PHYTOPLANKTON PHYSIOLOGY IN A NEARSHORE REGION OF THE SOUTHERN BENGUELA CURRENT

by

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Signed by candidate

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ABSTRACT

Physiological investigations on phytoplankton communities revealed that upwelling in an inshore region off the Cape Peninsula may be divided into three biological stages. In Type 1 water, temperatures were <10°C and concentrations of inorganic nutrients were high. Type 2 water was characterised by temperatures >10°C and nitrate concentrations varying between 2 and 15 μg-at NO_3-N.ℓ^{-1}, while Type 3 water contained low concentrations of nitrate (<2 μg-at NO_3-N.ℓ^{-1}) at temperatures >10°C.

During the summer of 1978-79 diatoms dominated the communities from October to January but microflagellates were dominant in February and March. Low concentrations of ATP, chlorophyll a, protein and carbohydrate were measured in Type 1 water with protein/carbohydrate ratios being >1. In Type 2 water concentrations of chlorophyll a, ATP and protein were high and the protein/carbohydrate ratio was >1. Concentrations of chlorophyll a and ATP remained high in Type 3 water but the protein/carbohydrate ratio decreased to <1 due to an increase in the concentration of acid-soluble glucan. It was concluded that the communities were in an active phase of growth in Type 1 and Type 2 water when adequate nutrients were available, but were in a slow-growing phase in Type 3 water when nitrate concentrations were low.

Correlation coefficients, simple linear regressions and stepwise multiple regressions between biochemical and environmental variables confirmed that nitrate was the most important nutrient influencing the biochemical composition of phytoplankton. Using linear regression equations of biochemical variables on glucan it was estimated that chlorophyll a existed in a ratio of approximately 1 : 1 between living phytoplankton and bacteria/detritus, while the percentage of ATP was high in the phytoplankton component of Type 1 water but low in that of Type 2 water. The percentage of protein in detritus was greater than in living phytoplankton, and the carbohydrate content of living phytoplankton increased as the upwelled water matured from Type 1 and Type 2 to Type 3.

The patterns of carbon assimilation into the major end-products of photosynthesis were studied during the summer of 1979-80 using ^{14}C-bicarbonate as a tracer. Time-series experiments on microflagellate communities indicated that the rate of carbon-14 assimilation increased approximately linearly between 0.5 and 4 hours, while the distribution of the label between the ethanol-soluble, TCA-soluble and TCA-insoluble fractions showed no significant variation

with an increase in time. It was concluded that a 4-hour incubation period was suitable for carbon-assimilation studies. Total assimilation of the carbon-14 and the percentage detected in the polysaccharide (TCA-soluble) fraction was high in the upper part of the euphotic zone, but this decreased with increasing depth and decreasing light intensity. The percentage of carbon-14 in the protein (TCA-insoluble) fraction increased with an increase in depth.

An approximately equal distribution of the label between polysaccharide and protein at the 50% light intensity was recorded in Type 1 water, but at the 1% intensity greater activity was detected in the ethanol-soluble fraction. In Type 2 and Type 3 water more carbon-14 was incorporated into polysaccharide at the 50% light intensity than at the 1% intensity, but the percentage incorporation was greater in Type 3 water when low nitrate concentrations prevailed. In both types of water radioactivity in the protein fraction was greater at 1% than at 50% light intensity.

The development of a phytoplankton bloom was studied by placing a drogue in a patch of cold upwelled water and following the water mass for 4 - 5 days. Chaetoceros compressus and Skeletonema costatum dominated the bloom which reached its peak in three days. In this period chlorophyll a concentrations increased by 19.2 $mg.m^{-3}$ in the euphotic zone while the concomitant decrease in nitrate concentration was 18.7 $mg\text{-}at.m^{-3}$. There was an overall increase in the concentration of protein with the highest concentration being measured just prior to the peak of the bloom. Carbohydrate concentrations increased significantly during the day but decreased at night. The pattern of carbon-14 assimilation at the 50% light intensity was characterised by high activity in the polysaccharide fraction as the bloom developed, but at the peak of the bloom a greater percentage of the label was found in the ethanol-soluble fraction. The percentage incorporation into protein was greater at night than during the day. These physiological changes are related to the growth pattern of the bloom.

CHAPTER 1

INTRODUCTION

The coastal waters off the west coast of South Africa are known to be fertile and to support a high level of phytoplankton production due to the rapid chemical and biological changes that occur in these waters. These changes occur as a result of a dynamic upwelling system that prevails off the coast during the summer months and this system is driven predominantly by south-easterly winds that blow at that time of the year (Andrews & Hutchings, 1980). Under the influence of these winds surface waters are moved offshore and water from lower depths moves up to replace the displaced surface water. This cold upwelled water brings a high concentration of inorganic nutrients into the euphotic zone and, with high light intensities prevailing, the environmental conditions are such that rapid growth of phytoplankton takes place when the upwelled water moves offshore and warms up. Consequently, abundant food becomes available to other participants in the marine food web over an extended period.

Other important upwelling systems occur off Peru, California and North-West Africa, with the most intensive research programmes having been undertaken off Peru and North-West Africa. While the processes of upwelling are similar, each area has its own individual characteristics with regard to chemical and biological changes (Table 1).

Environmental variables influencing phytoplankton growth

Nutrients

The two most common sources of nitrogen for algal growth are nitrate and ammonium ions, with ammonium-N being utilized preferentially when both nutrients are supplied (Morris, 1974). When nitrate is utilized it must be reduced in the cells to ammonia for the synthesis of organic nitrogen compounds. The reductive pathway involves the initial reduction of nitrate by nitrate reductase with cofactor NADPH or NADH as an electron donor. Further reduction to ammonia is carried out by nitrite reductase with reduced ferredoxin being the probable cofactor. As the reactions are endothermic, energy is required and this may be supplied by adenosine triphosphate. The preferential uptake of ammonium conforms to the energetics of the reductive processes since this form can be used directly

TABLE 1

Concentrations of chemical and biological variables measured in three major upwelling systems

Variable	Peru	N.W. Africa	Southern Benguela Current
Temperature, °C	10-16	10-17	10-18
Nitrate, μg-at NO_3-N.ℓ^{-1}	20	10-20	20
Silicate, μg-at SiO_3-Si.ℓ^{-1}	20	10-20	16
Phosphate, μg-at PO_4-P.ℓ^{-1}	2.5	1.5	2
Chlorophyll *a*, μg.ℓ^{-1}	10-15	5-10	15-20
Primary production, g C. m^{-2}.d^{-1}	10	1	10-20
References :	Beers *et al.*, 1971 Hobson *et al.*, 1973 Ryther *et al.*, 1971 Strickland *et al.*, 1969 Walsh *et al.*, 1971	Coste & Slawyk, 1974 Cruzado, 1974 Estrada, 1974 Fiala & Jacques, 1974 Grall *et al.*, 1974	Andrews & Hutchings, 1980

in the synthesis of amino acids (Morris, 1974; Raymont, 1980).

The preferential utilization of ammonium-N is related to the control of nitrate assimilation by feedback inhibition when ammonium is assimilated (Morris, 1974). It is thought that the reaction catalysed by nitrate reductase is the rate-limiting step and that the activity of the enzyme is stimulated by light and regulated by the ratio of nitrate to ammonia, rather than by the absolute concentration of nitrate (Packard et al.,1971; Blasco & Packard, 1974; Packard & Blasco, 1974).

Another important nutrient for phytoplankton growth is silicate. Diatoms require this nutrient to construct their cell walls and, since diatoms are the major algal component of upwelling systems, silicate plays a critical role in diatom productivity (Goering, 1974). The cell wall consists of two parts: an inner silicon shell and an outer organic casing that is tightly bound to the shell (Darley, 1974). Silicate uptake is apparently limited to the cell wall formation stage of the division cycle and once taken up is rapidly incorporated into the growing shell. Diatoms do not accumulate and store silicate for later use and only living cells can take up the nutrient.

Phosphorus has a significant role in cellular processes, particularly with regard to generating and transforming metabolic energy (Kuhl, 1974). This element appears to be taken up by algae in the form of orthophosphate only, and uptake is an active process for which energy may be supplied by photosynthesis and respiration. Phytoplankton are able to accumulate excess phosphate which is stored within the cells in the form of inorganic polyphosphate (Fogg, 1975). The reserves resulting from luxury consumption may then support growth in the absence of any further external supply.

Light and temperature

Light penetration into the ocean is a complex phenomenon and it is sufficient here to consider the effects of light on plankton growth. Photosynthesis takes place in two stages: light reactions and dark reactions. In the light reactions photosynthetically active radiation between 380 nm and 720 nm (Strickland, 1958) is trapped or collected in various pigments and converted into the chemical energy of ATP and $NADPH_2$ (Govindjee & Braun, 1974). The dark reaction involves the reduction of CO_2 by $NADPH_2$ with the formation of carbohydrate and the release from ATP of energy for the reaction (Raven, 1974).

As light penetrates the ocean the intensity decreases rapidly, depending on the concentration and nature of particulate matter. In summer the light intensity at the surface may be such that some inhibition of photosynthesis might take place, but at lower depths the light intensity decreases and greater levels of production occur. The bottom of the euphotic zone is the depth at which CO_2 exchange is zero (the compensation point) and the lowest levels of production would be expected at this depth. In field work the depth at which the light intensity is 1% of the surface intensity is usually taken as an approximation of the compensation point (Parsons et al., 1977).

Numerous studies on the effect of light intensity on phytoplankton production have been conducted, and Jassby & Platt (1976) formulated the following hyperbolic tangent expression for the light-saturation curve for light fluxes lower than the threshold of photoinhibition :

$$P^B = P^B_M \tanh(\alpha I/P^B_M) - R^B$$

where P^B = photosynthesis per unit chlorophyll biomass (mg C.mg Chl $\underline{a}^{-1}.h^{-1}$)

P^B_M = specific production rate at optimal light intensity - the assimilation number (mg C.mg Chl $\underline{a}^{-1}.h^{-1}$)

α = initial slope of the light saturation curve at low light levels (mg C.mg Chl $\underline{a}^{-1}.h^{-1}.W^{-1}.m^{-2}$)

I = irradiance ($W.m^{-2}$)

R^B = intercept at zero irradiance (mg C.mg Chl $\underline{a}^{-1}.h^{-1}$)

The value α is related to the quantum efficiency of photosynthesis, characterised by the photochemical reactions, and appears to be independent of temperature. P^B_M is thought to depend on the dark reactions of photosynthesis, controlled by rate-limiting enzyme reactions, and to be temperature dependent (Unesco, 1981).

As both temperature and light decrease with depth it might be expected that the effects of these two factors would be additive. However, while it may generally be accepted that higher temperatures lead to fast growth rates the problem is not simple. Most algal populations have adapted to specific temperature regimes and adaptive phenomena vary from species to species. Some species may, in fact, grow fast at low temperatures that would otherwise inhibit the growth of most species. The physiology of this phenomenon is poorly understood (Yentsch, 1974).

Biochemical constituents of phytoplankton cells

Chlorophyll and ATP

The analysis of photosynthetic pigments has received considerable attention in plankton ecology because the quantity of pigments has been used as a measure of phytoplankton biomass in productivity studies. The colorimetric method of measuring pigments has been improved by the adoption of spectrophotometric and fluorimetric techniques (Strickland & Parsons, 1972). With the trichromatic method, spectrophotometric readings at particular wavelengths are used to calculate the concentration of chlorophyll a, b, and c in terms of mg or µg dry pigment per volume of water. Carotenoids can be estimated only collectively and they are reported in more or less arbitrary units. A comparison of pigment quantities, determined by different scientists, is complicated by a wide divergence in the specific absorption coefficients that are used. Chlorophyll estimates are also in error when the samples contain chlorophyll degradation products which absorb at the same red absorption band as the parent chlorophyll. As a result of this unsatisfactory situation there has been a tendency to standardise measurements at the absorption band of chlorophyll a (665 nm) and to disregard other pigments (Strickland & Parsons, 1972).

A considerable amount of chromatographic research has been undertaken on the pigments of phytoplankton. Jensen & Liaaen Jensen (1959), Jeffrey (1961; 1965; 1968; 1974), Garside & Riley (1969), Jensen & Sakshaug (1973) and Hallegraeff (1976; 1977) have all used paper or thin-layer chromatography to separate chlorophylls a, b and c, the carotenoids and the chlorophyll degradation products of chlorophyllide, phaeophytin and phaeophorbide. Separation of the various carotenoids present in phytoplankton were also obtained and it was demonstrated that fucoxanthin was present in high concentration when diatoms were dominant, but when dinoflagellates dominated the community peridinin was more abundant than fucoxanthin (Jensen & Sakshaug, 1973). The concentration of chromatographically separated chlorophyll a (C-Chl a) was always lower than spectrophotometrically determined chlorophyll a (S-Chl a), and during the active phase of growth the percentage of C-Chl a to S-Chl a was high. When growth declined the percentage of C-Chl a declined and chlorophyll degradation products were detected.

For routine analysis chromatography is slow and few samples can be analysed per day compared to the spectrophotometric method. To separate active chlorophyll a from its inactive forms and avoiding the use of chromatography, Jeffrey (1974) suggested phase separation techniques. Using hexane to separate chlorophyll a and phaeophytin a from chlorophyllide a and phaeophorbide a, Whitney & Darley (1979) measured active chlorophyll a spectrophotometrically by a slight modification of the method of Lorenzen (1967). This phase separation technique may find application in measuring active chlorophyll a in eutrophic waters and in sediments where chlorophyll degradation products are usually abundant.

