

# LIMNOLOGY AND OCEANOGRAPHY

July, 1961

VOLUME VI

NUMBER 3

## MEASUREMENTS OF PRIMARY PRODUCTION IN COASTAL SEA WATER USING A LARGE-VOLUME PLASTIC SPHERE

C. D. McAllister, T. R. Parsons, K. Stephens  
and J. D. H. Strickland

Fisheries Research Board of Canada,  
Pacific Oceanographic Group, Nanaimo, British Columbia, Canada

### ABSTRACT

A free-floating 20-ft diameter thin transparent sphere, with its center 5.5 m below the sea surface, was filled with nutrient rich water filtered free from plants and animals and inoculated with a natural population of coastal phytoplankters. The resulting phytoplankton "bloom" was then studied in this well-mixed water mass under near-natural conditions of temperature and illumination with the minimum of complications arising from grazing, sinking or lateral transport of the plant cells.

Daily measurements were made of temperature, light and photosynthesis and the water was analyzed for oxygen, carbon dioxide and all common micronutrients. The production of plant material was followed by cell counts and pigment values and by analyses for particulate carbon, nitrogen, phosphorus, carbohydrate, protein and fat.

The results have a bearing on nearly all aspects of marine primary productivity and are discussed in detail. In particular, the growth kinetics and chemical composition of a natural mixed crop have been related to the environment with the minimum of ambiguity. Growth was finally limited by nitrogen deficiency, although the plant biomass nearly doubled after nitrate became depleted, with a shift of metabolism to carbohydrate and fat synthesis. The composition of the plankton at various stages in a bloom is summarized by ratios involving carbon and chlorophyll *a*.

Differences between the results for photosynthesis obtained by the oxygen and C<sup>14</sup> method have emphasized the importance of knowing photosynthetic quotient values and possible unlabeled sources of plant carbon. During an initial period of low illumination there was strong evidence for heterotrophic growth.

The principal species found in the "bloom" were *Skeletonema costatum*, *Thalassiosira nordenskiöldii*, *T. rotula*, *Gyrodinium fulcum*, *Glenodinium danicum*, *Nitzschia delicatissima* and *Asterionella japonica*. The last two grew very slowly and the two dinoflagellates only appeared after the nitrate was depleted.

### INTRODUCTION

It is unnecessary to stress the difficulty of studying photosynthetic productivity in sea water that arises because of the impossibility of observing any one discrete body of water for a sufficient length of time. Valuable information can be obtained by observing the behavior of pure cultures of marine phytoplankton but such work is no adequate

substitute for the study of the growth and decay processes that occur with mixed populations under natural conditions.

An obvious solution to the problem would be to impound sea water in a tank and valuable exploratory experiments of this nature were undertaken by Edmondson and Edmondson (1947) and Edmondson (1956). However, the use of a tank has several disadvantages unless it is very deep. The light-

ing of the water mass as a whole can be unrealistically high, and undesirable temperature increases usually occur. Furthermore, no precise "balance" of dissolved oxygen or carbon dioxide can be readily computed when there is a large surface area of water in contact with the atmosphere.

The thin plastic tube experiment proposed by Margalef (1956) would avoid some of this trouble, but in order to reduce wall-effects (the undesired growth of epigenous organisms, especially bacteria) one must have as large a volume to surface area ratio as possible. It is now common experience that a wall-effect from bacteria can be noticed within a very few hours in a small bottle and we may therefore assume that a container must be many feet in diameter before wall-effects can be neglected for a period of many days. A large container has the additional advantage that errors from accidental contamination are reduced.

To overcome most of the difficulties outlined above we have constructed a free-floating thin plastic sphere, 20 ft in diameter, suspended with its center a constant distance (5.5 m) beneath the sea surface. This plastic bag was connected to the surface by a comparatively narrow neck through which samples could be taken. The state of the water contained in the sphere approximated closely that of the surrounding sea water, both in temperature and in the conditions of illumination. An account of the construction of this equipment has already been published by Strickland and Terhune (1961). It was used in Departure Bay ( $49^{\circ}20' \text{ N}$ ,  $123^{\circ}50' \text{ W}$ ), near Nanaimo, on the east coast of Vancouver Island, British Columbia.

The purpose of the present paper is to describe a phytoplankton bloom which was observed in the plastic sphere under these near-natural conditions. The production of such a bloom has enabled growth conditions to be studied without any of the ambiguities normally introduced by water movement. Further, the detailed analysis of the resulting crop has given the first extensive set of results applicable to detritus-free marine phytoplankton grown under natural conditions, that has so far been reported. Phenomena such as the succession of species and the effect on phytoplankton of nutrient environment were studied with the minimum of complications.

In one experiment the plastic sphere was torn after only 5 days but in the other (June–July 1960) 22 days of growth and decay were recorded before the equipment commenced to leak and this experiment forms the basis of most of the results reported in the present communication. We propose to repeat the experiment at least once more, and expect to be able to prolong it for many more weeks so that the decay processes of an initial bloom can be studied in greater detail. There is really no end to the number of such experiments that could be undertaken as each time different species of plant cells would doubtless develop. The present work, however, measures the production of a diatom crop which is quite typical of the spring bloom in temperate coastal seas.

In this experiment some difficulty was encountered with the stirring mechanism and it was not possible to keep all dead and dying plant cells in uniform suspension within the bag. In these circumstances one cannot equate the formation of soluble metabolites to the decrease of particulate matter sampled at the center of the bag. However, this limitation applied only to the last few days of the decay processes which followed peak phytoplankton production.

The authors would like to record their thanks to L. D. B. Terhune and others at the Pacific Biological Station, Nanaimo, for their work in designing, assembling and launching the equipment. We also wish to acknowledge the assistance given by the undergraduate student assistants, Wing Wai and John Yu, in the analytical program.

#### PROCEDURE AND METHODS

##### *General procedure*

The plastic bag was filled with water taken near the sea floor, about 350 ft from the low-tide shore line. The depth of the water at the intake varied with the tide between some 15 and 20 m. The water was pumped to land, filtered through a cloth-plate filter coated with diatomaceous earth,

and returned to the location of the bag (250 ft out in 11–16 m of water) by an all-plastic pipe system. Volumes were recorded with a water meter and a total of 125 m<sup>3</sup> of sea water was required to fill the bag.

After preliminary analyses the contents of the bag were "inoculated" with 4 m<sup>3</sup> of the surrounding sea water drawn from a depth of about 4 m. This water was pumped through an all-plastic assembly, and was strained through 320- $\mu$  mesh nylon netting to remove all but the smaller zooplankters.

The initial water in the bag was entirely free from animals and contained few if any plant cells. The chlorophyll *a* content of about 0.15 mg/m<sup>3</sup> was probably detrital, although there could have been a few microflagellates present. The inoculum was high in pigment (an average chlorophyll *a* content of 11 mg/m<sup>3</sup>) and undoubtedly introduced some animal population into the bag. However, no significant zooplankton population developed during the course of the experiment and no chitin was detected analytically at any time.

The initial "background" level of particulate organic matter was undesirably high. The material consisted mainly of protein and we cannot explain its presence. Most probably there was a leak of diatomaceous earth at one stage in the filling, or detrital material of near-colloidal dimensions may have escaped filtration. The amount was very much less than in unfiltered sea water.

The inorganic nutrient content of the bottom water used to fill the plastic sphere was large and representative of the winter levels of concentration found in this area, which is one of the most fertile coastal regions of the world. Therefore, no artificial fertilization with silicate, nitrate, or phosphate was attempted.

As a daily routine, the depth of the plastic sphere was adjusted to a constant level by the suitable addition or removal of air from the buoyancy bell. The temperature and salinity of the water at the center of the bag and at the same depth in the sea alongside the bag was measured first thing each morning. A plastic Nansen reversing bottle with protected thermometer was employed to

measure temperature and to take samples for salinity determination.

At about 0900 hr each day samples were taken in a plastic Van Dorn bottle and used for water analyses. The water was filtered at once through a Millipore HA membrane filter and suitable aliquots of the filtrate stored at -20°C in the dark until ready for analysis. Separate samples were drawn for oxygen, pH and alkalinity determinations, which were performed immediately.

Measurements of the attenuation of light in the sea and in the bag were carried out in the mid-morning and mid-afternoon. At about 1030 hr an 8-L sample was taken in Van Dorn bottles and well mixed in a polyethylene aspirator. Nitrogen was passed through the water, if necessary, to reduce the concentration of dissolved oxygen to below the saturation level and then the water was passed into 300-ml B.O.D. bottles for the measurement of photosynthesis. Some bottles were placed in a stainless steel holder suspended at a point 2 ft above the center of the bag on the stirring line and were left in the bag for 24 hr (1200 to 1200). Other bottles were illuminated in a laboratory incubator under constant (artificial) light conditions for a period of less than 6 hr.

At 1330 the water in the bag was sampled for the analysis of particulate matter. Samples were taken at the mid-point of the plastic sphere, using a Van Dorn bottle, and were mixed and stored in a 20-L polyethylene bottle. Filtration of this sample was commenced without delay.

Occasionally samples were taken from several depths and analyzed for pigment and dissolved oxygen, *etc.* in order to check on the uniformity of distribution of metabolites within the sphere.

#### *Determination of dissolved substances*

Full details of all analytical methods are given by Strickland and Parsons (1961). The following brief summary indicates the general techniques that were used.

Salinity was measured by an electrical conductivity salinometer, oxygen by the Winkler method, pH using the Beckman

model GS meter and a glass electrode standardized by standard phosphate buffer at pH 6.87, alkalinity by the Anderson-Robinson method (Anderson and Robinson 1946), silicate by the method of Mullin and Riley (1955a), nitrite by the method of Bend-schneider and Robinson (1952), nitrate by a modification of the procedure described by Mullin and Riley (1955b), and phosphate-phosphorus by a modification of the method of Robinson and Thompson (1948). Total soluble phosphorus was determined as phosphate after wet oxidation with perchloric acid (*cf.* Hansen and Robinson 1953). Soluble organic phosphorus was measured as the difference between the total phosphate in a sample passed through a Millipore HA filter and the inorganic phosphate in the same sample. Particulate and soluble reactive iron and ammonia were estimated as outlined by Strickland and Austin (1959). The method used for manganese was based on the catalyzed oxidation of the leucobase of malachite green by periodate, as developed by one of the present authors (*see also* Yuen 1958). Copper was determined as the colored diethyldithiocarbamate complex which was extracted directly into carbon tetrachloride. Soluble organic nitrogen was estimated by evaporating 25 ml of filtered water and subjecting the residue to Kjeldahl oxidation. The ammonia in the residue was then determined absorptiometrically, as above, and the result corrected for any ammonia found in the initial sample.

