probably due to a competition among carbon dioxide, nitrite, and nitrate for the reducing potential of photosynthesis, rather than a change in the type of metabolism. A deficiency in the nitrogen source produced either an increased or decreased PQ, depending upon the original N source. No change in PQ was found for S. costatum grown on sulfur at various oxidation states due to insufficient instrument sensitivity.

Even though the PQ and the DOC production were found to be relatively constant with continuous light of different qualities and intensities, a rapid change from low light to high light decreased the PQ; whereas, a rapid change from high light to low light increased the PQ. Similar changes in PQ might be expected to occur in the environment during a clear day.

Other factors not examined in this study, such as the species composition of a population, might also be expected to influence the

PQ of populations because not all organisms photosynthesize the same proportions of metabolic products.

In consideration of the factors which could influence the PQ and RQ values of marine phytoplankton populations, if at all possible, it would be desirable to measure PQ's and RQ's directly. Perhaps, modifications of the methods presented here could be utilized for field measurements of PQ and RQ since the gas chromatographic analysis of oxygen approaches the sensitivity of the Winkler oxygen method. Nevertheless, some suggestions of PQ can be given for experimental use, if a population is predominantly composed of Skeletonema costatum, the light conditions relatively constant, and the nutrient conditions are known. Under these situations, suggested PQ's for populations growing on ammonia would be 1.00 ± 0.10 and for nitrate, 1.35 ± 0.15. However, further measurements are needed to make these suggested values statistically significant.

The RQ values for S. costatum were close to 0.75, indicating fatty acid respiration. Consequently, the 1.00 RQ value suggested by Ryther (1956) and Strickland (1965) would not be satisfactory for a population dominated by diatoms. The RQ is not simply the reciprocal of the PQ.

This work illustrates the application of gas chromatography for the study of photosynthetic and respiratory metabolism of marine phytoplankton. Gas chromatography approaches the sensitivity of the

Winkler method for analyzing oxygen, but it also has the added capability of analyzing many other substances such as carbon dioxide, nitrogen and dissolved organic carbon. It overcomes the "iodine error" of the Winkler method as experienced by Eppley and Sloan (1965). Myers' (1951) objection to manometric PQ measurements is resolved. It can yield a net production value for either oxygen or carbon dioxide, give an estimation of the predominant metabolic product, and, possibly, indicate the type of nutrient nitrogen source being utilized. Although gas chromatography is probably not sensitive enough at the present time for field work, it at least has the potential for productivity studies.

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