Another biomass parameter that may be measured is adenosine triphosphate (ATP) (Holm-Hansen & Booth, 1966; Holm-Hansen, 1969). ATP is present in all organisms but since it has an extremely short survival on cell death it is not associated with non-living material. Holm-Hansen & Booth (1966) developed an assay for ATP based on the measurement of the bioluminescence emitted from the reaction between luciferin and luciferase where ATP is required as an energy source (Strehler, 1968). The number of quanta of emitted light is proportional to the quantity of ATP added to the reaction mixture and thus a very sensitive and rapid assay for ATP has been made available.

Holm-Hansen (1970) investigated the cellular content of ATP relative to cell size and cellular organic carbon in 30 different species in culture. He found that ATP concentration remained fairly uniform under normal environmental conditions and averaged 0.35% of the cellular organic carbon content. The steady state levels of ATP in the cells were similar in light or in the dark, although there was a short fluctuation in concentration upon any sudden change in light conditions. Holm-Hansen (1970) concluded that the ATP/carbon ratio in diverse microbial cells was sufficiently uniform that reliable biomass estimates may be obtained from ATP measurements. A ratio of carbon to ATP of 250:1 was determined to be a reliable ratio to convert ATP concentration to carbon concentration for "live" matter. However, studies by Mayzaud & Taguchi (1979) suggests that, for naturally occurring populations, there is not a constant carbon to ATP ratio but rather an upper and lower limit. It was concluded that a serious underestimation may result if a single factor is used to estimate "living" carbon from ATP values.

In addition to measuring biomass a modification of the ATP method has been developed to assess the physiological state of cells. The concept of the adenylate energy charge (EC) was formulated by Atkinson (Atkinson & Walton, 1967; Atkinson, 1979) and defined in molar concentrations as

$$EC = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$$

Falkowski (1977) and Karl & Holm-Hansen (1978) measured adenylate energy charge in a *Skeletonema costatum* culture and in natural environmental samples respectively, and found that high EC values were associated with healthy active cells while low EC values indicated a degree of stress. Factors inducing stress were low nutrient concentrations, photoinhibition, low light intensity or a combination of these factors.

ATP has also been used in conjunction with chlorophyll *a* for estimating the physiological state of phytoplankton. Sakshaug (1977) and Sakshaug & Holm-Hansen (1977) found that with increasing temperature the chlorophyll/ATP ratio increased, but this ratio decreased with an increase in light intensity due to an increase in the ATP pool relative to chlorophyll. An increase in the growth rate of plankton cells led to an increase in the chlorophyll/ATP ratio but the ratio decreased under conditions of nitrogen deficiency. A close correlation was found between organic phosphorus and ATP, while ATP concentrations were reported to be higher in diatoms than in non-silicified algae.

Protein and carbohydrate

The chemical composition of eleven different species of phytoplankton, harvested during the exponential phase of growth, was determined by Parsons *et al*., (1961). The protein content varied from 30% - 51% dry weight, total carbohydrate accounted for between 5% and 35% dry weight, while lipid content was 2% - 18% dry weight, the lipid fractions for dinoflagellates being higher than for diatoms. Fractional anaylsis of the carbohydrates showed that glucose was always the predominant sugar unit in the polysaccharides. It was concluded that marine phytoplankton has very similar organic composition when grown under similar physical and chemical conditions, regardless of the size of the organism or the class to which it belongs. A similar study conducted by Mayzaud & Martin (1975) on a natural phytoplankton population dominated by dinoflagellates yielded protein and carbohydrate levels in the range reported by Parsons *et al*. (1961) for their dinoflagellate species.

A detailed study of carbohydrates in marine particulate matter in the Pacific Ocean near Japan indicated that carbohydrates were removed from particulate matter more rapidly than proteins in the euphotic zone, while proteins were removed preferentially in deep water (Handa & Tominaga, 1969;

Handa & Yanagi, 1969). Water-soluble and insoluble fractions isolated from particulate matter from a depth of 20 metres showed that the water-soluble extract was composed mainly of 1,3-glucans. The rapid decrease in the glucose content of the cells in the upper layers of the sea indicated that 1,3-glucan decays very quickly as particulate matter sinks into layers where light conditions are unfavourable for photosynthesis. Such a decrease was assumed to be due mainly to algal respiration. The insoluble fraction had a monosaccharide composition almost identical to deep-water particulate matter and it was concluded that structural polysaccharides remained very resistant to biological attack.

Myklestad & Haug (1972), Myklestad _et al_. (1972) and Myklestad (1974, 1977) conducted extensive studies into the protein, cellular carbohydrate and extracellular carbohydrate content of diatom cultures. They found that the chemical composition of diatoms grown in batch culture was markedly influenced by the concentration of nutrients in the medium. In the exponential phase of growth the content of cellular glucan was relatively low, but in the stationary phase the glucan content showed a rapid increase which coincided with the depletion of nitrate from the growth medium. Protein concentrations did not increase when nitrate became depleted and therefore pronounced variations in the ratio of protein to carbohydrate were observed. This ratio was considered to be a sensitive and convenient index for characterising the physiological state of diatom cells. During exponential growth protein content was higher than the carbohydrate content and thus the protein/carbohydrate ratio was high. In the stationary growth phase the ratio decreased as the total carbohydrate content increased due to glucan synthesis. Marked differences were observed, though, between various diatom species in their production of glucan. Some species produced much higher levels of glucan than other species even though all the cells were grown under identical conditions.

Similar culture studies conducted on a dinoflagellate by Sakshaug _et al_. (1973) showed that the protein/carbohydrate ratio could not be used as a physiological index for dinoflagellates. No sign of increased carbohydrate content was shown in late growth phases and the ratio of protein to carbohydrate was relatively constant, being independent of the growth phase. During nitrogen limitation in the growth medium protein content was lowest while carbohydrate content was highest.

Linked to these culture studies are the findings of Haug et al. (1973) on the chemical composition of natural phytoplankton populations in the Trondhemisfjord. Samples were analysed for carbohydrate, protein and lipid, and while the lipid content was generally less than 10% dry weight, the protein/carbohydrate ratio varied widely. Carbohydrates were conveniently divided into three fractions: (1) an acid-soluble fraction, (2) an alkali-soluble fraction and (3) an insoluble fraction. A marked difference in carbohydrate content was found between samples rich in dinoflagellates and those containing predominantly diatoms. In the former, the amount of insoluble carbohydrate was always high, probably corresponding to material in the cell walls. In diatom dominated populations the insoluble carbohydrate fraction was usually below 10%. Further differences between diatoms and dinoflagellates were revealed by the monosaccharide composition of the alkali-soluble polysaccharides. Diatoms were characterised by the fraction having a complex monosaccharide composition, but this fraction was virtually absent in dinoflagellate samples; glucose being the dominant component of the very small fraction obtained.

The protein/carbohydrate ratio was shown by Haug et al. (1973) to be a good physiological index for a diatom dominated community. For dinoflagellate samples the protein/carbohydrate ratio was always low due to the high content of insoluble cell wall material. The variation in the protein/carbohydrate ratio for diatoms resulted mainly from variations in acid-soluble carbohydrates which consisted mainly of a β-1,3 glucan. A high protein/carbohydrate ratio indicated a rapidly growing population, while depletion of available nutrients leading to a stationary phase in the growth of the population was indicated by a rise in the glucan content and a corresponding decrease in the protein/carbohydrate ratio.

An investigation of carbohydrates in particulate matter in an upwelling region was undertaken by Hitchcock (1977) off North-West Africa. Vertical profiles of carbohydrate/chlorophyll a ratios showed a decrease with increasing depth. It appears that this was due to a decrease in the intracellular reserve carbohydrate within the top 40 metres, but below this depth the decrease was attributed to degradation of plant material with a consequent decrease in particulate carbohydrate. These conclusions were supported by the fact that the dilute-acid fractions were generally 30-50% of the total carbohydrate in surface waters, but only accounted for 15-20% of total carbohydrates in deeper water. The dilute-alkali fraction contributed 30%

of the total carbohydrate concentration in surface waters and 15% in deeper samples. The residual carbohydrate fraction generally increased with depth and accounted for more than 50% of the total carbohydrate content in deep water samples.

Patterns of carbon-14 assimilation

Steemann-Nielsen (1952) originally introduced the carbon-14 method for measuring primary productivity in the ocean. Its value came from the precision, the sensitivity and the convenience with which it permitted measurements of photosynthesis in the sea. However, an understanding of the physiological state of natural populations from this approach was not easy to assess; one reason being that the overall rate of photosynthesis by algae can remain approximately constant over wide differences in physiological state of the organisms. A possible way of using carbon-14 to indicate physiological state is to measure the gross pattern of photosynthetic carbon fixation as well as the overall rate.

The primary reactions involved in carbon dioxide fixation were elucidated by Calvin and his co-workers (Calvin & Bassham, 1962) and it has generally been assumed that the mechanisms apply to all algae. However, there have been few studies on the way in which pathways of photosynthetic carbon dioxide fixation are influenced by environmental conditions. Fogg (1956) studied the pattern of photosynthesis in the diatom <u>Navicula pelliculosa</u> and observed that in rapidly growing cells having a high nitrogen content a large proportion of the carbon was assimilated into protein. In cells with a low nitrogen content a major portion of assimilated carbon accumulated in the lipid fraction.

More recently Wallen & Geen (1971a, b) investigated light quality in relation to photosynthesis. They observed increased incorporation of carbon-14 into the ethanol-insoluble fraction (primarily protein) in two species of marine algae in blue and green light compared to white light. In natural populations it was found that for surface phytoplankton the greatest proportion of carbon-14 was in the ethanol-soluble fraction, while the proportion of the label in the insoluble fraction increased with depth. As blue-green light predominates in the lower part of the euphotic zone it was concluded that the increase in the relative rate of protein synthesis with increasing depth was due to a change in light quality.

Patterns of photosynthesis in natural freshwater populations by Olive & Morrison (1967) revealed that carbon-14 uptake was greater in ethanol-soluble extracts than insoluble fractions in the upper photic zone. In the middle and lower euphotic zones increasingly larger amounts of carbon-14 were detected in the insoluble fraction. Olive *et al*. (1969) observed high rates of carbon-14 fixation into carbohydrate, but low incorporation into protein, in a *Coscinodiscus* population under moderate illumination. Steady-state *Aphanizomenon* and *Microcystis* communities showed a similar fixation pattern under high illumination. Carbon-14 labelled protein fractions exceeded labelled carbohydrate fractions in rapidly growing *Aphanizomenon* and *Microcystis* communities under moderate illumination and at the peak of a blue-green algal bloom under low illumination. Besides changing environmental conditions Olive *et al*. (1969) concluded that species successional patterns accounted for much of the change in the pattern of photosynthesis during their study.

A simple and convenient method for determining the gross pattern of photosynthetic carbon dioxide fixation in marine phytoplankton was developed by Morris *et al*. (1974), using differential solvent extraction. The effects of various environmental factors on the pattern of photosynthesis by the marine diatom *Phaeodactylum tricornutum* (Bohlin) was investigated using this fractionation technique. Low light intensity and increasing degrees of nitrogen limitation in a chemostat culture markedly increased the relative rates of protein synthesis. Growth of the diatom at low temperatures also increased the proportion of carbon incorporated into the protein fraction. The increased protein synthesis was generally found to be at the expense of the polysaccharide fraction.