The change of total carbonate-carbon in the bag was obtained from precise measurements of the change in pH, knowing the total alkalinity of the system, and using a modification of the Buch tables given by Strickland and Parsons (1961) with graphical interpolations. As only changes in carbonate concentration were being measured in an isolated system, a very precise alkalinity determination was not necessary (*cf.* Strickland 1960).

#### *Determination of particulate matter*

Details of all methods (with the exception of the enumeration of phytoplankton cells) are described by Strickland and Parsons (1961). A brief summary of the procedures

will be given here for the convenience of the reader.

All sea water samples were first filtered through a nylon net with a mesh size of 300  $\mu$  to remove any large particles of extraneous detritus introduced during sampling. The concentration of the particulate matter was accomplished by filtering the water through a Millipore type AA membrane filter, previously treated with magnesium carbonate (Parsons and Strickland 1959). When sufficient sea water had been filtered the Millipore filter was removed and the concentrated particulate material was washed from the surface with a small quantity of 3.0% saline. The suspended particulate matter and magnesium carbonate were then further concentrated by centrifugation and the supernatant liquid was discarded. The resulting residue was used for the determination of carbon, carbohydrate, crude fibre carbohydrate, protein, fat, nitrogen, and phosphorus.

#### *Plant pigments*

Chlorophyll *a*, *b*, *c* and carotenoids were measured as described by Richards with Thompson (1952) using the Millipore filter technique to concentrate the phytoplankton cells described by Creitz and Richards (1955). Extinctions were measured in 10-cm cuvettes. A correction for turbidity was made using the extinction at a wave length of 7,500 Å, multiplied by a predetermined factor to allow for increasing light scatter at decreasing wave lengths.

#### *Carbon*

Particulate carbon was determined by wet oxidation with potassium dichromate in concentrated sulphuric acid by a modification of the procedure described by Johnson (1949). The decrease in the extinction of dichromate solutions at a wave length of 4,400 Å served as a measure of the amount of oxidizable carbon.

#### *Protein and nitrogen*

Particulate protein was determined by two methods. The first involved Kjeldahl digestion of the sample and an estimation of the ammonia using Nessler's reagent, Johnson (1941). Nitrogen values determined by this

method were converted to protein values by multiplying by a factor of 6.25. The second method involved the hydrolysis of the particulate protein with 6 N HCl at 100°C for 6 hr, followed by a colorimetric determination of the hydrolyzed protein using 2,5-hexanedione and Ehrlich's reagent, as described by Keeler (1959). The method was standardized using pure dry casein. Nitrogen values were obtained by dividing by a factor of 6.35. Unlike the tyrosine methods for protein, the Keeler technique involves the reaction of 8 amino acids and ammonia in a hydrolysate. However, results still depend on the amino acid spectrum of the algal proteins which differs from species to species and which cannot be expected to resemble that of casein exactly.

#### Carbohydrate

Particulate carbohydrate was determined by the anthrone reaction using the reagents employed by Hewitt (1958) and measuring the light absorption of the colored solution developed at a wave length of 6,200 Å. Additional absorptions were measured at wave lengths of 5,500 Å and 6,500 Å in order to determine, qualitatively, the presence of hexuronic acids and pentoses. The method was standardized with glucose. "Crude Fibre Carbohydrate" was also determined by the anthrone reagent on a concentrated residue after consecutive treatments with 1.25% sulphuric acid and 1.25% sodium hydroxide in a boiling water bath.

#### Fat

The amount of fat present in the particulate matter was determined by a modification of the method described by Mukerjee (1956). The method specifically measures fatty acids. Results obtained by the use of this method may be less than values obtained by an ether extraction technique. The method was standardized with stearic acid.

#### Particulate phosphorus

Particulate phosphorus was determined as phosphate after a wet oxidation with perchloric acid (*cf.* Hansen and Robinson 1953). The particulate phosphorus is defined as that retained on a Millipore HA filter.

#### Enumeration of phytoplankton cells

Phytoplankton cells were counted using a Unitron inverted biological microscope. Samples of sea water were preserved in 2% neutral formalin and counts were made on 5- or 10-ml aliquots of sea water without prior concentration of the cells. Samples were counted at magnifications of 50×, 100×, and 400×. Duplicate counts of 20 fields made at equal intervals across the diameter of the cylinders (2.6 cm in diameter) normally agreed within 10%. An approximate estimate of the average cell volume of each of the principal species present was made by direct microscopical measurement of the dimensions and shapes of the cells. All cells were counted, living or dead, provided that they were sufficiently intact for ready identification.

Phytoplankton cell numbers, as such, were of limited interest because of the enormous size differences between one species and another. For better comparison total cell numbers were converted to total cell volume ( $\mu^3$ ) by the following experimentally determined factors: *Nitzschia delicatissima*, 500; *Skeletonema costatum*, 1,900; *Glenodinium danicum*, 3,600; *Thalassiosira nordenskiöldii*, 11,000; *Thalassiosira rotula*, 35,000, and *Gyrodinium fulvum*(?), 150,000. (In the other experiment, of only 5 days duration, the following additional factors were used: *Coscinodiscus radiatus*, 12,500; *Coscinodiscus centralis*, 7,350,000.) To convert the resulting algal volumes to organic carbon a factor of 0.1 was employed (*cf.* Strickland 1960). The evaluation of total carbon from cell counts by the above procedure is, at best, very approximate. However, results of cell counts alone can be most misleading unless some such attempt is made to translate cell numbers into a measure of biomass.

#### Measurement of radiant energy

Incident radiation was measured with an Eppley pyrheliumeter situated on a hill about 400 yd from the plastic sphere. Photosynthetically active radiation (3,800–7,200 Å) was assumed to be 50% of that measured by the pyrheliumeter, and is reported in cal/cm<sup>2</sup>/min (ly/min, *cf.* Strickland 1958).

Mean daily light intensities are the averages of the values recorded between sunrise and sunset.

Optical extinction coefficients of the water in the bag and the surrounding sea were determined from measurements with an underwater light meter and deck cell. Both cells were fitted with 2 mm of Schott BG. 12 blue glass, giving a maximum response of photocell-filter combination near to a wave length of 4,300 Å. The attenuation of photosynthetically active light was estimated from these extinction coefficients using the data collected by Jerlov (1951).

Mean daily light intensities at the center of the plastic bag were calculated using the extinction coefficient of the surrounding water through the first 3 m, and then the extinction coefficient of the water in the sphere through a further 3 m. When the morning and afternoon extinction coefficients differed markedly their mean value was used. It must be emphasized that the radiation intensities obtained by this method are only very approximate but relative values from day to day are strictly comparable.

#### *Measurement of photosynthesis*

Measurements of photosynthesis were carried out by both the light and dark bottle oxygen method and a modification of Nielsen's  $C^{14}$  technique (see Strickland and Parsons 1961). All rates were measured using duplicate "light" bottles and a single "dark" bottle. The initial oxygen concentration was determined so that the net oxygen changes and respiration values in dark bottles could be evaluated.

The constant light intensity incubator used in this work held the B.O.D. bottles lying horizontally in black painted trays and cooled by running tap water. Samples were illuminated from above by blue and green fluorescent lamps emitting 0.1 ly/min of radiation as measured by a pyrheliometer.

No account was taken of possible periodicity effects in photosynthesis as these are thought to be quite small at latitude 49°N (Doty 1959).

One would suppose from the work of Fogg (1958) and others that  $C^{14}O_2$  uptake experi-

ments of more than about 6-hr duration would give errors due to the secretion of labelled material. It was impracticable, however, to conduct a series of short exposures in the sphere throughout each day and extrapolation from a single 6-hr period to a full 24 hr of light and dark was too inaccurate to be of use. Therefore we resorted to 24-hr radiocarbon experiments, on the assumption that at low light intensities the results would be tolerably accurate, as already reported by Rodhe (1958). We confirmed this experimentally by comparing the sum of the uptakes in two half-day exposures with that of a full 24-hr exposure in the bag. At the higher light intensity in the incubator there was very poor agreement between the rates found with 6- and 24-hr exposures, again supporting the findings of Rodhe (1958). At this higher light intensity plants were metabolizing many times more rapidly in the incubator than in the bag, and dividing once or twice in the 24-hr period of continuous illumination. Under such conditions the secretion of metabolites and "light fatigue" of the plant cells would be expected to be pronounced.

Experiments to measure the relative rate of photosynthesis as a function of illumination were carried out with the  $C^{14}$  method and the wire-screen attenuators described by McAllister and Strickland (1961). Natural daylight was used as a source of illumination during the period between about 1000 and 1400 on cloudless days. The light intensity over this period was nearly constant.

#### RESULTS

The salinity of the water in the bag was 27.00‰ and its temperature initially 11°C. The transmission of light through the polyvinyl chloride plastic and its supporting coarse-mesh nylon netting was greater than 75%, excluding any effects from direct shading by the supporting buoyancy bell and cradle. Temperature changes in the water inside the sphere closely followed those of the surrounding sea water. This is illustrated by Figure 1 which shows the total daily amount of solar radiation at the sea surface, the temperature at 5 m depth in the sea, and

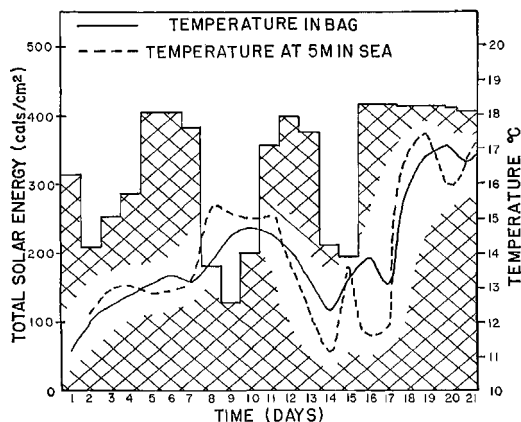


FIG. 1. Total solar radiation and the water temperature inside and outside the plastic sphere.

the temperature at 5.5 m in the bag. It is interesting to note the delay of some 4 days before the sea temperature showed the influence of large changes of incoming radiation. The temperature of the water in the bag followed that of the sea outside with a lag of only about one day.