In natural communities in the Gulf of Maine, diurnal studies showed continuing incorporation of carbon-14 into protein during the hours of darkness (Morris & Skea, 1978). This was accompanied by a concomitant decrease in the proportion assimilated into polysaccharide. A seasonal investigation indicated that during the winter approximately 10 - 20% of the fixed carbon was incorporated into protein, while this value increased to 22 - 35% during the summer. A transient high value of 37 - 47% of the fixed carbon entering protein coincided with the spring bloom. The proportion of carbon incorporated into protein during photosynthesis also increased markedly at reduced light intensities and the extent of this increase depended on the nutritional state of the phytoplankton. For example, summer populations

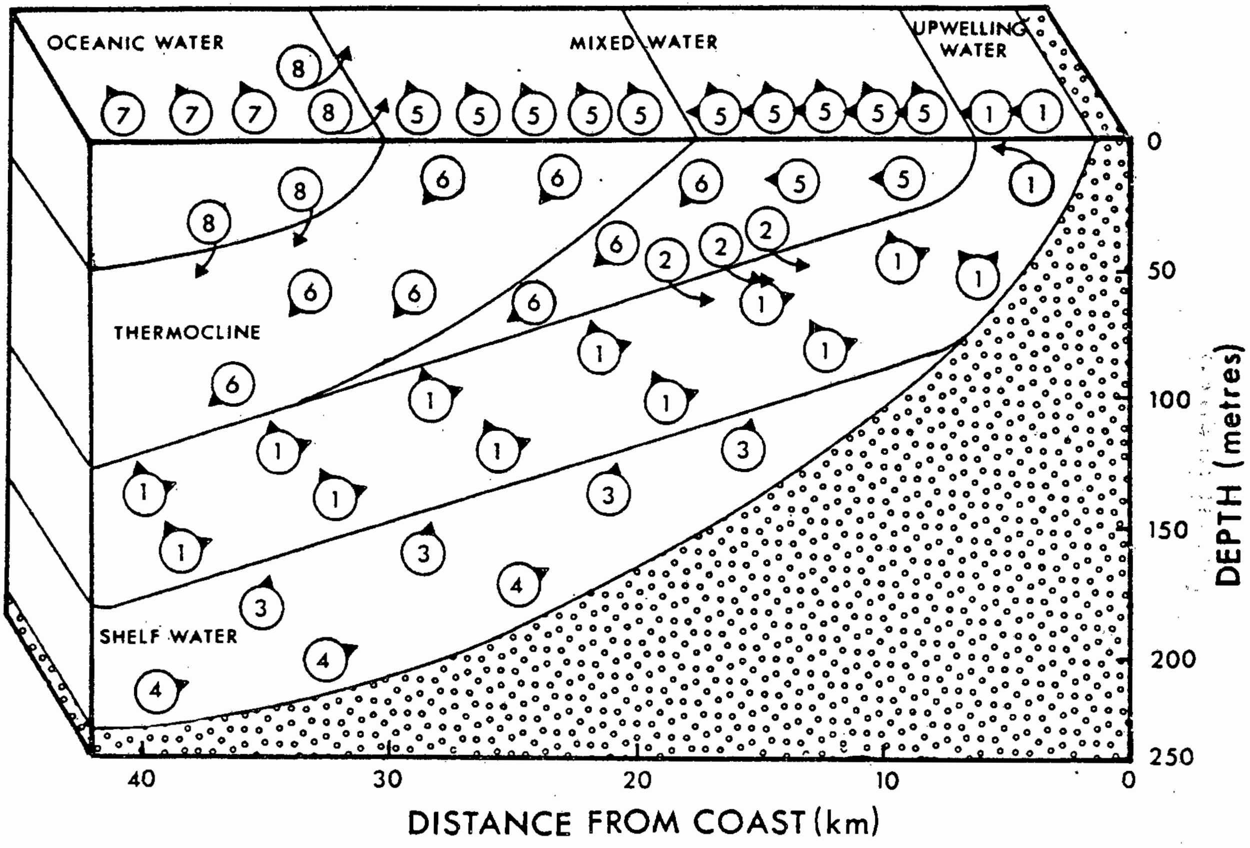


Fig. 1. The relation of water types found off the Cape Peninsula during upwelling (Andrews & Hutchings, 1980).

from water containing low concentrations of inorganic nutrients responded less dramatically to reduced light intensities than did populations from nutrient-rich waters.

Studies in the Benguela upwelling system

An investigation of upwelling in the Benguela system by Andrews & Hutchings (1980) revealed that definite trends existed in the relation between temperature and nutrient, chlorophyll a and oxygen concentrations. All the data were, therefore, classified according to temperature in order to categorise the water masses in the Southern Benguela region into four water types as shown in Fig. 1. Oceanic Water was defined as water of temperature >18°C lying outside the Oceanic front and above the thermocline. Upwelling water was defined as water of temperature between 8° and 10°C and upwelling into the euphotic zone, while Shelf Water was defined as water of temperature <8°C. Mixed water (10° - 18°C) formed the greatest proportion of all water encountered and developed through the mixing of Oceanic and Upwelling Waters.

Diatoms are usually the dominant species in upwelling regions (Raymont, 1980) and Olivieri (in prep.) has estimated that *Nitzschia seriata* and *Nitzschia pungens* accounted for 50% or more of the total cell count in selected samples collected by Andrews & Hutchings (1980) between September, 1972 and February, 1973. In a preliminary investigation of phytoplankton physiology in the local upwelling region (Appendix 1) *Thalassiosira aestivalis* and *Skeletonema costatum* were the dominant species in samples collected during November and December, 1977 at a nearshore station.

Unfortunately there has been very little detailed research into the ecology of phytoplankton in South Africa. Phytoplankton production was measured in Langebaan lagoon by Henry *et al*. (1977), but only recently has regular measurement of primary production been made in the Cape Peninsula upwelling region (Henry, pers. comm.; Brown, 1980). A study of the succession of species in local waters was also recently initiated by Olivieri (in prep.).

Objectives of the present investigation

With a view to complementing the investigations of Brown (1980) and Olivieri (in prep.) a research programme was developed to study the physiology of natural phytoplankton communities. The succession of blooms that occur off the Cape Peninsula during the summer months provides a "natural laboratory" where rapid changes in the growth cycle of phytoplankton are continuously exhibited.

A preliminary investigation (Barlow, 1980 in Appendix 1) was undertaken during the summer of 1977-78 to establish whether chlorophyll _a_, ATP, protein and carbohydrate were suitable indicators of physiological change in phytoplankton communities in the Cape Peninsula upwelling region. During active upwelling temperatures <10°C, high nutrient concentrations and low concentrations of the biochemical constituents were measured. When upwelling lessened and conditions stabilised temperatures increased and blooms of phytoplankton appeared. High concentrations of chlorophyll _a_ and ATP and a high protein/carbohydrate ratio were then recorded. At very low nutrient levels chlorophyll _a_ and ATP concentrations remained high, but an increase in the acid-soluble carbohydrate content and a corresponding decrease in the protein/carbohydrate ratio was observed. It was concluded that the ratio of protein to carbohydrate was a suitable indicator of physiological ageing in the local upwelling region.

The objective of the investigations reported in this thesis was to determine the pattern of growth of phytoplankton in response to the rapid environmental changes that occur in the upwelling system. This pattern was monitored by measuring the concentrations of chlorophyll _a_, ATP, protein and carbohydrate and the assimilation of carbon-14 into the major end-products of photosynthesis, under varying conditions of light, temperature and concentration of nutrients. These determinations were performed on different bodies of water initially, and then co-ordinated into a study of the development of a bloom in a singlemass of water, followed by means of a drogue.

CHAPTER 2

METHODS

A nearshore region off the Cape Peninsula was selected as a suitable study area (Fig. 2). Two fixed stations were established, one at the base of an upwelling plume, 2 kilometres off Oudekraal (33°58'S;18°20.6'E), and another, further offshore, 2 kilometres north of Robben Island (33°46.4'S; 18°22.3'E). To investigate the development of a phytoplankton bloom in a single mass of water (Chapter 5), a 2m x 2m tetrahedral drogue was placed in a patch of upwelled water near Oudekraal and tracked for a few days. The track followed by the drogue is shown in Fig. 2.

Sampling procedure

At the Oudekraal and Robben Island stations the transparency of the water was estimated using a Secchi disc and appropriate light depths were calculated using the formula

$$d = \ell n \frac{I_d}{I_o} D/1.7$$

where D = Secchi disc depth in metres

d = light depth in metres

I_o = light intensity at the surface (100%)

I_d = light intensity at depth d (% of surface intensity).

This formula was derived from the expression

$$I_d = I_o e^{-kd} \quad \text{(Parsons \underline{et} \underline{al}., 1977)}$$

where k = average extinction coefficient in the water column

$= 1.7/D$.

During the drogue study light depths were determined by measuring the decrease in light intensity down the water column with a Lambda quantum meter.

Temperatures were recorded with a Nansen-Petterson bottle or with a bathythermograph and a hand held thermometer.

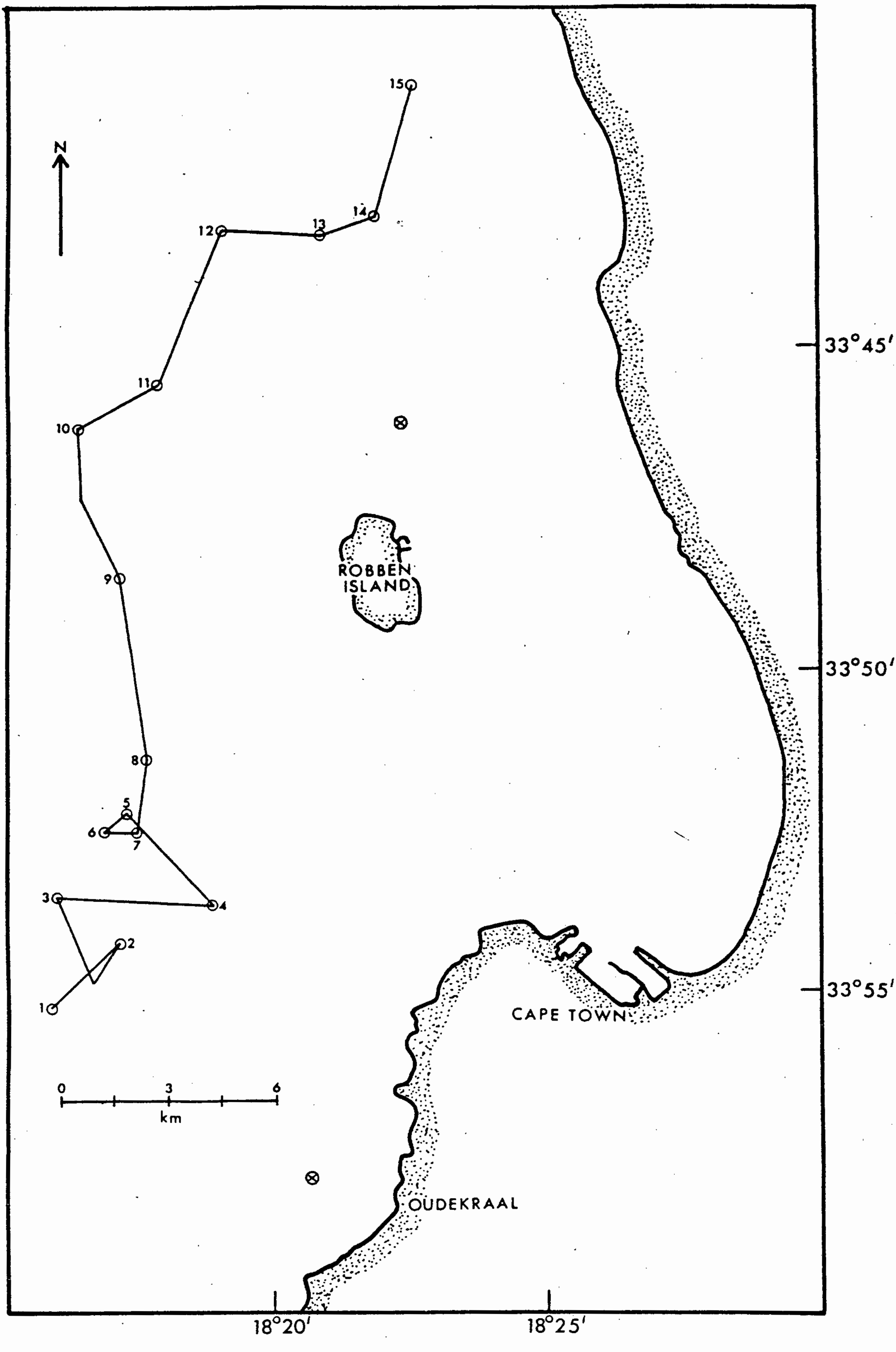


Fig. 2. Map of the study area showing the fixed stations (⊗) and the stations sampled while following a body of water during December, 1979 (○——○).

Water samples were drawn from within the euphotic zone at intervals corresponding to the 100, 50, 25, 10 and 1% light levels in 5 or 7ℓ N.I.O. bottles. Sub-samples were drawn off for analysis of nutrients, chlorophyll a, ATP, protein, carbohydrate and for carbon-14 assimilation studies. A further sub-sample, fixed in 1% formalin, was utilized for species identification and cell counts using a 2 mm squared counting chamber on a Zeiss light microscope.

Samples for nutrient anaylsis were frozen and stored at -20°C until analysed. Chlorophyll a, ATP, protein and carbohydrate samples were filtered through 4.7 cm Whatman GF/F glass fibre filters and frozen in liquid nitrogen immediately after filtering. Chlorophyll a and ATP filters were stored in liquid nitrogen until analysed, while protein and carbohydrate filters were freeze-dried before being analysed.

Nutrient analysis

Analyses were performed on a Technicon Autoanalyser using the methods of Strickland & Parsons (1972) for determining phosphate and nitrate (including nitrite), and Grasshoff (1965) for silicate. Ammonia was analysed according to Slawyk & MacIsaac (1972) but the results were found to be unreliable and are not used here.

Chlorophyll a analysis

The method used to determine total chlorophyll a is described in Appendix 2. This method was developed to speed up and increase the accuracy of the chlorophyll assay. Storing samples in liquid nitrogen, ultrasonic agitation, and the calculation of chlorophyll a concentrations using a calibration curve prepared with pure chlorophyll a were the steps introduced to modify the method of Strickland & Parsons (1972). The modifications are discussed in Appendix 2.

ATP analysis

The method described by Strickland & Parsons (1972) was modified to utilize a liquid scintillation counter to measure the luminescence emitted in the assay procedure. The extraction of ATP into Tris buffer immediately after filtering was found to be impractical at sea, therefore all samples were frozen and stored in liquid nitrogen until analysed. The procedure adopted and the reasons for modifying the original method are described in Appendix 3.