Most of the data obtained during the present experiment are summarized graphically, and generally the limits of variation are indicated by vertical lines or by the size of the plotted points. The confidence limits embrace the 95% probability region or are thought to approximate this value. Certain important ratios of metabolites and of metabolites to photosynthetic rates, *etc.* are collected in the tables and will be discussed later. The precision of these ratios varies greatly but their significance may generally be judged by the number of significant figures quoted. Some other results appear to have little obvious significance and will be summarized briefly in this section.

No chlorophyll *b* was detected in any analyses and the level of animal carotenoid (astacin) pigments was very low and erratic. The apparent presence of the latter may have been an analytical artifact. Copper was present in a concentration of  $0.048 \mu\text{g-at Cu/L}$  which did not change throughout the three weeks of the experiment. The concentration of reactive manganese varied between  $0.01$  and  $0.04 \mu\text{g-at Mn/L}$  with little obvious pattern, although there was a

maximum just before the main phytoplankton growth period started (day 8). Soluble reactive iron changed little in concentration being in the range  $0.07$  to  $0.12 \mu\text{g-at Fe/L}$ . The amount of particulate reactive iron was quite large. Initially present at  $0.7 \mu\text{g-at Fe/L}$  it decreased in quantity to one-third of this value by day 12. The concentration thereafter increased to the initial level. The significance of this behavior, if any, is not clear. The decrease roughly paralleled that of soluble nitrogen but more experimental work is necessary before definite conclusions are possible.

The initial concentration of soluble organic phosphorus in the sphere was appreciable ( $0.3 \mu\text{g-at/L}$ ) and seems to have increased slowly during the growth of the phytoplankton crop and then more rapidly as the crop decayed, reaching a value as high as  $0.55 \mu\text{g-at/L}$ . Three anomalous results were obtained in this series of determinations and more work is required before any definite pattern can be stated. The concentration of soluble organic nitrogen showed a similar increase throughout the experiment (about 2 up to  $7 \mu\text{g-at N/L}$ ) but there was no marked correlation with the algal growth behavior. The precision of this method is relatively low ( $\pm 0.8 \mu\text{g-at N/L}$  or worse) which may have masked any subtle relationships.

In a well-stirred conserved system the decrease of total dissolved nitrogen or phosphorus must be equal to the increase found in the particulate matter. The analytical results of losses and gains in the present work gave tolerable agreement until after day 17. Then serious discrepancies resulted which showed that stirring was insufficient to keep dead and dying cells in suspension. The decrease of particulate matter after day 17, therefore, represents both the true dissolution of solids and their removal from the bag center by settling. Although analytical results on the particulate material, *per se*, are still valid, no conclusions can be drawn from *changes* of concentration (as sampled at the center of sphere) for the last 5 days of the experiment.

The useful life of the plastic bag was ter-

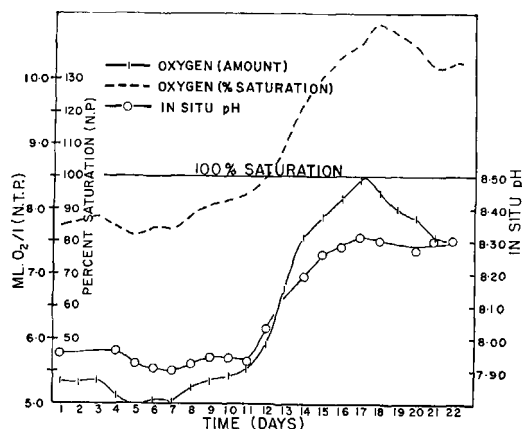


FIG. 2. Changes in pH and dissolved oxygen.

minated by tears near the neck (at day 5 in one trial and day 23 in the main experiment). As the salinity of the surrounding sea water was generally 2 or 3‰ less than the water in the bag the entry of outside water was easily detected by precise (conductivity) salinity measurements.

#### DISCUSSION

The net production of organic matter during the course of the main experiment is illustrated by Figure 2, which records the change of pH and oxygen concentration in the plastic sphere. The percentage saturation of oxygen (at atmospheric pressure) shows the striking degree of supersaturation often reported in the literature and attributed to the effect of photosynthesis. A value of nearly 150% was recorded in the present work. The subsequent decrease of oxygen concentration (after day 17) was mainly due to physical desaturation processes as no corresponding decrease of pH occurred.

The main phytoplankton species present at various stages in the experiment are shown in Figure 3. The use of algal weight (derived from algal volume assuming a mean density of unity) enables one to comprehend the relative contribution of each species to the overall primary production. The total cell count exceeded that of the named species by up to 25% but the unnamed species represented a much smaller addition to the biomass than numbers alone would indicate.

*Asterionella japonica* was the most conspicuous of the minor constituents.

The growth rate of each species was not uniform but over the period of most constant growth mean division times could be evaluated. Values for the diatoms *Skeletonema costatum*, *Thalassiosira rotula*, *Thalassiosira nordenskiöldii*, and the dinoflagellate *Glenodinium danicum* were very similar (30–38 hr). The dinoflagellate *Gyrodinium fulvum* (?) was noticeably more rapid in growth (22 hr) and for an initial period *T. rotula* increased with a doubling time of only 19 hr. The cell numbers of all species eventually showed a decrease which we attribute to the rapid sinking of dead or dying cells (ref., e.g., Gross and Zeuthen 1948; Steele and Yentsch 1960). *T. rotula* passed its peak of metabolic efficiency somewhere between days 8 and 11. This could reflect either a marked sensitivity to the concentration of nitrate or be the result of an increase of water temperature from 12 to 15°C. One cannot rule out the possibility of an inhibition by the increasing concentration of *Skeletonema* cells. *Nitzschia delicatissima* was the only organism to grow to any degree under markedly unfavorable conditions (a doubling time of over 80 hr) and this species commenced to die off soon after the depletion of nitrate at the end of day 13 (Fig. 4). *Asterionella japonica* persisted in small numbers throughout most of the experiment but was obviously living under conditions so unfavorable as to bring cell division almost to a standstill.

The growth rate of *S. costatum* decreased as soon as nitrate disappeared from solution but, statistically, the cells were capable of one further division. *T. nordenskiöldii* grew more vigorously and divided twice before the cells commenced to die. By contrast the two dinoflagellates only began to increase rapidly after the nitrate was exhausted and *Gyrodinium fulvum* survived in fair condition until the end of the experiment. The above observations give a striking illustration of the kind of conditions that bring about a "succession of species" in nature (cf. Conover 1956; Margalef 1956). We are unable to explain the kinks in the curves for *T.*



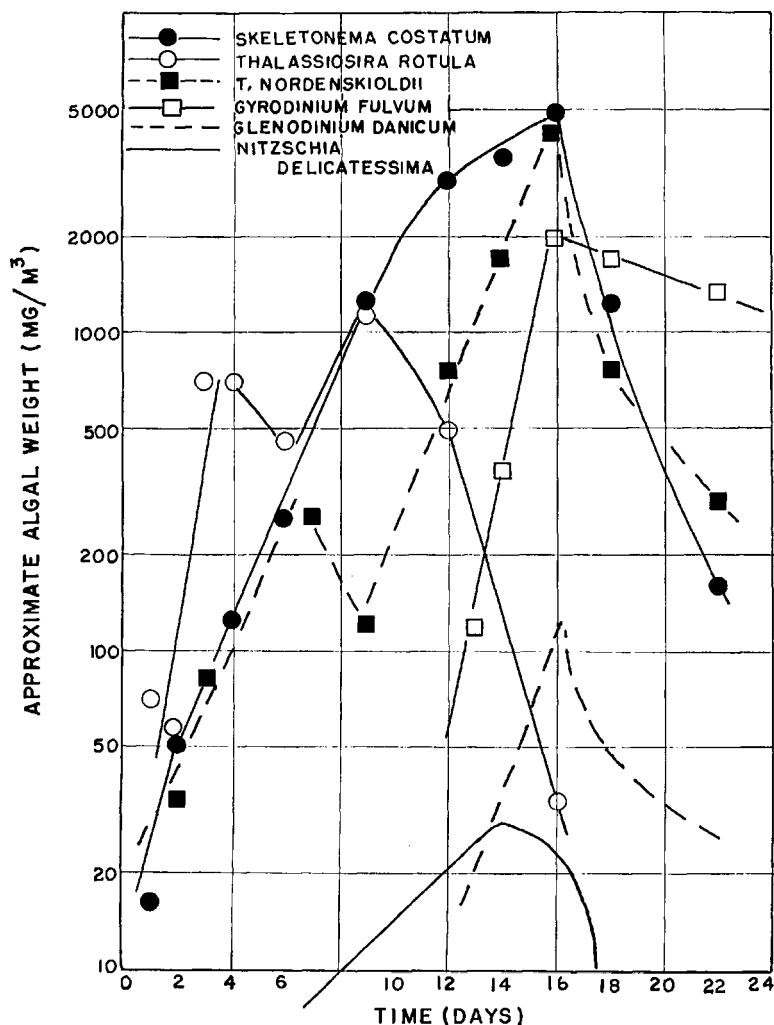


FIG. 3. Changes in the algal weight of the main phytoplankton species.

*rotula* and *T. nordenskiöldii* (there was a similar very high point for *Skeletonema* at day 3, not plotted). There must have been an error either in sampling or in counting brought about by a change in mean chain length, and hence a contagion factor, for the species concerned.

Iron, copper, and manganese were clearly not limiting nutrients for plant growth and reactive silicate was initially present at such a concentration that it never became depleted, despite the crop being predominantly diatoms. One would infer from our results that the initial Si:N ratio in sea water would have to be less than 2:1 (on an atom basis)

for silicon to become a limiting nutrient in a diatom bloom. It is interesting to note the small but significant rise in silicate concentration after day 16 which indicates that a fraction of the silicon in a diatom is very labile (*cf.* Jørgensen 1955a). Subsequently there was only very slow remineralization. In considering this fact and the remineralization of nitrogen (see later) it must be remembered that the present experiment is unrepresentative of nature in that no regeneration processes could occur as a result of grazing (*cf.* Cushing 1959).