Protein analysis

The procedure used for determining protein concentration is described in Appendix 4. The extraction step was based on the technique of Mayzaud & Martin (1975), but modified to use a more dilute concentration of the extracting solution and a shorter extracting time. This was found to give a higher yield of protein. The method of Lowry et al. (1951) was used for assaying the amount of protein in the extracts, but as numerous substances interfere with the assay, proteins were separated from the interfering material by precipitation with sodium deoxycholate and trichloroacetic acid according to Bensadoun & Weinstein (1976). However, the final concentration of the precipitating reagents had to be increased approximately two-fold to ensure complete precipitation of the proteins. The reasons for modifying various steps in the procedure are discussed in Appendix 4.

Carbohydrate analysis

Carbohydrates were fractionated into acid-soluble, alkali-soluble and insoluble fractions according to Haug et al. (1973), and the carbohydrate content in each fraction was assayed by the phenol-sulphuric acid method of Dubois et al. (1956). However, the original method had to be modified to adapt the procedure for analysis of samples collected on filters. Haug et al. (1973) determined total carbohydrate on net samples by hydrolysing weighed portions with 29 N sulphuric acid. In the present investigations an underestimate of the total carbohydrate content was made when filters were hydrolysed in this manner. The procedure adopted, therefore, was to extract the acid-soluble and alkali-soluble fractions first and then hydrolyse the insoluble fraction with 29 N sulphuric acid. The total carbohydrate concentration was calculated as the summation of the carbohydrate content in each fraction. A description of the modified method and the reasons for introducing the modifications are presented in Appendix 4.

Carbon-14 assimilation experiments

The method of Morris et al. (1974) was used to determine the pattern of carbon-14 assimilation into the end-products of photosynthesis. Essentially, the fixation of the label into ethanol-soluble, TCA-soluble (polysaccharides) and TCA-insoluble (proteins) fractions can be determined by the procedure, but a few modifications were necessary to adapt the method to local conditions.

On completion of carbon-14 incubations Morris _et al_. (1974) stored their filters in 80% ethanol at -14°C until fractionating the samples. In this study it was more convenient to freeze-dry the filters and then undertake the differential solvent extraction on dry samples. In a further modification the evaporation of aliquots of the solvent fractions to dryness before counting was avoided by using Picofluor-30. This scintillant could absorb 30% by volume of the ethanol and TCA solutions and was also suitable for determining the activity of the TCA-insoluble fractions on the filters. Appendix 5 describes the procedures used and the modifications which were introduced.

CHAPTER 3

THE BIOCHEMICAL COMPOSITION OF PHYTOPLANKTON COMMUNITIES

The biochemical composition of phytoplankton has been used extensively in taxonomic studies (Lewin, 1974), but investigations of changes in composition can provide valuable information about their ecology and physiology. This knowledge may lead to a better understanding of primary production as well as of particular substances which may be of special significance in marine food chains. The nutritive value of phytoplankton is also of interest as different nutritive qualities may influence the feeding and breeding activities of zooplankton.

In a dynamic ecosystem such as the Cape Peninsula upwelling system, phytoplankton production can be high, 700 mg $C.m^{-2}.h^{-1}$ (Brown, 1980), and physiological changes can be expected to be rapid. Chlorophyll a, ATP, protein and carbohydrate are all important biochemical constituents of phytoplankton cells (Raymont, 1980) and were selected as suitable variables to monitor the response of communities to changes in the environment. Chlorophyll a is unique to plant cells while ATP is only present in living organisms (Holm-Hansen & Booth, 1966). Protein and carbohydrate are metabolites whose concentration in algal cells have been found to vary in accordance with the physiological state of the cells (Myklestad, 1978).

Diatoms are usually the dominant species in upwelling ecosystems (Parsons, 1979) and important research has been undertaken on the biochemical composition of cultured diatoms at the Institute of Marine Biochemistry, University of Trondheim. Myklestad (1978) has reviewed this work and concluded that the content of protein, total cellular carbohydrate and acid-soluble carbohydrate in all the diatoms investigated was dependent on the composition of the growth medium. The acid-soluble fraction was usually a major one and contained glucose as the predominant sugar. It was concluded that the fraction was a glucan of the β-1,3-linked type as previously determined for the marine diatom Phaeodactylum tricornutum (Ford & Percival, 1965) and, furthermore, this glucan was only present in living phytoplankton.

Biochemical ratios have been used to relate change in biochemical composition to changes in the environment. Nutrient concentration, light and temperature affect the chlorophyll/ATP ratio (Sakshaug, 1977; Sakshaug & Holm-Hansen, 1977), while nutrient limitation may be indicated by changes in the protein/carbohydrate ratio (Myklestad, 1978). The importance of these ratios is that they indicate a direction of change in biochemical composition, which reflects a changing physiological state of the community. In this study the following ratios were selected to convey the most useful information : -

protein/carbohydrate
glucan/ATP
chlorophyll/ATP
glucan/chlorophyll.

Variation in nutrient concentration, particularly nitrate, has a marked affect on the biochemical composition of phytoplankton as shown in a preliminary study (Barlow, 1980 in Appendix 1) and by Antia et al. (1963), Myklestad & Haug (1972), Haug et al. (1973), Sakshaug (1977) and Sakshaug & Holm-Hansen (1977). On the basis of these reports the following hypothesis is proposed for changes in the biochemical composition of phytoplankton communities in response to changes in nutrient supply in an upwelling region :

When sufficient nutrients are available the chlorophyll/ATP and protein/carbohydrate ratios are high due to active production of chlorophyll a, ATP and protein. The glucan/ATP and glucan/chlorophyll ratios are low since glucan synthesis proceeds at a slow rate. When nutrient levels decrease and low nitrate concentrations are reached, glucan synthesis is enhanced at the expense of the synthesis of other cell constituents. Under these conditions the chlorophyll/ATP and protein/carbohydrate ratios are low, while the glucan/ATP and glucan/chlorophyll ratios are high.

This chapter reports the investigations that were undertaken in a nearshore region off the Cape Peninsula to test this hypothesis.

METHODS

Samples were collected at the Oudekraal and Robben Island stations during the 1978-79 summer season. Daily sampling was conducted between 0930 and 1130 hours, over 7-11 day periods, each month from October to March. This sampling strategy resulted in different bodies of water being monitored at various depths as they moved past these stations.

Each day, light and temperature measurements were made initially and then single samples were drawn from various depths within the euphotic zone for analysis of nutrients, ATP, chlorophyll a, protein, carbohydrate and species composition. The sampling procedure and methods of analysis that

TABLE 2

Characteristics of water types off the Cape Peninsula: Type 1, recently upwelled water; Type 2, maturing upwelled water; Type 3, aged upwelled water (n = number of observations)

	Type 1	n	Type 2	n	Type 3	n
Temperature, °C	8-10	42	10-15	138	12-16	32
Nitrate, μg-at NO_3-N.ℓ^{-1}	15-30	42	2-15	138	0-2	32
Silicate, μg-at SiO_3-Si.ℓ^{-1}	10-30	42	2-22	138	2-12	32
Phosphate, μg-at PO_4-P.ℓ^{-1}	1.2-3.5	42	0.5-2.5	138	0.5-1.5	32
Chlorophyll a, μg.ℓ^{-1}	0-1	42	1-20	138	5-30	32
Phytoplankton density, cells x 10^3.ℓ^{-1}	35-120	4	32.5-2162.5	20	120-4145	7

were used have been outlined in Chapter 2 and detailed in Appendix 2 (chlorophyll a), Appendix 3 (ATP) and Appendix 4 (protein and carbohydrate).

RESULTS

Water types

A total of 212 sets of observations were made in the study area and, using the environmental measurements as a basis, the water in the region was classified into three types (Table 2). In Type 1 water, temperatures were <10°C while the concentrations of nitrates, silicates and phosphates were high. These measurements indicated that there was active upwelling of cold water. When upwelling lessened and conditions stabilised temperatures increased to >10°C and high or adequate concentrations of nutrients for phytoplankton growth prevailed (Type 2 water). In Type 3 water nitrate concentrations were low with temperatures still >10°C.

These water types followed sequentially after water had upwelled and merged with other water bodies as described by Andrews & Hutchings (1980). Type 1 water was similar to the upwelling water type of Andrews & Hutchings (1980), while Type 2 and Type 3 water were two types of Mixed Water (Fig. 1). Type 2 water prevailed on most days during the study, but Type 3 was encountered on only a few occasions when environmental conditions had been stable for a number of days.

Species composition

Analysis for phytoplankton species composition revealed that the same species were present at all the depths sampled on a particular day. The species detected at the surface or at the 50% light intensity depth on each sampling day are presented in Table 3, together with the biochemical composition of the communities. The dominant species accounted for over 50% of the total number of cells in each sample.

During the months of October, November, December and January diatoms dominated the communities, with *Skeletonema costatum* being most prominent during the first 3 months and *Thalassiosira aestivalis* and *Chaetoceros compressus* dominating during January. However, diatoms were not significant during February and March as the communities appeared to be composed almost entirely of microflagellates. No cell numbers are reported for these flagellate communities since it was extremely difficult to obtain acceptably accurate counts of these cells.

TABLE 3

Phytoplankton species composition, total cell numbers and the biochemical composition of samples collected at the surface or from depths corresponding to the 50% light intensity level (Chloro. = Chlorophyll a; Prot. = Protein; Carbo. = Total Carbohydrate)

Date	Depth (m)	Dominant species	Other genera	Water type	Cell nos. (Cells x $10^3.\ell^{-1}$)	Chloro. ($\mu g.\ell^{-1}$)	ATP ($\mu g.\ell^{-1}$)	Prot. ($\mu g.\ell^{-1}$)	Carbo. ($\mu g.\ell^{-1}$)
17-10	3	Skeletonema costatum	Thalassiosira; Nitzschia	3	555.0	22.63	0.968	486.8	1509.0
18-10	1.5	S. costatum	Thalassiosira; Asterionella	2	376.0	18.75	0.968	662.9	351.5
20-10	2.5	S. costatum	Thalassiosira	3	472.5	30.18	0.800	863.5	1108.5
21-10	2.5	S. costatum	Thalassiosira; Nitzschia; Chaetoceros	3	605.0	13.21	0.872	732.5	842.2
22-10	2.5	S. costatum	Thalassiosira; Nitzschia; Chaetoceros; Hemiaulus	3	122.5	9.06	0.488	443.6	765.5
25-10	5	S. costatum	Coscinodiscus	2	60.5	1.04	0.096	129.2	77.9
26-10	7	S. costatum	Coscinodiscus; Thalassiosira	2	72.5	1.57	0.152	119.0	74.0
6-11	7	Thalassiosira rotula; Thalassiothrix frauenfeldii		1	35.0	0.48	0.056	44.0	36.7
7-11	0	Thalassiosira rotula	Thalassiosira	1	75.0	0.37	0.056	49.7	16.7
9-11	0	S. costatum	Thalassiosira; Nitzschia; Hemiaulus	2	1220.0	6.74	0.348	342.0	79.7
9-11	12	S. costatum	Thalassiosira; Nitzschia; Hemiaulus; Rhizosolenia; Asterionella	2	220.0	1.39	0.140	191.9	51.7
10-11	1.5	S. costatum	Thalassiosira; Nitzschia; Hemiaulus; Chaetoceros	3	4145.0	14.15	0.640	962.3	1962.7
11-11	1.5	S. costatum	Thalassiosira; Nitzschia; Chaetoceros	2	2162.5	12.88	0.392	701.2	515.0
12-11	4	S. costatum	Thalassiothrix; Nitzschia	3	120.0	8.35	0.656	652.9	750.5
13-11	3.5	S. costatum	Thalassiosira; Nitzschia; Chaetoceros; Hemiaulus; Rhizosolenia; Eucampta; Asterionella	2	1782.5	10.07	0.808	613.8	200.0
14-11	7	S. costatum	Thalassiosira; Nitzschia; Chaetoceros; Hemiaulus	2	365.0	3.03	0.248	180.2	49.2
1-12	12	S. costatum	Thalassiosira; Chaetoceros	1	120.0	0.34	0.036	36.0	28.4
6-12	8.5	S. costatum	Thalassiosira; Nitzschia; Chaetoceros	2	435.0	1.11	0.188	125.6	54.0
7-12	6	S. costatum	Hemiaulus; Nitzschia; Coscinodiscus	2	855.0	2.46	0.248	217.8	79.6
14-12	5	S. costatum	Thalassiosira; Nitzschia; Hemiaulus; Chaetoceros	2	762.5	2.12	0.328	280.5	190.0

TABLE 3 (continued)