The initial ratio of nitrogen to phosphorus in solution was 9 (on an atom basis) or 10 if

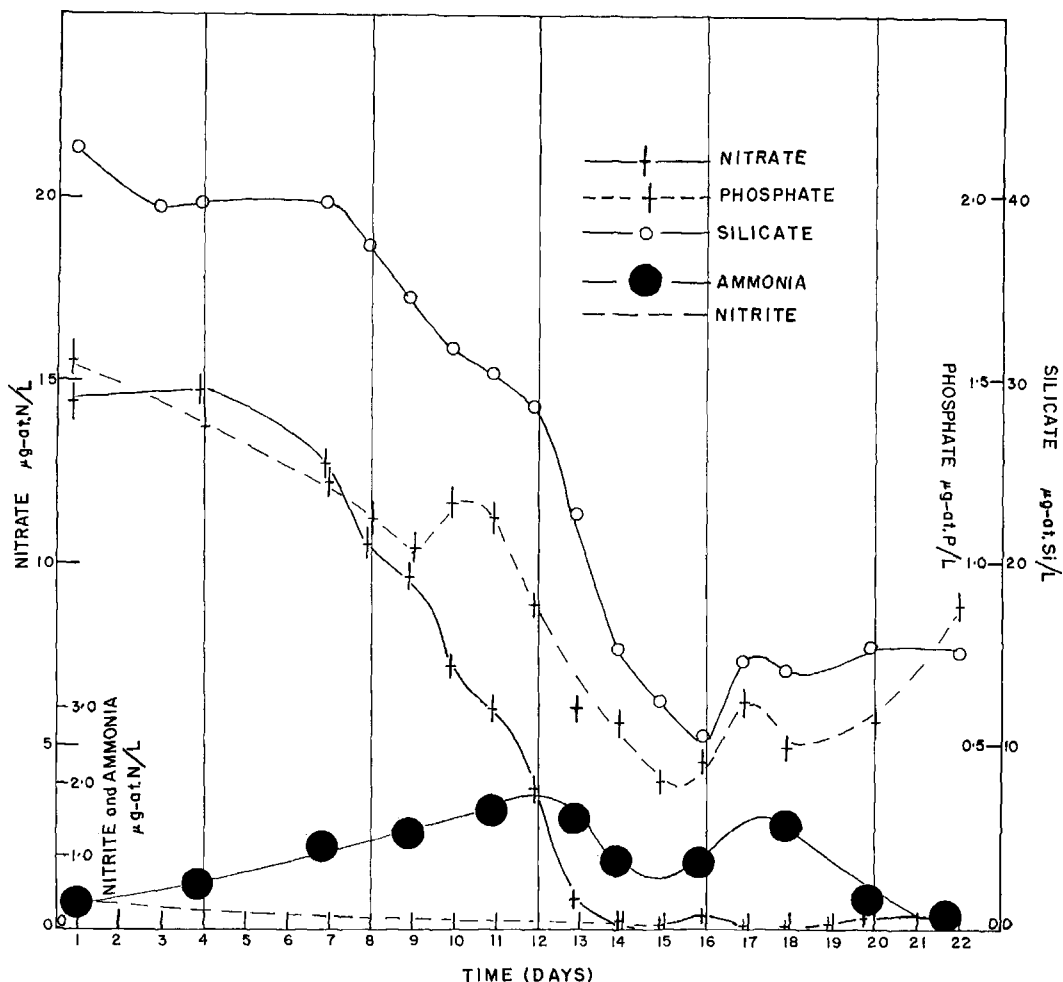


FIG. 4. Changes in the concentration of nitrate, nitrite, ammonia, reactive phosphate and reactive silicate.

we consider only nitrate and inorganic phosphate. On the assumption that growing plants withdraw these elements from sea water in the mean atomic ratio of 15:1 (Cooper 1937, 1938) one would expect to find nitrate removed before phosphate, as was indeed the case (Fig. 4). Furthermore, the tendency for phosphate to continue to decrease in concentration after all the nitrate has gone, as found in cultures of open Pacific Ocean water by McAllister, Parsons, and Strickland (1960), was also shown in this experiment with coastal water. It will be seen from Figure 4 and from the analytical data given in Table 2, that the ratio of nitrogen to phosphorus in the growing cells was

variable, being quite low during the first part of the experiment and then increasing to approach the "classical" 15:1 value.

Phosphorus was not a "limiting nutrient." The comparatively labile nature of its incorporation in phytoplankton (Gest and Kamen 1948; Goldberg, Walker and Whisenand 1951; Rice 1953; Rigler 1956) and the rapidity with which it can leave dead cells (Hoffmann 1956; Golterman 1960) is reflected by irregularities in the curve in Figure 4. In particular there was a small but definite *increase* in the amount of the element in solution between days 9 and 10. Cells appear to have stored an abnormally large amount of phosphorus (see Table 2), some of which

TABLE 1. Ratios involving photosynthetic rates

Day	Ratio: Rate. (O <sub>2</sub> gross) Rate. (C <sup>14</sup> ) (PQ=1)		Ratio: Rate. mg C/hr (O <sub>2</sub> gross) mg chlorophyll <i>a</i> (PQ=1.3)	Productivity index <sup>2</sup> from gross O <sub>2</sub> (PQ=1.3)
	Bag	Incubator		
1	—	—	1.3 <sup>1</sup>	—
2	—	—	1.2 <sup>1</sup>	—
5	—	3.3	1.65	—
6	2.4	—	—	—
7	1.8	—	—	—
8	2.1	3.3	1.6	—
9	2.7	—	—	—
10	1.9	2.9	1.75	0.9
11	1.9	3.3	1.6	0.75
12	1.9	3.3 Mean 3.3	1.45	0.75
14	1.7	—	1.6	0.75
15	2.2	2.0	1.65	0.75
16	1.5	1.7	1.05	0.55
17	2.0	1.4	1.05	0.4
18	1.5	1.9 Mean 1.75	0.95	0.2
19	1.7 Mean 1.95	—	—	—

<sup>1</sup> Values using carbon-14 rate measurements.<sup>2</sup> Carbon measured by direct chemical method.

was then liberated during a period of low illumination and increasing temperature. Phosphorus was obviously lost more rapidly than nitrogen from dead cells and this is also shown by the ratios given in Table 2.

Nitrite nitrogen, initially present at 0.35  $\mu\text{g-at N/L}$  decreased to one-fifth of this value in the first 14 days. It is not possible to say whether or not nitrite was incorporated into phytoplankton (*cf.* Harvey 1953; Bongers 1956) or oxidized by bacteria.

Whereas nitrate nitrogen was consumed rapidly and in a more or less regular fashion it is interesting to see that there was an increase in ammonia concentration (Fig. 4) until day 13 and a significant increase in the amount of soluble organic nitrogen. Ammonia was clearly not used in preference to nitrate when the latter was in great excess (*cf.* Harvey 1940) although when nitrate became depleted there was some indication that ammonia could be scavenged from solution and might, in this instance, have been providing *Gyrodinium fulvum* with its most readily accessible supply of nitrogen. There was little or no indication of any nitrogen remineralization, but this is not surprising considering the time scale suggested by the work of Von Brand, Rakestraw and Renn

(1939), Spencer (1956) and others and the fact that some plant cells were still living and removing available forms of the element until the end of the experiment.

As noted by several workers (*e.g.*, Harvey 1953; Rodhe 1957) the growth rate of phytoplankton can be quite rapid even when all nitrate has been removed from a medium and cells exist for one or more divisions on their reserves of protein. The present work gives striking illustration of this fact as nearly half of the final organic matter in the phytoplankton bloom was produced after nitrate depletion. Any attempt to predict the kinetics of natural population growth using the concept of a "limiting concentration" of nitrogen (or phosphorus) therefore has little meaning unless the past history of the phytoplankton crop is also known. There may, of course, be conditions where the supply of nutrients is just balancing consumption but for how long such an unstable equilibrium could exist in nature, especially in coastal regions, remains to be shown.

There is a shortage in the literature of direct comparisons between the oxygen and C<sup>14</sup> methods of measuring photosynthesis and many of the results quoted are for pure cultures. In the present work ratios were

measured by the two methods whenever practical, both at the center of the plastic bag for 24 hr and in the constant light incubator for shorter periods. In Table 1 the ratios between gross oxygen liberation and radioactive carbon uptake are presented with no assumptions as to what is being measured.

The ratios are disconcertingly large, but they can be reduced if a large photosynthetic quotient ( $PQ$ ) is allowed and if the  $C^{14}O_2$  method is assumed to measure net photosynthesis in cells which have a high respiration rate. The highest  $PQ$  and respiration values normally used are about 1.35 and 15% respectively which would give a ratio of 1.6 at moderate light intensities. (This subject is fully reviewed by Strickland 1960.)

The ratios measured by the bottles at the center of the bag averaged 1.95. They fell into two distinct groups in the incubator, where there was from 3 to 10 times more illumination. Values before day 14 (and nitrate depletion) averaged 3.3 and the four values after day 14 had a mean of 1.75. It is generally supposed that nitrogen deficient cells have a high respiration rate and hence low rate of net photosynthesis (Ketchum, *et al.* 1958) but these last four results do not indicate that the  $C^{14}$  results are usually low, unless  $PQ$  values are also very small. The ratio of 3.3 in a nitrate rich medium is even less easy to explain. One is compelled to assume very high values for the  $PQ$  of diatom cells taken from a nitrate rich medium at low illumination and exposed for a few hours to comparatively high light intensities. The whole question of a correct  $PQ$  value is of much greater importance in marine studies than is generally admitted, especially if one wishes to make an accurate estimate of carbon uptake from oxygen measurements.

It is interesting to note that the depletion of nitrate had no significant effect on the 24-hr ratios determined in the plastic bag. These values may be slightly high due to the prolonged period of  $C^{14}O_2$  uptake.

With all these results we suspect that ratios are greater than they should be, even having regards to likely experimental errors and high  $PQ$  and respiration values. It is

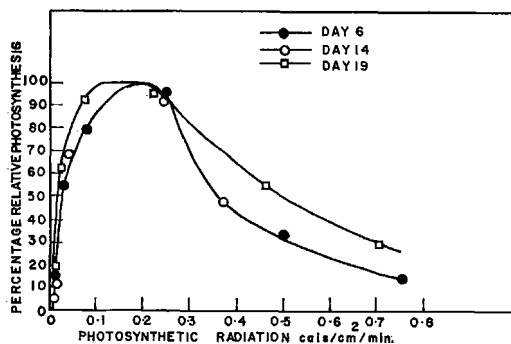


FIG. 5. Relative rate of  $C^{14}$  assimilation as a function of radiation intensity.

possible that in coastal water the  $C^{14}$  method is giving low results due to the photosynthetic assimilation of non-labeled forms of carbon (Smith, Tatsumoto and Hood 1960; and see also Strickland 1960 for a discussion).

The relative photosynthetic rates ( $f$ ) vs. illumination values ( $I$ ) for the populations in the sphere on days 6 and 14 have been plotted on the same curve in Figure 5. This is very similar to that given by Ryther (1956). At day 19, when *Gyrodinium fulvum* comprised about half the population, the values lay on a slightly different curve. There was then the greater tolerance to high light intensities to be expected from a dinoflagellate (*e.g.*, Ryther 1956) but the curve at lower intensities still resembled that for the diatom population. It will be seen that the light intensity of 0.1 ly/min used in the incubator was at approximately the optimum value. Mean light intensities at the center of the bag did not exceed 0.055 ly/min and photosynthesis was therefore always roughly proportional to light intensity.

Back extrapolation of  $f$  vs.  $I$  curves to zero light intensity should indicate the amount of plant respiration, if the assumptions made by Nielsen and Hansen (1959) are justified. The values so obtained, about 10%, are reasonable.

Figure 6 shows the increase in carbon in the plastic bag from day 1 to day 22, as measured by both oxygen and  $C^{14}$  methods, again with no assumption of  $PQ$  values or the significance of the  $C^{14}$  measurements. The increase on day 13, and hence the total for all subsequent days, may be low as there

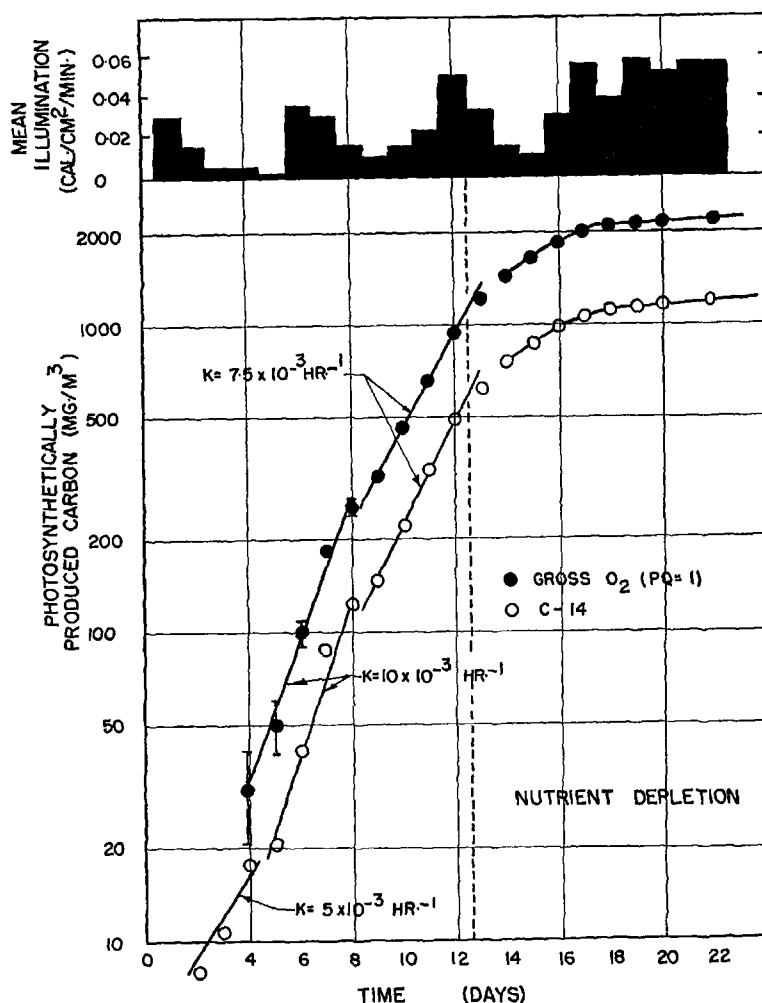


FIG. 6. Photosynthetic carbon fixation in the plastic sphere as a function of time under natural lighting.

was poor stirring that day with a temporary loss of plant cells. The curves are parallel and their slope can be used to evaluate logarithmic growth constants. The values given in Figure 6 (base 10) probably reflect changes in temperature and light intensity (see the histogram for mean daily illumination at the bag center). After the end of day 15, severe nitrogen shortage brought about a reduced growth rate and results no longer have much meaning, because cells were sinking below the sampling point in the bag.

The most fruitful way to examine the effect of nutritional changes on the photosynthetic metabolism of algal cells is to

expose them to constant light intensity in an incubator and find either the rate of carbon increase per unit of chlorophyll *a*, or the productivity index, defined by Strickland (1960) as:

$$\frac{\text{hourly rate of carbon increase}}{(\text{amount of plant carbon}) \times (\text{illumination in ly/min})}$$

Both these values are given in Table 1, using the gross photosynthetic rate measured by oxygen evolution and an assumed *PQ* of 1.3. There was no significant change in the photosynthetic potential of the cells from day 1 to day 15, having regards to the general magnitude of experimental errors.

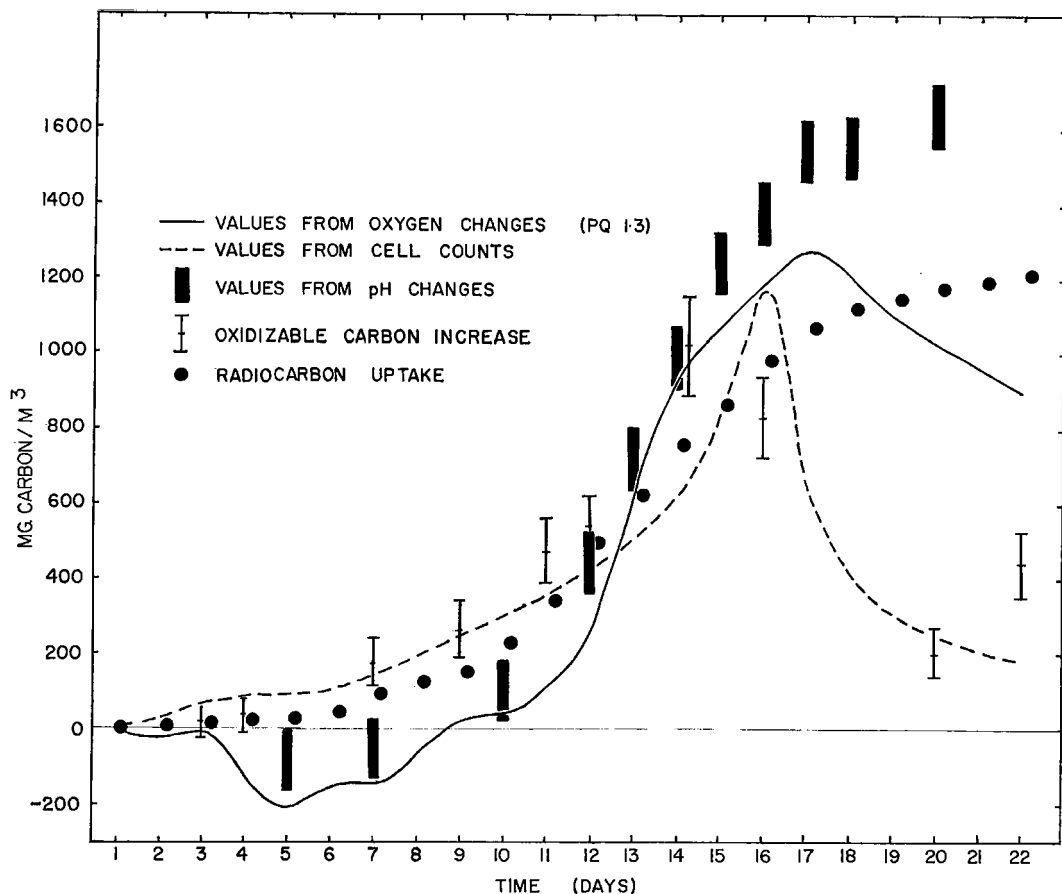


FIG. 7 Net production of carbon as measured by five independent methods.

The gross rates, per unit of chlorophyll *a* at optimum lighting, were very much lower than the rates reported by Ryther and Yentsch (1957), although they were within the range found in the literature (Strickland 1960) and comparable to the values found by McAllister, *et al.* (1960) in the open Pacific Ocean.

These productivity index values are the first of their kind to be reported, and are near to many of the values inferred from the literature (Strickland 1960). The high value at day 10 may be significant but was not reflected in the corresponding figure for the rate per unit of chlorophyll *a*. Similarly the decrease of productivity index at the end of the experiment was much more pronounced than the corresponding decrease in the chlorophyll ratios, but this would be expected

because a large amount of detrital carbon was accumulating. In particular it should be noted that the loss of nitrate at the end of day 13 had no effect on either ratio. Only at day 16, when the mean nitrogen content per cell was probably less than a half of its peak value, did the photosynthetic processes, as reflected by these ratios, become seriously impaired. At the same time cells commenced to settle out rapidly (Fig. 3).

There was no evidence in our experiment for the situation reported by Ryther, *et al.* (1958) where the growth of a diatom bloom in nitrogen deficient water was static as the result of a sizable amount of photosynthesis during the day being compensated for by respiration during the hours of darkness.