Date	Depth (m)	Dominant species	Other genera	Water type	Cell nos. (Cells x $10^3.\ell^{-1}$)	Chloro. ($\mu g.\ell^{-1}$)	ATP ($\mu g.\ell^{-1}$)	Prot. ($\mu g.\ell^{-1}$)	Carbo. ($\mu g.\ell^{-1}$)
10-1	5.5	Thalassiosira aestivalis	Skeletonema; Nitzschia	2	205.0	5.40	0.552	391.0	183.5
11-1	5.5	T. aestivalis		2	1470.0	6.38	0.736	540.3	232.5
12-1	5	T. aestivalis		2	190.0	5.30	0.640	537.0	317.7
13-1	6.5	T. aestivalis		2	1190.0	3.02	0.496	296.7	144.5
15-1	9	T. aestivalis		2	32.5	1.92	0.352	205.1	105.2
23-1	0	S. costatum	Thalassiosira; Chaetoceros	1	52.5	0.40	0.064	72.3	41.6
24-1	0	Chaetoceros compressus	Thalassiosira; Chaetoceros	3	185.0	4.82	0.456	353.9	531.5
25-1	0	C. compressus	Nitzschia; Chaetoceros; Hemiaulus; Bacteriastrum; Rhizosolenia	2	307.5	4.32	0.424	252.7	199.7
26-1	0	C. compressus	Nitzschia; Chaetoceros; Hemiaulus; Stephanopyxis; Rhizosolenia	2	465.0	7.89	0.560	364.0	190.0
27-1	0	C. compressus S. costatum		2	95.0	2.64	0.148	208.9	140.7
28-1	0	Rhizosolenia stolterfothii Hemiaulus sinensis		2	37.5	1.99	0.400	152.2	98.2
5-2	6.5	Microflagellates		2		1.13	0.344	151.5	93.2
6-2	8	Microflagellates		2		1.88	0.116	105.4	87.1
7-2	6	Microflagellates		2		1.41	0.264	136.6	85.5
8-2	9	Microflagellates		2		1.39	0.868	129.2	65.6
9-2	6	Microflagellates		1		0.48	0.048	57.5	31.2
11-2	3.5	Microflagellates		2		4.54	0.528	427.8	156.5
12-2	3	Microflagellates		2		4.94	0.488	378.5	143.2
13-2	3.5	Microflagellates		2		8.34	0.744	539.3	205.0
14-2	3	Microflagellates		2		10.33	1.136	520.8	198.5
15-2	3.5	Microflagellates		2		12.47	0.872	688.8	358.7
16-2	2	Microflagellates		2		17.43	1.000	826.7	286.0
8-3	4	Microflagellates		2		2.10	0.376	219.0	62.5
12-3	3	Microflagellates		2		7.95	0.704	511.6	383.2
13-3	2	Microflagellates		2		19.85	1.264	849.1	470.5
14-3	3.5	Microflagellates		2		8.08	0.568	526.4	340.0
15-3	2	Microflagellates		3		25.26	1.256	457.4	561.0
16-3	2	Microflagellates		2		16.87	1.080	674.9	266.2

TABLE 4

Statistically significant correlation coefficients between environmental and biochemical variables for all types of water and communities (values significant at the 1% level for 212 observations; N.S.- not significant i.e. $P > 0.05$)

	ATP	Chlorophyll *a*	Protein	Carbohydrate
Light	N.S.	N.S.	N.S.	N.S.
Temperature	0.650	0.721	0.774	0.760
Nitrate	-0.531	-0.732	-0.684	-0.851
Phosphate	-0.235	-0.408	-0.429	-0.505
Silicate	N.S.	-0.273	-0.281	-0.429

TABLE 5

Statistically significant correlation coefficients between biochemical variables for all types of water and communities (values significant at the 1% level for 212 observations)

	ATP	Chlorophyll *a*	Protein
Chlorophyll *a*	0.842		
Protein	0.828	0.936	
Carbohydrate	0.709	0.867	0.870

TABLE 6

Statistically significant correlation coefficients between biochemical ratios for all types of water and communities (values significant at the 1% level for 212 observations; N.S.- not significant i.e. $P > 0.05$)

	Protein/Carbohydrate	Glucan/ATP	Chlorophyll/ATP
Glucan/ATP	-0.591		
Chlorophyll/ATP	-0.252	0.484	
Glucan/Chlorophyll	-0.633	0.843	N.S.

No flagellates were detected in formalin preserved specimens and yet high concentrations of chlorophyll a were determined in many of the samples. Unpreserved samples, kept cold in a refrigerator at 10°C, were brought back to the laboratory for examination and the microflagellates were then observed. Their diameter ranged between 5 and 10 μm. Only a small percentage remained intact and the rest were badly damaged. It appeared that these flagellates had a short survival time when removed from their natural environment. The best solution would have been to count the cells at sea immediately after collecting the samples, but it was not possible to use a mircroscope on board the small vessel used. In addition to preserved samples, unpreserved samples were brought back to the laboratory and merely examined for the species present without attempting cell counts.

The biochemical composition of the various communities in Table 3 showed marked differences between samples drawn from different types of water. Samples from Type 1 water had low concentrations of the biochemical variables, while these concentrations increased with a change to Type 2 and Type 3 water. It may be observed that there is a considerable range in the biochemical composition of samples drawn from Type 2 water, with cell numbers generally varying in accordance with this range.

Relationship between variables

To investigate relationships between the various variables the product-moment correlation coefficient (r) was calculated between all pairs of transformed $[\log_{10}(x+1)]$ variables. These coefficients are given in Tables 4, 5 and 6. No significant correlation was found between light and the biochemical variables, but a positive correlation was obtained between temperature and the variables. The negative correlations between nutrients and the biochemical variables were expected and it is noteworthy that nitrate gave higher correlations with the variables than the other nutrients. The correlations between biochemical variables are statistically very significant, particularly those of chlorophyll a with the other variables. The correlations between the protein/carbohydrate ratio and the other biochemical ratios suggested that the glucan/ATP and glucan/chlorophyll ratios could be considered as indicators of the physiological state of the phytoplankton communities as has been established for the protein/carbohydrate ratio (Appendix 1).

Distribution of variables in the euphotic zone

Examples of the temperature, the nutrient content and the biochemical

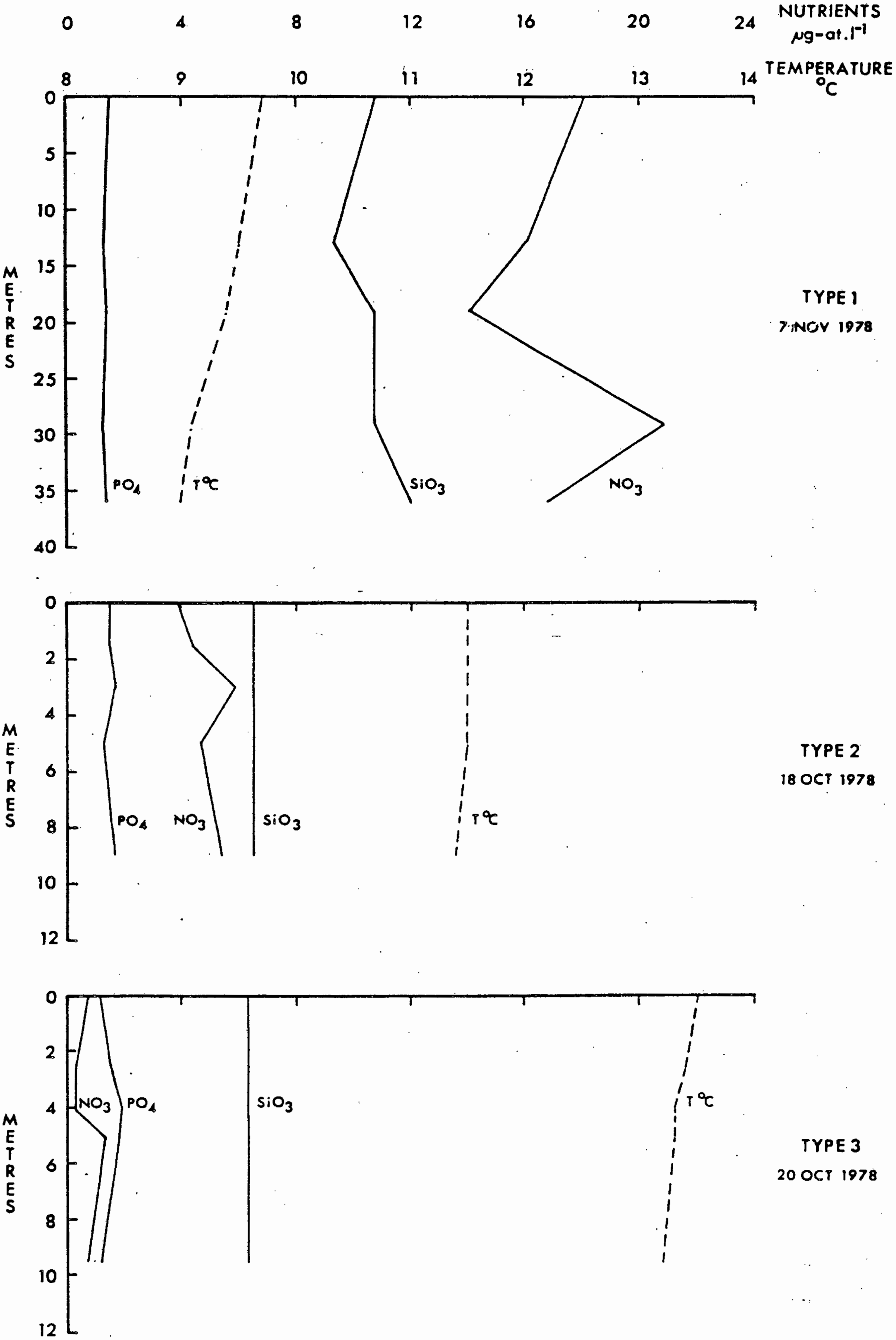


Fig. 3. Vertical distribution of temperature and nutrients in Type 1, Type 2 and Type 3 water.

composition of samples from the three types of water are presented (Fig. 3 - 7). Each type is exemplified by environmental data (Fig. 3) and biochemical data (Figs. 4 and 5) collected on the same day; each water type occurred on a different day. The graphs in Figs. 6 and 7 are related in a similar manner. The concentrations of silicate and phosphate did not vary greatly between water types, but differences in temperature and in the concentration of nitrate were marked. Nitrate concentrations were $>15\mu g$-at NO_3-N.ℓ^{-1} in Type 1 water but in Type 3 water the concentrations were $<1\mu g$-at NO_3-N.ℓ^{-1}.

For diatom communities the concentrations of all the biochemical variables were low in Type 1 water (Fig. 4). The protein/carbohydrate ratio was >1 (Fig. 5), while the glucan/chlorophyll and glucan/ATP ratios were <32 and <350 respectively. In Type 2 water ATP and chlorophyll a concentrations were mainly $>0.7\mu g.\ell^{-1}$ and $>16\mu g.\ell^{-1}$ respectively. Protein concentration ($>600\mu g.\ell^{-1}$) was greater than the carbohydrate concentration ($<400\mu g.\ell^{-1}$) giving a protein/carbohydrate ratio of >1. The glucan/chlorophyll and glucan/ATP ratios were low as found in Type 1 water.

ATP and chlorophyll a concentrations remained high in Type 3 water, with the carbohydrate concentration being greater than the protein concentration (Fig. 4). This resulted in a low protein/carbohydrate ratio (<1) (Fig. 5). The glucan/ATP and glucan/chlorophyll ratios were variable in the ranges 400 - 2300 and 18 - 70 respectively.

The vertical distribution of biochemical variables for communities dominated by microflagellates showed trends similar to that observed for diatom communities (Figs. 6 and 7). While the glucan/ATP and glucan/chlorophyll ratios did not show significant differences between water types the protein/carbohydrate ratio was lower in Type 3 water (<1) than in Type 1 or Type 2 water (>1).

The mean concentrations and values of the biochemical variables and ratios for all the observations made in this study are presented in Table 7. Chlorophyll a, ATP, protein, total carbohydrate and glucan concentration were low in Type 1 water but were greater in Type 2 and Type 3 water; the highest concentrations were found in Type 3 water. The protein/carbohydrate ratio was high (>1) in Type 1 and Type 2 water but low (<1) in Type 3 water, while the chlorophyll/ATP ratio was greater in Type 3 water than in Type 1 and Type 2. The glucan/ATP and glucan/chlorophyll ratios were <200 and