The net production of organic particulate carbon in the plastic bag was estimated by

five methods and the results are shown together for comparison in Figure 7. The full line shows the values calculated from changes of dissolved oxygen in the bag, assuming a  $PQ$  of 1.3. Results are probably low after day 15 or 16 due to physical losses of oxygen from the supersaturated system. The dotted line is a measure of plant carbon derived from cell counts and, as mentioned earlier, is only very approximate. The bars show the range of individual results estimated from changes of total carbon dioxide content. This is the most direct method of all but unfortunately not as precise as could be wished. The solid circles mark uncorrected data calculated from the uptake of radiocarbon. These results were used in preference to the gross oxygen photosynthesis data because they formed a more complete set, and did not involve assumptions as to respiration rates and photosynthetic quotients. However, it is by no means certain that  $C^{14}$  data represent true net photosynthesis; they may well be too low. Finally, there are the experimentally measured oxidizable carbon results corrected for initial detritus. These results are entered as horizontal dashes with the 95% confidence limits indicated by vertical lines. Any excretion of soluble organic compounds during plant growth would make these results lower than they should be and this would also influence the ratios in Table 2.

Ignoring the measurements derived from dissolved oxygen and pH, the agreement between the other three estimates was as good as could be expected for much of the time. The two direct methods, carbon analysis and algal volume, finally showed decreases due to the settling of cells, although there was appreciable net synthesis going on inside the bag until at least day 20.

The various measurements of carbon during the first few days of this experiment gave the most anomalous results and they merit closer investigation. The dissolved oxygen content of the bag first *decreased*, representing the consumption of over 200 mg C/m<sup>3</sup>, but there was no corresponding decrease of particulate carbon. A wall effect would not be expected so soon in equipment of this size

(in the other experiment the oxygen decreased steadily from the first day) so presumably, much of the oxidation was by non-proliferating bacteria in suspension, using a substrate of dissolved organic carbon. This was borne out by the high but fairly constant respiration values found in the dark bottles used in photosynthesis experiments.

Not only was there no decrease of particulate matter, corresponding to the net consumption of oxygen in the bag, but the increase in photoplankton carbon, as estimated by cell volumes and changes of chlorophyll (see later) was appreciable and very rapid during the first 4 to 5 days. This increase, mainly in *T. rotula* and *Skeletonema*, could not be accounted for by photosynthesis, as the radio carbon uptake data predicted very much smaller values for carbon (one quarter or less). The most likely explanation, therefore, is that during the period of very low illumination at the start of this experiment (see Fig. 6) heterotrophic growth from dissolved organic carbon was a large fraction of the total growth. The effect was not detected in the 5 days of the second experiment, when the illumination was quite high. But at the beginning of the main experiment, a very heavy bloom of flagellates in the surrounding water prevented light entering the bag for several days. It is interesting to note that what little photosynthesis did occur during this period took place in a normal manner. The photosynthetic rate, per unit of chlorophyll *a*, in the incubator had about the same value as found during the rest of the experiment (Table 1).

Heterotrophic growth of algae is well established (Saunders 1957) and has been studied in marine diatoms by Lewin and Lewin (1960). Conversion of dissolved substrate to algal carbon can be high if the behavior of *Chlorella* is representative (Myers and Johnston 1949; Samejima and Myers 1958). Several workers have stressed the probable importance of heterotrophy in phytoplankters (Rodhe 1955; Wood 1956; Bernard 1958) but we believe the present work to be the most direct demonstration of its occurrence in marine phytoplankton under

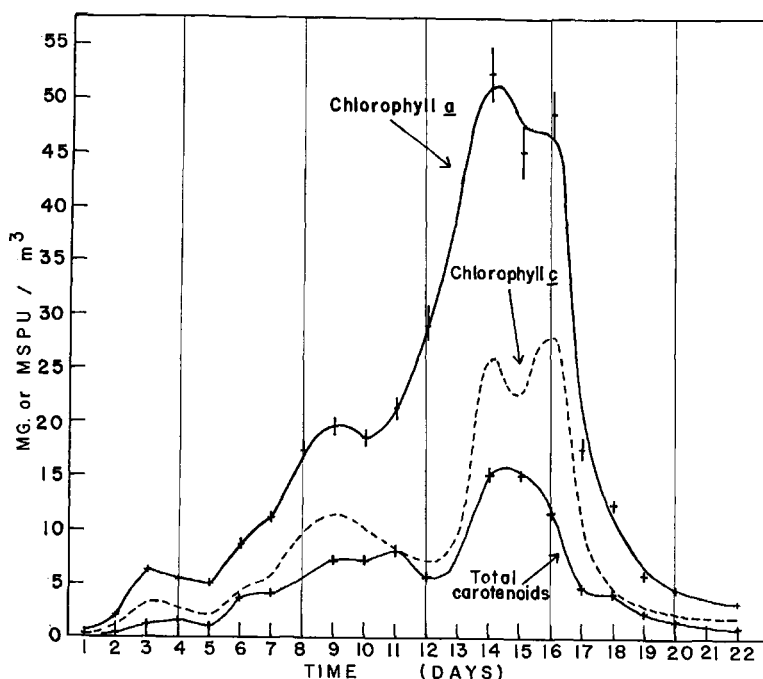


FIG. 8. Changes in the concentration of plant pigments.

conditions resembling those found at the base of the euphotic zone.

Variations of chlorophyll *a*, chlorophyll *c*, and total carotenoid concentration at the center of the bag are shown in Figure 8. The chlorophyll *c* curve roughly parallels that for chlorophyll *a* for most of the experiment, giving values about 50–60% of the chlorophyll *a*, in terms of MSP units and milligrams respectively. Between days 11 and 13, however, the ratio of chlorophyll *c* to chlorophyll *a* was much smaller, decreasing to 25%. This period coincided with the predominance of *Skeletonema costatum* in the bloom and we have found (unpublished work using pure cultures) that this species has what is probably an abnormally high chlorophyll *a* content and a low chlorophyll *c* to chlorophyll *a* ratio for a diatom. The large chlorophyll *a* content of *Skeletonema* would explain the very high maximum value found in the present experiment (50 mg/m<sup>3</sup>). In the open ocean, a natural culture in water of essentially the same initial nitrate concentration bloomed to only 26 mg/m<sup>3</sup> chlorophyll *a* at

its peak (McAllister, Parsons and Strickland 1960).

The first few days of the experiment are particularly interesting. In 3 days the chlorophyll *a* concentration increased from 0.4 to 6 mg/m<sup>3</sup>. If we estimate carbon from cell volumes, the ratio of carbon to chlorophyll *a* is found to change from 25 to 11 in the first 3 days, which is reasonable for a surface population recovering its pigment content in a nutrient-rich, relatively dark environment. The remainder of the chlorophyll increase must be associated with a rapid development of cells during this initial period. Ratios calculated using photosynthetically produced carbon data were impossibly low which, as mentioned earlier, supports the suggestion of some heterotrophic growth.

The sudden decrease of chlorophyll after day 16 was striking and much more than would be expected from a decrease of cell numbers alone. The possible presence of "detrital chlorophyll" in nature is a matter of some importance in field studies. Odum, McConnel and Abbott (1959) could find no



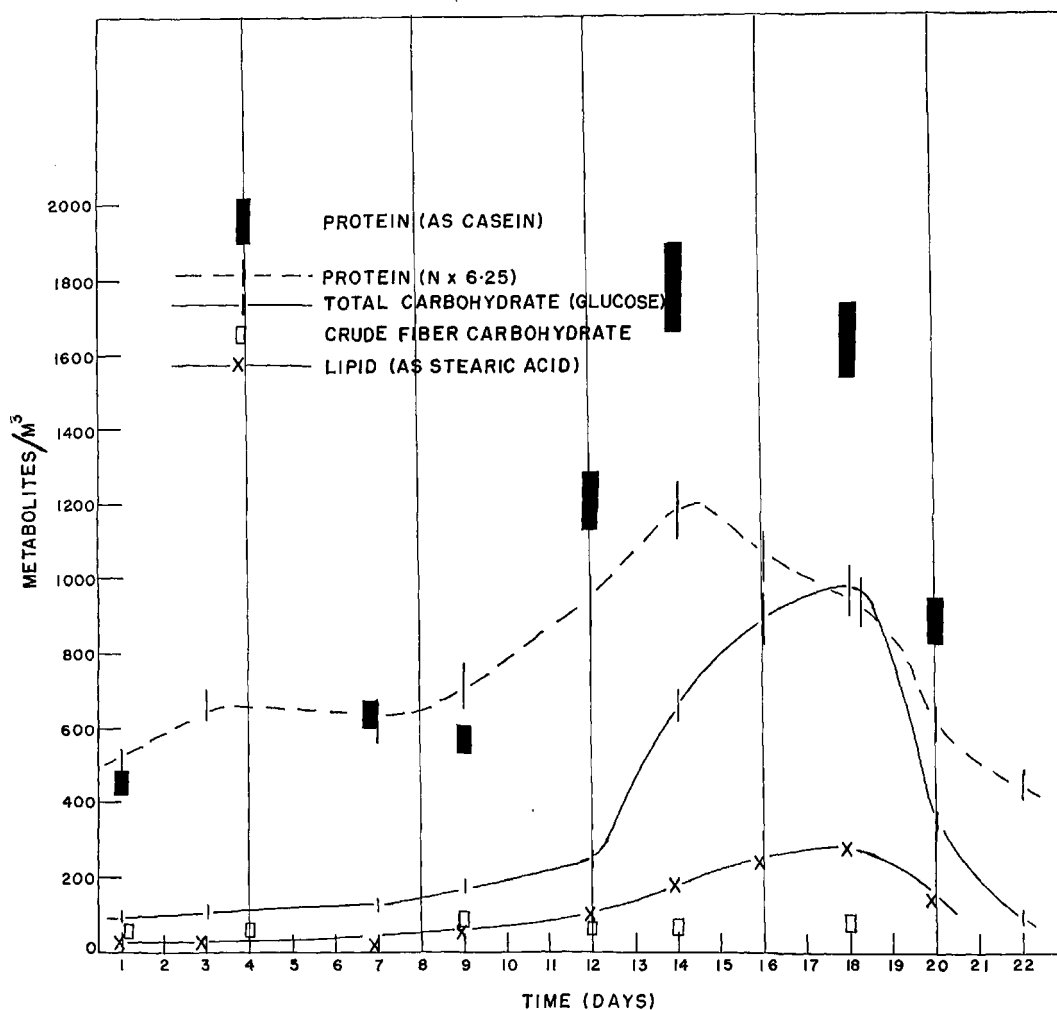


FIG. 9. Changes in the concentration of major metabolites.

spectrophotometric indication of such material. The present experiment seemed to be an excellent opportunity to investigate the matter. We measured the complete pigment spectrum, in 90% acetone, of the populations on day 12 when cells were growing with full vigor and day 18 when many cells were dead or dying but found no evidence for phaeophytin or phaeophorbide pigments. There was a noticeable broadening of the long-wave-length chlorophyll *a* peak in the extract made on day 18, and a slight shift to shorter wave lengths (6,650 Å to 6,630 Å). We have seen this effect in old laboratory cultures and it is reminiscent of the *in vivo*

behavior reported by French and Elliott (1958). There is little likelihood, however, that such small changes would have any practical significance in marine pigment analysis. However, chlorophyllides, which could conceivably occur in decomposing plant tissue, have similar absorption spectra to chlorophylls and it also remains to be proven whether the detrital chlorophyll from faecal pellets is in the form of phaeopigments and hence may give misleading results in nature.

The composition of the particulate matter in the sphere, in terms of major metabolites, is summarized in Figure 9. In order to esti-

TABLE 2. Ratios involving carbon

Day	Si <sup>1</sup> C	Protein C	Protein <sup>2</sup> C	Carbohydrate C	Lipid C	C P	C N	C N <sup>2</sup>	N <sup>2</sup> (atoms) P
7	—	0.66	0.89	0.2	—	19	9.5	7	6
9	1.0	0.72	0.76	0.3	—	17	9	8.5	5
12	0.6	0.78	1.4	0.3	0.15	23	8	4.5	11 <sup>3</sup>
15	0.65	0.65	1.3	0.7	0.2	28	10	5	12.5 <sup>3</sup>
18	—	0.65	1.8	1.35	0.4	40	10	4	25
20	—	0.43	1.9	0.7	0.55	35	15	3.5	23
Value suggested by Strickland (1960)	0.8	—	—	—	—	40 ± 15	—	6 ± 2	14.5

<sup>1</sup> Calculated from the decrease of reactive silicate.<sup>2</sup> Values from the absorptiometric protein method, standardized with casein.<sup>3</sup> The ratio had a maximum of about 15 on day 13.

mate the amounts of carbon, protein, carbohydrate, phosphorus, *etc.* in living phytoplankton it was necessary to subtract from the amounts found on a given day, the amounts of each metabolite present initially in the water used to fill the sphere and, further, to assume that this initial "background" concentration of detrital material did not change throughout the experiment. Almost certainly such an assumption is not fully justified, although there is reason to suppose that settling of detritus was comparatively slow. Errors from this source were most significant in the earlier part of the experiment and ratios such as those given in Tables 2 and 3 had little precision until at least day 7 or 9.

There was a considerable discrepancy between the amount of protein obtained by the two chemical methods. Some of this difference undoubtedly arises because the amino acid spectrum of algal protein is not the same as that of casein, but we would not expect from our work on pure phytoplankton cultures (unpublished) that the differences would have been so great. Evidence from the "balance" between dissolved and particulate nitrogen in the bag pointed to the Kjeldahl nitrogen values being too low. A similar poor balance between nitrate lost from solution and nitrogen found in growing algae has been reported by Yentsch and Vaccaro (1958) but we are at a loss to explain the matter and more work is desirable. In the meantime, we have reported both sets of data in Figure 9, Table 2 and Table 3 and suggest that the colorimetric protein values

are probably nearer to the "correct" results until at least day 12.

Crude fibre carbohydrate in the bag never exceeded a few per cent of the total phytoplankton carbohydrate. This confirms our (unpublished) findings for pure phytoplankton cultures and for natural coastal populations at the time of heavy diatom blooms. A quite different analysis is found for water containing much detritus, when as much as 80% of the total carbohydrate may be present as crude fibre. (There was about 60% in the detrital carbohydrate initially in the water of the present experiment.) An analysis for crude fibre is one of the best rapid chemical indicators for detritus, as distinct from living plant cells, in the sea.

The most striking feature shown by Figure 9 is the way in which the lipid and the carbohydrate (mainly glucose) in the plant cells increased when nitrate became depleted from solution on day 13 (*cf.* Spoehr and Milner 1949; Bongers 1956; Fogg 1957). Between about days 13 and 17 fats and sugars were almost the sole products of photosynthesis.

The classical conception of diatoms containing large reserves of fat is not borne out by the present work. When cells were in a dying condition in nutrient depleted water their lipid content was higher than earlier in the experiment, but was still less than 20% of the dry weight (see Table 2, assuming about one-third of the dry weight of a diatom is carbon). In the sea, near the surface, nitrate depletion doubtless occurs frequently and

TABLE 3. Ratios involving chlorophyll *a*

Day	C chl. <i>a</i>	C Carotenoids <sup>2</sup>	N chl. <i>a</i>	N <sup>1</sup> chl. <i>a</i>	P chl. <i>a</i>	Carotenoids <sup>2</sup> chl. <i>a</i>	Protein chl. <i>a</i>	Protein <sup>1</sup> chl. <i>a</i>	Carbohydrate chl. <i>a</i>	Lipid chl. <i>a</i>
7	15	43	1.6	2.2	0.81	0.36	10	14	3	—
9	13	37	1.5	1.6	0.80	0.36	11	10	4	—
12	19	100	2.4	4.2	0.83	0.19	15	27	5.5	3
15	22	70	2.2	4.5	0.78	0.31	14	28	15	4
18	56	160	5.9	16	1.4	0.36	37	100	76	22
20	49	150	3.3	15	1.4	0.33	21	93	33	27
Values suggested by Strickland (1960)	30	40	—	7±3	0.75±0.2	—	—	—	—	—

<sup>1</sup> Values from the absorptiometric protein method, standardized with casein.<sup>2</sup> Total carotenoids in MSPU.

hence diatoms collected by net tows may often be expected to have their maximum lipid content (*cf.* Barker 1935). However, it is difficult to believe from the present work that the presence of fat assists diatoms in maintaining their buoyancy to any great extent.

The ratios of elements and metabolites to carbon and chlorophyll *a* at various stages in the phytoplankton bloom are given in Tables 2 and 3 respectively. The ratios involving carbon (the direct oxidizable carbon data were used) illustrate the relative changes in composition of the phytoplankton with time. The values involving chlorophyll *a* are important mainly because this pigment is the only convenient measure of plant material in sea water, as distinct from animals and detritus. Data such as those in Table 3 show the limits within which the plant carbon, nitrogen, metabolites, *etc.* can be estimated, knowing the pigment content of a water sample. Clearly each possible mixed algal population in nature, at every stage of its growth, will give rise to different ratios so that the value of any particular one is limited. Nevertheless, as pointed out by Strickland (1960) there are still very few relevant data of this kind available for marine phytoplankton. The results given here are a useful beginning, especially as they apply to a mixed diatom population growing under near-natural conditions. For comparison, the approximate mean values for marine crops suggested by Strickland (1960) are given at the bottom of each table.

It is unfortunate that the precision of the ratios measured during the early part of the experiment is not as high as could be desired, but the data for days 7 and 9 are approximately correct for a diatom population growing under low illumination in nutrient rich waters. The data for day 12 apply to a vigorously growing and rapidly dividing bloom, about to strip the nutrients from the water. On day 15 the results apply to algae at the end of their growth period in nitrogen-deficient water. By day 18 there were many dead and dying cells, and on day 20 little living material was present at the bag center, except dinoflagellates.

The Si:C ratios given in Table 2 are some of the few to be reported for a live natural population and are of the order to be expected from results cited in the literature. The decrease from day 9 to day 12 may be real, supporting the observations made by Jørgensen (1955b).

Most of the other ratios in Tables 2 and 3 are self-explanatory and illustrate the trends that have already been discussed. The increase in the ratios involving chlorophyll *a* after day 15 reflects the rapid loss of this pigment from dead or dying plant material, a loss more nearly paralleled by that of phosphorus and total carotenoid, than by any other of the measured variables.

The co-variation of chlorophyll *a* and the carotenoids is indeed remarkable and supports the detailed observations made by Haslkin (1941) on *Chlorella* and our own (unpublished) work on pure marine cultures.

Except for day 12, when there was a preponderance of *Skeletonema costatum*, which we have found to have a relatively low carotenoid content, the ratios were very constant and similar to the values found for mixed coastal populations in Departure Bay at all times of the year. There was no evidence of either the particulate nitrogen values or the ratios in Table 1 being functions of the carotenoid-to-chlorophyll ratio, as has been reported by Ketchum, *et al.* (1958) and Yentsch and Vaccaro (1958). Why there should be this discrepancy between their well-established laboratory results and the present large-scale experiment is not clear. Of course, conditions were not identical and this emphasizes the dangers of extrapolating from laboratory-scale results or even, to a lesser extent, from the present experiment to phenomena taking place in the sea under completely natural conditions.