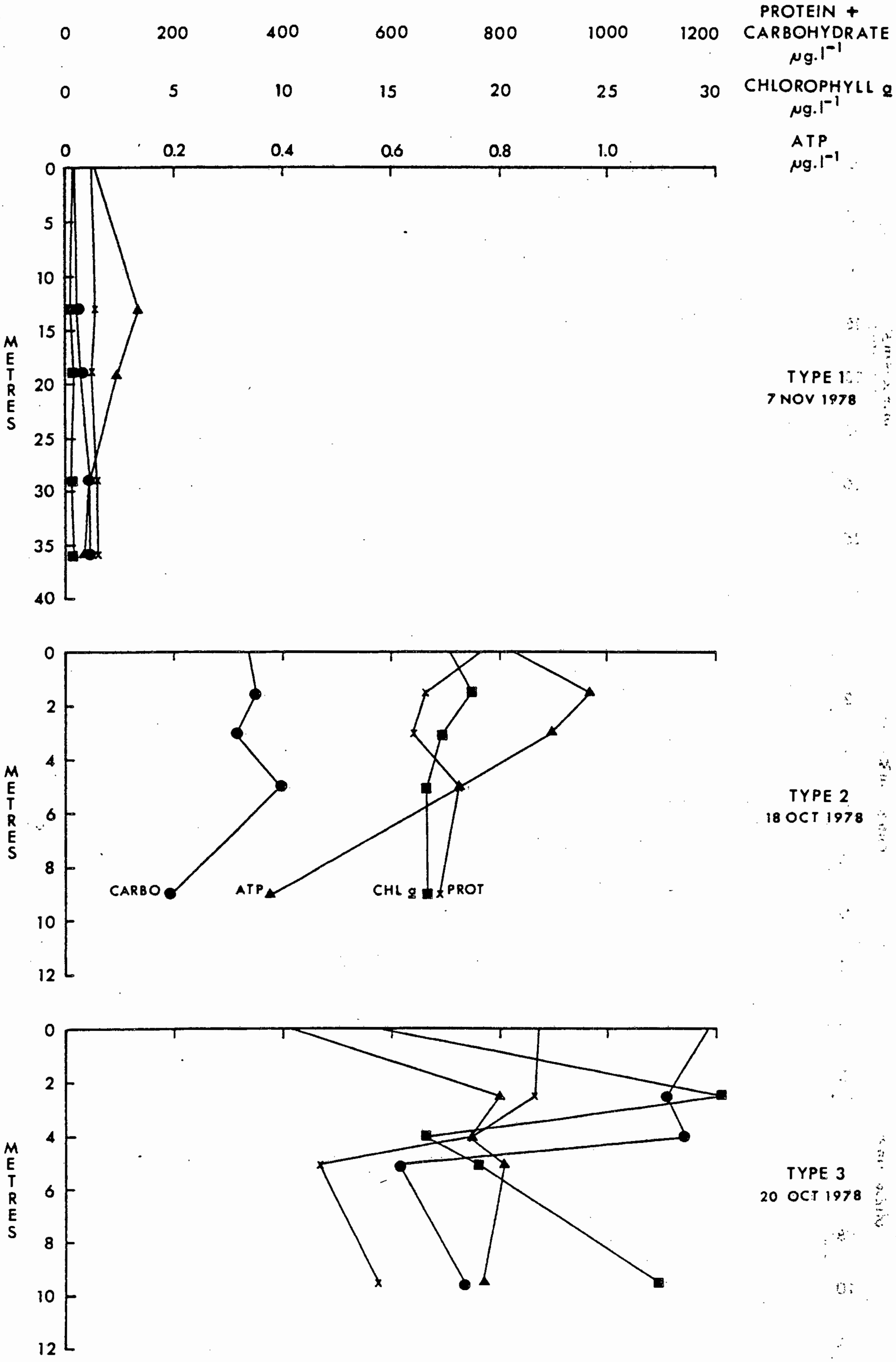


Fig. 4. Vertical distribution of ATP, Chlorophyll a (CHL a), Protein (PROT) and Carbohydrate (CARBO) in Type 1, Type 2 and Type 3 water for diatom communities.

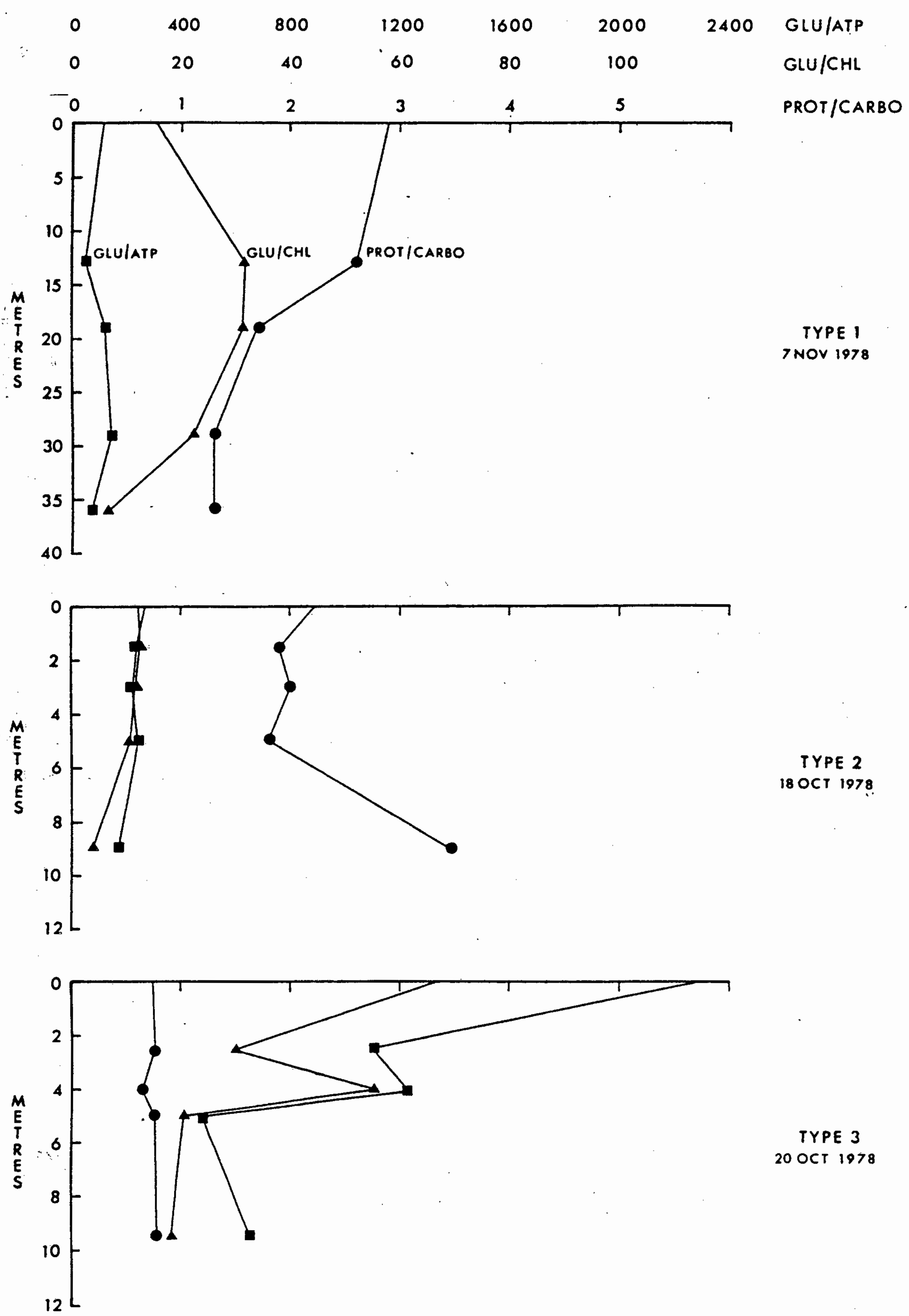


Fig. 5. Vertical distribution of the protein/carbohydrate (PROT/CARBO), glucan/chlorophyll (GLU/CHL) and glucan/ATP (GLU/ATP) ratios in Type 1, Type 2 and Type 3 water for diatom communities.

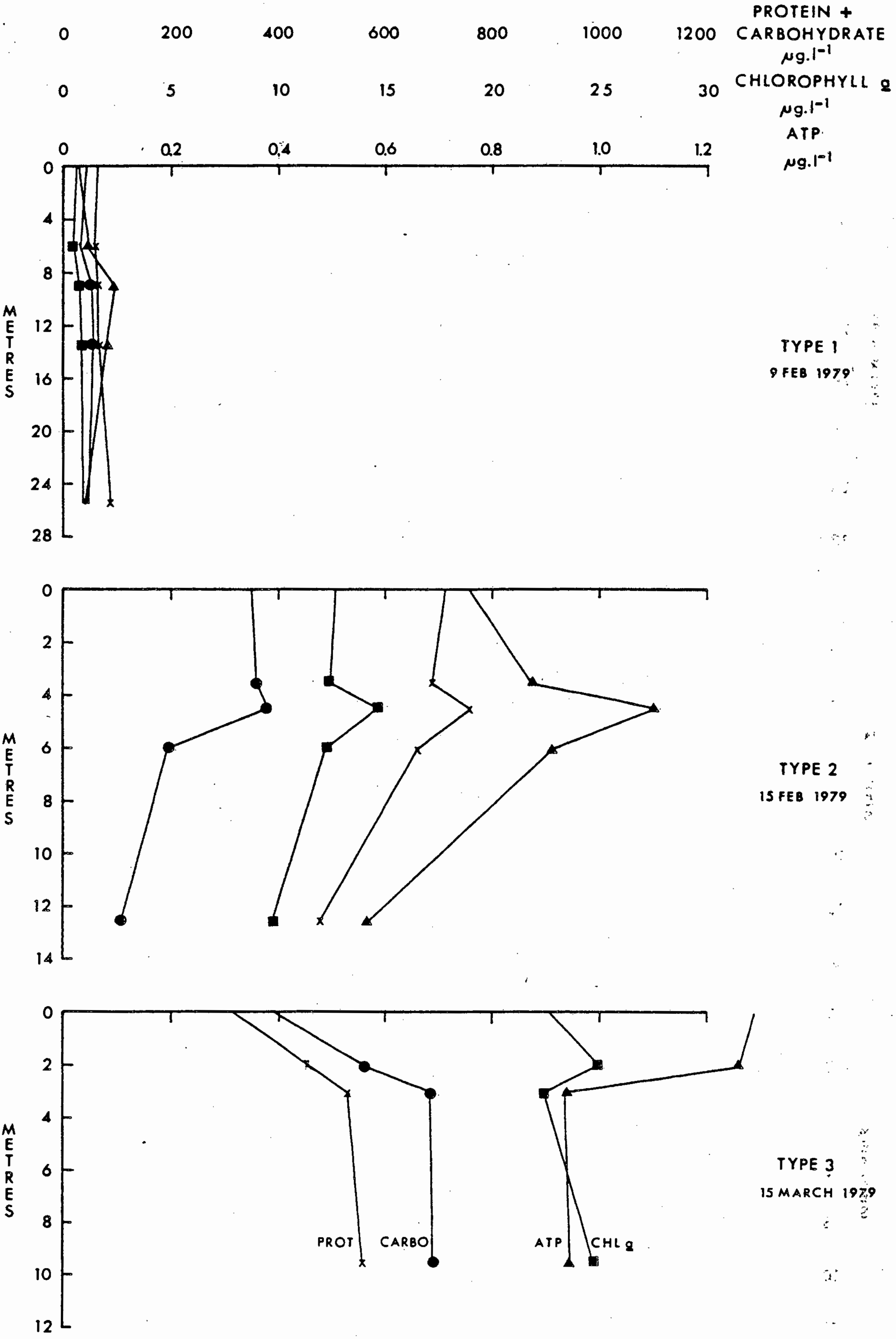


Fig. 6. Vertical distribution of ATP, Chlorophyll a (CHL a), Protein (PROT) and Carbohydrate (CARBO) in Type 1, Type 2 and Type 3 water for microflagellate communities.

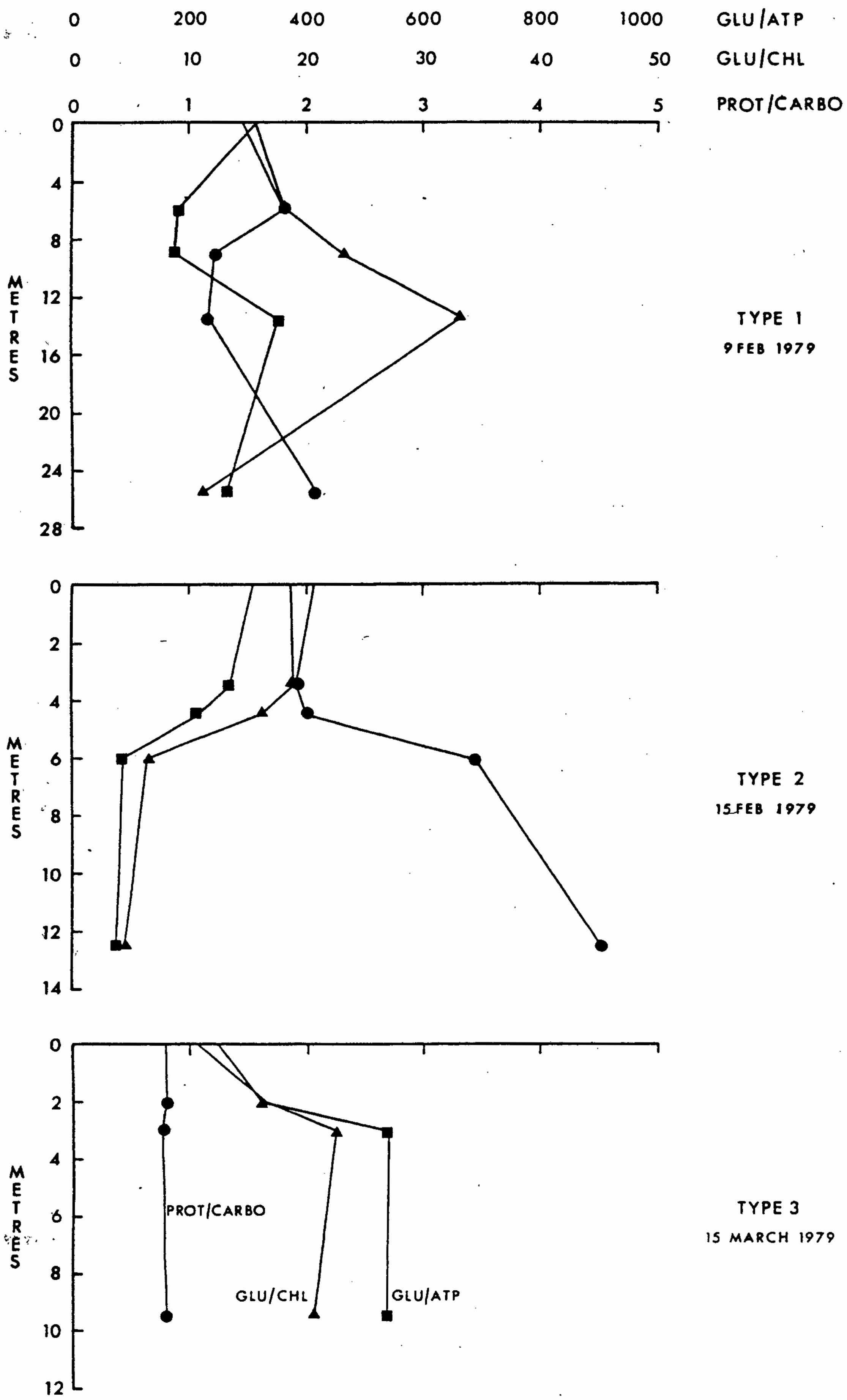


Fig. 7. Vertical distribution of the protein/carbohydrate (PROT/CARBO), glucan/chlorophyll (GLU/CHL) and glucan/ATP (GLU/ATP) ratios in Type 1, Type 2 and Type 3 water for microflagellate communities.

TABLE 7

Mean concentrations and values of biochemical variables and ratios in three types of water on all communities. Standard errors of the mean (±) are given in paranthesis and n = number of observations

	Type 1 (n=42)	Type 2 (n=138)	Type 3 (n=32)
Chlorophyll a, $\mu g.\ell^{-1}$	0.66 (0.1)	7.45 (0.5)	15.00 (1.18)
ATP, $\mu g.\ell^{-1}$	0.143 (0.032)	0.566 (0.029)	0.726 (0.042)
Protein, $\mu g.\ell^{-1}$	69.9 (4.9)	423.6 (20.8)	579.5 (30.8)
Total carbohydrate, $\mu g.\ell^{-1}$	32.4 (2.8)	188.8 (11.3)	928.9 (87.8)
Glucan, $\mu g.\ell^{-1}$	9.2 (1.4)	99.4 (8.2)	748.0 (87.2)
Protein/Carbohydrate	2.66 (0.23)	2.5 (0.08)	0.68 (0.03)
Chlorophyll/ATP	9.6 (1.5)	12.8 (0.5)	20.5 (1.2)
Glucan/ATP	112.1 (19.1)	183.8 (12.9)	1186.5 (163.4)
Glucan/Chlorophyll	14.7 (1.6)	15.7 (0.8)	58.8 (7.3)

TABLE 8

Soluble and insoluble fractions of carbohydrates for diatom and microflagellate communities in three types of water

Date	Species	Water type	Depth (m)	Total carbohydrate ($\mu g.\ell^{-1}$)	Acid-sol. fraction ($\mu g.\ell^{-1}$)	Alkali-sol. fraction ($\mu g.\ell^{-1}$)	Insoluble fraction ($\mu g.\ell^{-1}$)	Acid-sol./ Total
7 Nov 1978	Diatoms	1	0	16.7	6.2	0	10.5	0.37
			19	29.0	11.5	1.5	16.0	0.39
			36	44.8	2.5	0	42.3	0.05
18 Oct 1978		2	0	338.2	217.5	55.5	62.2	0.64
			3	315.2	201.0	38.0	76.2	0.64
			9	194.7	64.5	31.0	99.2	0.33
20 Oct 1978		3	0	1183.0	968.0	135.5	79.5	0.82
			4	1145.6	918.0	143.0	84.6	0.80
			9.5	734.0	503.0	134.0	97.0	0.68
9 Feb 1979	Microflagellates	1	0	43.0	10.0	2.5	30.5	0.23
			9	51.1	16.7	3.5	30.9	0.33
			25.5	41.7	10.5	6.0	25.2	0.25
15 Feb 1979		2	0	350.2	235.0	67.0	48.2	0.67
			4.5	380.2	238.0	96.0	46.2	0.63
			12.5	106.0	42.0	43.0	21.0	0.39
15 Mar 1979		3	0	396.5	281.0	81.5	34.0	0.71
			3	687.5	506.0	107.0	74.5	0.74
			9.5	689.2	506.0	112.0	71.2	0.73

$<$20 respectively in Type 1 and Type 2 water but $>$1000 and $>$50 respectively in Type 3 water.

Carbohydrate fractions

The most dramatic change in the concentration of the biochemical variables was exhibited by the carbohydrates. Examples of the fractional analysis of the carbohydrates in phytoplankton are shown in Table 8. It may be observed that the acid-soluble fraction (glucan) contributed significantly to the variation in total carbohydrate concentration for both the diatom and microflagellate communities.

DISCUSSION

Community species composition

Species analysis revealed a surprising change in composition during the months of February and March. While microflagellates have been detected in local waters (D. Horstman, pers. comm.) it is unusual for them to dominate the community when environmental conditions are optimal for diatom growth (Table 3, Type 2 water). Silicate concentrations varied between 10 and 37 μg-at SiO_3-Si.ℓ^{-1} and phosphates between 0.5 and 3.5 μg-at PO_4-P.ℓ^{-1} at all sampled depths during February and March. Nitrate concentrations varied between 2 and 29 μg-at NO_3-N.ℓ^{-1} except for one day (15 March 1979) when concentrations were $<$1μg-at NO_3-N.ℓ^{-1}. Nanoplankton, such as microflagellates, usually proliferate under conditions of low nutrient availability and high temperatures (Durbin _et al_., 1975). Malone (1971) estimated that nanoplankton accounted for 60 - 99% of the observed productivity and standing stock both inshore and offshore in the California Current under conditions of high temperatures and low nutrient concentrations. Diatom productivity and standing stock exceeded that of the nanoplankton only during periods of strong upwelling, characterised by low surface temperatures and high nutrient concentrations. Variations in seasonal productivity were due primarily to the diatoms, with the nanoplankton maintaining a comparatively stable background level.

Some microflagellates were observed in unpreserved diatom dominated samples in this study and it is possible that the phenomenon observed by Malone (1971) may occur in the local upwelling region. However, it is difficult to find an explanation for the domination of microflagellates during February - March, 1979. One possibility is that vitamins, or other organic

TABLE 9

Stepwise multiple regression statistics for biochemical variables on environmental variables for all types of water and communities (n = 212). Underlined values indicate highest partial correlation coefficients

Dependent variable	Free variable	Regression coeff.	Partial corr.coeff.	Multiple corr.coeff.	Coeff. of determination
ATP				0.762	0.571
	Y-Intercept	-0.088	-0.024		
	Light	-0.002	-0.248		
	Temperature	0.050	0.173		
	Nitrate	-0.044	-0.444		
	Phosphate	0.098	0.163		
	Silicate	0.028	0.470		
	Ammonia		0.079		
Chlorophyll *a*				0.794	0.624
	Y-Intercept	25.279	0.375		
	Light		-0.104		
	Temperature	-1.188	-0.246		
	Nitrate	-1.362	-0.670		
	Phosphate	2.692	0.246		
	Silicate	0.442	0.436		
	Ammonia		0.019		
Protein				0.799	0.634
	Y-Intercept	577.51	0.832		
	Light	-0.618	-0.147		
	Temperature		0.064		
	Nitrate	-38.809	-0.784		
	Phosphate		0.040		
	Silicate	16.387	0.447		
	Ammonia		-0.038		
Carbohydrate				0.647	0.413
	Y-Intercept	1212.46	0.291		
	Light		0.090		
	Temperature	-44.37	-0.154		
	Nitrate	-42.15	-0.519		
	Phosphate		-0.015		
	Silicate		0.132		
	Ammonia		-0.116		

compounds essential for algal growth (Provasoli & Carlucci, 1974) were not present in sufficient quantities in the water for diatom growth to take place, even though adequate macro-nutrients were available.

Environmental effects on biochemical variables

The correlations obtained between the environmental and biochemical variables (Table 4) provide an indication that a relationship existed between changes in the environment and changes in biochemical composition. The positive correlations between temperature and the biochemical variables support the observed increase in the concentration of the biochemical variables with increasing temperature. Significant correlations between chlorophyll a and ATP, protein and carbohydrate (Table 5) indicate that the latter variables are present in large concentrations in plant material, although this gives no indication of the nature of this material.

While the results in Table 4 have indicated that nitrate has an influence on the biochemical composition of phytoplankton, the simple correlations give no indication of the importance of this in the presence of the other environmental variables. To test this, stepwise multiple regressions were performed on each biochemical variable with the environmental variables. The results presented in Table 9 show that nitrate gave a higher partial correlation coefficient with chlorophyll a, protein and carbohydrate than the other environmental variables. For ATP, silicate gave the highest partial correlation coefficient, but the coefficient for nitrate was significant. It may be noted that the nitrate partial correlation coefficients are negative and the silicate coefficients are positive. A possible explanation for this is that, as the concentrations of phytoplankton increased, nitrate was utilised at a faster rate than silicate resulting in different signs for the respective correlations in the multiple regression.

The fact that nitrate gave higher correlations with chlorophyll a than the other nutrients indicated that nitrate is important in determining the upper limit of phytoplankton biomass in the local upwelling region, complementing the findings of Andrews & Hutchings (1980). Bruno *et al*. (1980) also concluded that nitrate had a similar control on phytoplankton growth in the Peconic Bay Estuary, New York. The highly significant correlation between nitrate and carbohydrate (-0.851, Table 4) suggests further that low nitrate concentrations induce an increase in the production of carbohydrate. In theory, carbohydrate biosynthesis is not directly linked to nitrate availability since this nutrient is incorporated into the amino acids of the protein fraction. At low nitrate concentrations net primary production decreases and the continued assimilation of CO_2 is channelled into the carbohydrate fraction.

Growth phases

Very high concentrations of glucan and total carbohydrate were measured in Type 3 water, but protein concentration did not vary significantly between Type 2 and Type 3 water (Figs. 4 and 6, and Table 7). Consequently, the protein/carbohydrate ratio showed a distinctive variation between these water types (Figs. 5 and 7, and Table 7). The glucan/ATP and glucan/chlorophyll ratios varied widely when estimated for single communities (Figs. 5 and 7) and it appears that these two ratios are not useful in assessing physiological change. However, when the mean value for all communities is estimated (Table 7), these ratios increase as the water changes from Type 1 and 2 to Type 3.

Variations in the protein and carbohydrate composition of diatoms due to nutrient depletion have been indicated by other studies (Antia *et al.*,1963; Myklestad & Haug, 1972; Haug *et al.*, 1973). The protein/carbohydrate ratio could not be used as a physiological index for dinoflagellates (Sakshaug *et al.*, (1973) but the findings in this study indicate that this ratio can be used for assessing microflagellate communities. The results in Fig. 6 show that the protein concentration is higher than the carbohydrate concentration in Type 1 and Type 2 water, but is less than the carbohydrate concentration in Type 3 water. The actual concentrations of protein and carbohydrate in the microflagellate communities were lower than in the diatom communities even though the ATP and chlorophyll *a* concentrations were similar in both communities (cf. Figs. 4 and 6).

When adequate nutrients were available (Type 2 water) a protein/carbohydrate ratio of >1 indicated an active phase of growth (Haug *et al.*, 1973), while in Type 3 water, when nitrate levels were low, a ratio of <1 appeared to indicate a senescent phase of growth as found in culture studies (Myklestad & Haug, 1972; Myklestad, 1974; Myklestad, 1977). High concentrations of chlorophyll *a* and ATP were measured in Type 3 water (Figs. 4 and 6) although Jensen & Sakshaug (1973), Sakshaug (1977) and Sakshaug & Holm-Hansen (1977) have shown that chlorophyll *a* and ATP concentrations should decrease at low nutrient concentrations when senescence is reached. It appears that local phytoplankton populations had adequate intracellular reserves of nitrogen to maintain growth even though the protein/carbohydrate ratio indicated a change in physiological state.

It may be concluded that the communities were in a slow-growing phase in Type 3 water, intermediate between the active and senescent phases. On no occasions were low chlorophyll a and ATP concentrations measured in Type 3 water, thus the local phytoplankton communities did not reach a senescent phase of growth. Alternation between the active and slow-growing phases is the most likely growth pattern to be exhibited by phytoplankton in the local upwelling region during the summer, as frequent upwelling pulses maintain a regular supply of nutrients in the euphotic zone (Andrews & Hutchings, 1980).

The accumulation of carbohydrate in local communities was due mainly to the increase in the concentration of acid-soluble glucan (Tables 7 and 8). This has been shown to be a general characteristic of diatoms, as concluded from culture studies on nine different diatom species (Myklestad, 1974). However, marked differences were observed between different species in the production of glucan, Skeletonema costatum for instance accumulating a glucan content of 81% of organic dry matter. This diatom also exhibited a high growth rate (1.7 div.d^{-1}) and was able to divide under conditions of very low nutrient levels in culture. Skeletonema costatum often dominates local communities (Table 3; D. Horstman, pers. comm.) and this may be due to the diatom being able to rapidly accumulate adequate intracellular reserves of nitrogen and energy.

Phytoplankton and detritus

An important consideration in plankton studies is the contribution of detritus and bacteria to the total biomass. Lancelot-Van Beveren (1980) has determined that they can contribute as much as 50% to the total biomass in the southern bight of the North Sea. Investigations of bacteria by Mazure (1978) have indicated that a clear relation between the cycle of bacterial populations and the cycle of phytoplankton standing stock exists on the west coast of the Cape Peninsula.