## REFERENCES

- ANDERSON, D. H., AND R. J. ROBINSON. 1946. Rapid electrometric determination of the alkalinity of sea water using a glass electrode. *Industr. Eng. Chem. (Anal.)*, **18**: 767-769.
- BARKER, H. A. 1935. Photosynthesis in diatoms. *Arch. Microbiol.*, **6**: 141-156.
- BENDSCHNEIDER, K., AND R. J. ROBINSON. 1952. A new spectrophotometric method for the determination of nitrite in sea water. *J. Mar. Res.*, **11**: 87-96.
- BERNARD, M. F. 1958. Comparaison de la fertilité élémentaire entre l'Atlantique tropical africain, l'Océan Indien et la Méditerranée. *Compt. rend. Acad. Sci. Paris*, **247**: 2045-2048.
- BONGERS, L. H. J. 1956. Aspects of nitrogen assimilation by cultures of green algae. *Mededel. Landbouwhogeschool Wageningen*, **56**: 1-52. *C. A.*, **54**: 18662. 1960.
- CONOVER, S. A. 1956. Oceanography of Long Island Sound 1952-1954. IV Phytoplankton. *Bull. Bingham Oceanogr. Coll.*, **15**: 63-112.
- COOPER, L. H. N. 1937. On the ratio of nitrogen to phosphorus in the sea. *J. Mar. Biol. Ass. U.K.*, **22**: 177-182.
- . 1938. Redefinition of the anomaly of the nitrate-phosphate ratio. *J. Mar. Biol. Ass. U.K.*, **23**: 179.
- CREITZ, G. I., AND F. A. RICHARDS. 1955. The estimation and characterization of plankton populations by pigment analysis III. A note on the use of Millipore membrane filters in the estimation of plankton pigments. *J. Mar. Res.*, **14**: 211-216.
- CUSHING, D. H. 1959. On the nature of production in the sea. *Fish. Invest. Lond., Ser. 2*, **22**: 1-40.
- DOTY, M. S. 1959. Phytoplankton photosynthetic periodicity as a function of latitude. *J. Mar. Biol. Ass. India*, **1**: 66-68.
- EDMONDSON, W. T. 1955. Factors affecting productivity of fertilized salt water. *Deep Sea Res.*, **3**: (Suppl.) 451-464.
- , AND Y. H. EDMONDSON. 1947. Measurements of production in fertilized salt water. *J. Mar. Res.*, **6**: 228-246.
- FOGG, G. E. 1957. Relationships between metabolism and growth in planktonic algae. *J. Gen. Microbiol.*, **16**: 294-297.
- . 1958. Extracellular production of phytoplankton and the estimation of primary production. *Rapp. Cons. Explor. Mer*, **144**: 56-60.
- FRENCH, C. S., AND R. F. ELLIOTT. 1958. The absorption spectra of chlorophyll in various algae. *Ann. Rept. Dept. Plant Biology, Carnegie Institution of Washington Year Book 1957-1958*, pp. 278-286.
- GEST, H., AND M. D. KAMEN. 1948. Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis. *J. Biol. Chem.*, **176**: 299-318.
- GOLDBERG, E. D., T. J. WALKER, AND A. WHISENAND. 1951. Phosphate utilization by diatoms. *Biol. Bull.*, **101**: 274-284.
- GOLTERMAN, H. L. 1960. Studies on the cycle of elements in fresh water. *Acta. Bot. Neerl.*, **9**: 1-58.
- GROSS, F., AND E. ZEUTHEN. 1948. The buoyancy of plankton diatoms: a problem in cell physiology. *Proc. Roy. Soc. Lond. (Ser. B)*, **135**: 382-389.
- HANSEN, A. L., AND R. J. ROBINSON. 1953. The determination of organic phosphorus in sea water with perchloric acid oxidation. *J. Mar. Res.*, **12**: 31-42.
- HARVEY, H. W. 1940. Nitrogen and phosphorus required for the growth of phytoplankton. *J. Mar. Biol. Ass. U.K.*, **24**: 115-123.
- . 1953. Synthesis of organic nitrogen and chlorophyll by *Nitzschia closterium*. *J. Mar. Biol. Ass. U.K.*, **31**: 477-487.
- HASKIN, H. H. 1941. The chloroplast pigments in *Chlorella pyrenoidosa*. Doctorate Thesis, Dept. of Biology, Harvard University.
- HEWITT, B. R. 1958. Spectrophotometric determination of total carbohydrate. *Nature*, **182**: 246-247.
- HOFFMANN, C. 1956. Untersuchungen über die Remineralisation des phosphors im plankton. *Kiel. Meeresforsch.*, **12**: 25-36.
- JERLOV, N. G. 1951. Optical studies of ocean waters. *Rep. Swedish Deep-Sea Exped. 1947-1948*, **3**: 1-59.
- JOHNSON, M. J. 1941. Isolation and properties of a pure yeast polypeptidase. *J. Biol. Chem.*, **137**: 575-586.

- . 1949. A rapid method for estimation of non-volatile organic matter. *J. Biol. Chem.*, **181**: 707-711.
- JØRGENSEN, E. G. 1955a. Solubility of the silica in diatoms. *Physiol. Plantarum*, **8**: 846-851.
- . 1955b. Variations in the silica content of diatoms. *Physiol. Plantarum*, **8**: 840-845.
- KEELER, R. F. 1959. Colour reactions for certain amino acids, amines and proteins. *Science*, **129**: 1617-1618.
- KETCHUM, B. H., J. H. RYTHER, C. S. YENTSCH, AND N. CORWIN. 1958. Productivity in relation to nutrients. *Rapp. Cons. Int. Explor. Mer*, **144**: 132-140.
- LEWIN, J. C., AND R. A. LEWIN. 1960. Auxotrophy and heterotrophy in marine littoral diatoms. *Canadian J. Microbiol.*, **6**: 127-133.
- MCALLISTER, C. D., T. R. PARSONS, AND J. D. H. STRICKLAND. 1960. Primary productivity at Station "P" in the northeast Pacific Ocean. *J. Cons. Int. Explor. Mer*, **25**: 240-259.
- , AND J. D. H. STRICKLAND. 1961. Light attenuators for use in phytoplankton photosynthesis studies. *Limnol. Oceanogr.*, **6**: 226-228.
- MARGALEF, R. 1958. Spatial heterogeneity and temporal succession of phytoplankton. *Perspectives in Marine Biology*. U. of California Press, pp. 323-349.
- MUKERJEE, P. 1956. Use of ionic dyes in the analysis of ionic surfactants and other ionic organic compounds. *Analyt. Chem.*, **28**: 870-873.
- MULLIN, J. B., AND R. P. RILEY. 1955a. The colorimetric determination of silicate with special reference to sea and natural waters. *Analyt. Chim. Acta.*, **12**: 162-176.
- , AND J. P. RILEY. 1955b. The spectrophotometric determination of nitrate in natural waters with particular reference to sea water. *Analyt. Chim. Acta.*, **12**: 464-480.
- MYERS, J., AND J. A. JOHNSTON. 1949. Carbon and nitrogen balance of *Chlorella* during growth. *Plant. Physiol.*, **24**: 111-119.
- NIELSEN, E. STEEMAN, AND V. KR. HANSEN. 1959. Measurements with the carbon-14 technique of the respiration rates in natural populations of phytoplankton. *Deep Sea Res.*, **5**: 222-233.
- ODUM, H. T., W. MCCONNELL, AND W. ABBOTT. 1959. The chlorophyll *a* of communities. *Pub. Inst. Mar. Sci. (Univ. Texas)*, **5**: 65-96.
- PARSONS, T. R., AND J. D. H. STRICKLAND. 1959. The proximate analysis of marine standing crops. *Nature*, **184**: 2038.
- RICE, T. R. 1953. Phosphorus exchange in marine phytoplankton. *Fish. Bull. U.S. Fish and Wildl. Serv.*, **54** (80): 77-89.
- RICHARDS, F. A., WITH T. G. THOMPSON. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of phytoplankton pigments. *J. Mar. Res.*, **11**: 156-172.
- RIGLER, F. H. 1956. A tracer study of the phosphorus cycle in lake water. *Ecology*, **37**: 550-562.
- ROBINSON, R. J., AND T. G. THOMPSON. 1948. The determination of phosphates in sea water. *J. Mar. Res.*, **7**: 33-41.
- RODHE, W. 1955. Can plankton production proceed during winter darkness in subarctic lakes? *Verh. Int. Ver. Limnol.*, **12**: 117-119.
- . 1958. The primary production in lakes, some results and restrictions of the  $^{14}\text{C}$  method. *Rapp. Cons. Int. Explor. Mer*, **144**: 122-128.
- RYTHER, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. *Limnol. Oceanogr.*, **1**: 61-70.
- , AND C. S. YENTSCH. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. *Limnol. Oceanogr.*, **2**: 281-286.
- , C. S. YENTSCH, E. M. HULBURT, AND R. F. VACCARO. 1958. The dynamics of a diatom bloom. *Biol. Bull.*, **115**: 257-268.
- SAMEJIMA, H., AND J. MYERS. 1958. On the heterotrophic growth of *Chlorella pyrenoidosa*. *J. Gen. Microbiol.*, **18**: 107-117.
- SAUNDERS, G. W. 1957. Interrelations of dissolved organic matter and phytoplankton. *Bot. Rev.*, **23**: 389-410.
- SMITH, J. B., M. TATSUMOTO, AND D. W. HOOD. 1960. Carbamino carboxylic acids in photosynthesis. *Limnol. Oceanogr.*, **5**: 425-431.
- SPENCER, C. P. 1956. The bacterial oxidation of ammonia in the sea. *J. Mar. Biol. Ass. U.K.*, **35**: 621-630.
- SPOEHR, H. A., AND H. W. MILNER. 1949. The chemical composition of *Chlorella*; effect of environmental conditions. *Plant. Physiol.*, **24**: 120-149.
- STEELE, J. H., AND C. S. YENTSCH. 1960. The vertical distribution of chlorophyll. *J. Mar. Biol. Ass. U.K.*, **39**: 217-226.
- STRICKLAND, J. D. H. 1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. *J. Fish. Res. Bd. Canada*, **15**: 453-493.
- . 1960. Measuring the production of marine phytoplankton. *Bull. Fish. Res. Bd. Canada*, **122**: 1-172.
- , AND K. H. AUSTIN. 1959. The direct estimation of ammonia in sea water with notes on reactive iron, nitrate and inorganic phosphorus. *J. Cons. Int. Explor. Mer*, **24**: 446-451.
- , AND T. R. PARSONS. 1961. A manual of seawater analysis (with special reference to the more common micro-nutrients and to particulate organic material). *Bull. Fish. Res. Bd. Canada*, **125**: 1-185.
- , AND L. D. B. TERHUNE. 1961. The study of in-situ marine photosynthesis using a large plastic bag. *Limnol. Oceanogr.*, **6**: 93-96.

- VON BRAND, T., N. W. RAKESTRAW, AND C. E. RENN. 1939. Further experiments on the decomposition and regeneration of nitrogenous organic matter in sea water. *Biol. Bull.*, **77**: 285-296.
- WOOD, E. J. F. 1956. Considerations on productivity. *J. Cons. Int. Explor. Mer*, **21**: 280-283.
- YENTSCH, C. S., AND R. F. VACCARO. 1958. Phytoplankton nitrogen in the oceans. *Limnol. Oceanogr.*, **3**: 443-448.
- YUEN, S. H. 1958. Determination of traces of manganese with leucomalachite green. *Analyst*, **83**: 350-356.