The gross composition of phytoplankton on the one hand, and bacteria and detritus on the other, were evaluated statistically by Lancelot-Van Beveren (1980) using a regression analysis of the biochemical variables on chlorophyll a. The slope of the linear regression gives an estimate of the biochemical relationships in phytoplankton, while the Y-intercept estimates the average organic matter in detritus and non-phytoplanktonic living micro-organisms. However, chlorophyll a measurements include active and inactive forms of the pigment and the results of Lancelot-Van Beveren (1980) are, therefore, an estimate of the biochemical composition of living phytoplankton and fresh plant detritus together.

TABLE 10

Regression equations and correlation coefficients (*r*) of ATP, chlorophyll *a*, protein and carbohydrate on glucan for three types of water. Concentrations of biochemical variables are in μg.ℓ^{-1} (*n* = number of observations; *P* = significance level)

Water type	Regression equation (Y = c + bX)	*r*	*n*	*P*
1	ATP = 0.021 + 0.013 Glu	0.600	42	<0.01
	Chl *a* = 0.24 + 0.04 Glu	0.759	42	<0.01
	Prot = 45.7 + 2.63 Glu	0.771	42	<0.01
	Carbo = 18.19 + 1.54 Glu	0.789	42	<0.01
2	ATP = 0.398 + 0.002 Glu	0.503	138	<0.01
	Chl *a* = 3.48 + 0.04 Glu	0.650	138	<0.01
	Prot = 247.85 + 1.77 Glu	0.700	138	<0.01
	Carbo = 57.87 + 1.32 Glu	0.960	138	<0.01
3	ATP = 0.828 - 0.0001 Glu	-0.278	32	>0.05
	Chl *a* = 15.41 - 0.0006 Glu	-0.040	32	>0.05
	Prot = 389.5 + 0.25 Glu	0.719	32	<0.01
	Carbo = 179.32 + 1.00 Glu	0.996	32	<0.01

TABLE 11

Estimated percentages of ATP, chlorophyll *a*, protein and carbohydrate in living phytoplankton and bacteria/detritus for three types of water in the Cape Peninsula upwelling region. Estimates for living phytoplankton were made using regression coefficients (b) of Table 10 and expressed as a percentage of the mean total amounts of Table 7. Detrital percentages were estimated by difference; see text for example of calculations

Water type	% in living phytoplankton				% in bacteria/detritus			
	ATP	Chl *a*	Prot	Carbo	ATP	Chl *a*	Prot	Carbo
1	83.2	56.0	34.6	43.8	16.8	44.0	65.4	56.2
2	35.0	53.3	41.5	69.5	65.0	46.7	58.5	30.5
3			32.3	80.5			67.7	19.5

A method of separating the influence of detritus is to use a variable that is present in living phytoplankton, but not associated with detrital matter. Glucan is an important cell constituent that fulfils these requirements because of its biochemical properties and solubility in sea water (Myklestad, 1978). In this study the regression analysis of biochemical variables on glucan gave the regression equations presented in Table 10, and the regression coefficients (b) were used to estimate the composition of living phytoplankton (Table 11). For example, the level of ATP in living phytoplankton in Type 1 water (83.2%, Table 11) was estimated by multiplying the regression coefficient for ATP on glucan (0.013, Table 10) by the mean concentration of glucan in all Type 1 communities (9.2, Table 7), and expressing the result as a percentage of the mean ATP value (0.143, Table 7). The level of ATP in bacteria/detritus (16.8%, Table 11) was estimated as the difference between the total value and the estimated level in living phytoplankton, expressed as a percentage. The other results in Table 11 were estimated from the appropriate regression coefficients (Table 10) and the mean concentrations of the biochemical variables (Table 7) in the same way.

The results in Table 11 show that the bacteria/detritus fraction contains a significant proportion of the total amount of ATP, chlorophyll a, protein and carbohydrate. The percentage of ATP in living phytoplankton is high in Type 1 water but the high percentage of ATP in the bacteria/detritus fraction in Type 2 water indicates that a large bacterial population is associated with the phytoplankton standing stock. This complements the findings of Mazure (1978) that bacterioplankton density is related to phytoplankton density. Although the percentage of chlorophyll a is $>$50% in living phytoplankton, the large percentage in the bacteria/detritus fraction suggests that a considerable amount of plant detritus is present in local waters, an expected result for an upwelling region. In all three types of water the percentage of protein in the bacteria/detritus fraction is significantly greater than in living phytoplankton, and it appears that bacteria and detritus may be important sources of protein for other members of marine food chains. The percentage of carbohydrate in living phytoplankton increases as the water type matures from Type 1 and Type 2 to Type 3. This increase may be attributed to the rapid production of glucan (Tables 7 and 8), ensuring adequate intracellular reserves of energy for the cells to survive limiting nutrient conditions.

Conclusions

The protein/carbohydrate ratio appears to be the most useful indicator for assessing physiological change in local phytoplankton communities. The chlorophyll/ATP ratio was, however, very variable and not useful. The glucan/ATP and glucan/chlorophyll ratios can be used to assess the overall effect of nutrient supply on local communities, but due to their wide range in values they are of limited use in assessing the state of individual communities. Variation in the value of these ratios is due primarily to the accumulation of glucan. Since glucan is only associated with living cells it is an important variable for distinguishing living from detrital components in natural phytoplankton communities. This may have considerable application in the study of natural food chains in upwelling environments.

It may be concluded that when the protein/carbohydrate ratio is >1 and nutrient availability is not limiting, the communities are in an active phase of growth. Under these conditions the glucan/ATP and glucan/chlorophyll ratios are low. At low nitrate levels the glucan concentration increases with a concomitant decrease in the protein/carbohydrate ratio to <1, indicating that the community changes to a slow-growing phase. In this phase the glucan/ATP and glucan/chlorophyll ratios are high. The proposed hypothesis is, therefore, valid for the protein/carbohydrate, glucan/ATP and glucan/chlorophyll ratios. However, the results in Table 7 indicate that the hypothesis concerning the chlorophyll/ATP ratio is invalid since this ratio increases in Type 3 water when the communities are in a slow-growing phase. If the communities reach a senescent phase after prolonged exposure to low nitrate levels then it is likely that low chlorophyll/ATP ratios would be recorded when the chlorophyll a and ATP concentrations decrease (Sakshaug, 1977; Sakshaug & Holm-Hansen, 1977).

CHAPTER 4

PATTERNS OF CARBON-14 ASSIMILATION IN PHYTOPLANKTON COMMUNITIES

The investigations described in the previous chapter has shown that variations in nitrate concentrations induced significant physiological changes in local phytoplankton communities, but the role of light in influencing the biochemical composition was not established. It must, however, be noted that dead and living matter could not be distinguished. An attempt was made to determine the composition of living phytoplankton using a regression analysis of particular biochemical variables on glucan, a specific component of living phytoplankton cells. While some important results were obtained a more sensitive approach is needed to study physiological change.

An appropriate technique is to follow the pattern of carbon-14 assimilation into the major end-products of photosynthesis. Investigations of this nature have been undertaken on natural populations near Vancouver (Wallen & Geen, 1971b), in the Gulf of Maine (Morris & Skea, 1978), in the Southern Ocean (Smith & Morris, 1980a,b) and in the Caribbean Sea (Li et al., 1980). Variations in light intensity, nutrient availability and temperature were found to have an effect on the distribution of carbon-14 between various biochemical components.

Light is an important environmental variable influencing the rate of CO_2 fixation. At high light intensities, for example, additional synthesis of reserve polysaccharide usually occurs after other cell components have reached their maximum synthetic rates (Raven, 1974). Inorganic nitrogen is another variable that has an effect on the carbon pathway. When adequate nitrogen is available the flow of carbon into nitrogenous substances increases, at the expense of carbon assimilation into reserve polysaccharide (Raven, 1974). It appears that the light regime and the availability of inorganic nitrate have important influences on the carbon metabolism of phytoplankton cells.

Protein and carbohydrate were found to be important biochemical variables for assessing the physiological state of local communities (Chapter 3). To complement these studies the investigations reported in this Chapter were undertaken to determine the effects of light and nitrate concentration on the assimilation of carbon into protein and polysaccharide fractions.

TABLE 12

Total carbon-14 activity in 1ℓ surface water samples incubated at 100% light intensity with different amounts of radioactive bicarbonate for 4 hours

Amount of ^{14}C-bicarbonate (μCi.ℓ$^{-1}$)	Total activity (DPM.μg Chlorophyll a^{-1})
10	5087
10	5186
25	10344
25	9932
50	21936
50	21688
100	44367
100	45424

TABLE 13

Total radioactivity and distribution between fractions for 1ℓ water samples incubated at 1% light intensity with 100 μCi and 50 μCi ^{14}C-bicarbonate for 4 hours

Experiment no.	Amount of carbon-14 (μCi.ℓ$^{-1}$)	Total activity (DPM.μg Chl a^{-1})	Percentage carbon-14 in		
			Ethanol-sol. fraction	TCA-sol. fraction	TCA-insol. fraction
A	100	2164	23.9	40.9	35.2
	50	1690	26.7	39.0	34.3
B	100	2546	44.2	31.2	24.6
	50	2470	42.9	33.9	23.2

TABLE 14

Total carbon-14 activity determined directly and as the sum of the 3 fractions. Surface water samples (1ℓ) were incubated at 100% light intensity with 50 μCi of ^{14}C-bicarbonate for 4 hours

Experiment no.	Measured directly (DPM.μg Chl a^{-1})	Sum of the 3 fractions (DPM.μg Chl a^{-1})	Sum of the fractions/ Direct measure
C	40757	41928	102.8%
D	16239	15115	93.1%
E	88003	91596	104.1%
F	85441	91945	107.6%

METHODS

Experiments were undertaken at the Oudekraal and Robben Island stations during the 1979-80 summer season. Field work was conducted between 0930 and 1530 hours on selected days during the months of October, January, February and March. This sampling strategy resulted in experiments being performed on different water bodies as they moved past these stations.

Each day, light and temperature measurements were made initially and then samples were drawn from various depths within the euphotic zone for incubation with ^{14}C-bicarbonate, and analysis of nutrients, total chlorophyll a and species composition. The sampling procedure and methods of analysis that were used have been outlined in Chapter 2 and detailed in Appendix 2 (chlorophyll a) and Appendix 5 (carbon-14 assimilation).

RESULTS

Preliminary experiments were undertaken to determine the appropriate amount of radioactive bicarbonate to be used in incubations, and to compare the total activity measured directly with that calculated as the sum of the activities in each of the three fractions. Different amounts of radioactive bicarbonate (10, 25, 50 and 100 µCi) were incubated with 1ℓ surface water samples at 100% light intensity for 4 hours. The results presented in Table 12 reveal that samples incubated with 100 µCi of carbon-14 gave the highest activity in these experiments. However, incubations performed at 1% light intensities showed only small differences in activity between samples incubated with 100 or 50 µCi of ^{14}C-bicarbonate (Table 13). It was decided, therefore, that 50 µCi of ^{14}C-bicarbonate per 1ℓ of water sample was an adequate amount of the label to use in all experiments. Recovery values of between 93 and 108% were observed when total radioactivity, determined as the sum of the three fractions, was compared to that measured directly (Table 14).

Twenty-one experiments were performed under a variety of environmental conditions on communities dominated by three different species. The environmental conditions and dominant species prevailing during the experiments are presented in Tables 15 and 16. The water types encountered in this study were classified according to the criteria in Table 2. The dominant species accounted for 50% or more of the total number of species and Rhizosolenia sp. was either Rhizosolenia delicatula or Rhizosolenia fragilissima. Due to poor preservation of these cells it was difficult

TABLE 15

Environmental conditions and dominant species for investigations of the time-series (experiments 1 - 4) and depth profiles (experiments 5 - 9) assimilations of carbon-14

Expt. no.	Depth (m)	Light (% of surface intensity)	Temp. (°C)	Nitrate (μg-at NO_3-N.ℓ^{-1})	Phosphate (μg-at PO_4-P.ℓ^{-1})	Silicate (μg-at SiO_3-Si.ℓ^{-1})	Water Type	Dominant Species
1	0	100	14.3	5.26	3.28	16.07	2	Microflagellates
2	0	100	13.8	6.73	1.82	13.10	2	Microflagellates
3	0	100	14.2	5.32	2.51	25.73	2	Microflagellates
4	0	100	14.2	1.51	1.52	16.74	3	Microflagellates
5	0	100	13.0	8.41	1.15	6.03	2	Rhizosolenia sp.
	2.5	50	12.9	3.26	1.45	6.35	2	
	5.5	25	12.9	7.09	1.35	6.67	2	
	8.5	10	12.7	5.15	1.32	4.44	2	
	17.5	1	12.2	8.75	1.65	8.04	2	
6	0	100	13.2	2.75	1.31	6.49	2	Rhizosolenia sp.
	1.5	50	13.2	4.29	1.10	5.88	2	
	3	25	13.2	3.49	1.34	4.64	2	
	5.5	10	13.2	5.32	1.37	6.49	2	
	11	1	13.1	4.97	1.41	6.49	2	
7	0	100	14.6	0.24	1.26	1.97	3	Rhizosolenia sp.
	1.5	50	14.3	0.28	0.99	1.97	3	
	3	25	14.6	0.38	1.30	1.97	3	
	5.5	10	12.5	0.47	1.42	2.63	3	
	11	1	11.6	12.90	2.41	11.84	2	
8	0	100	14.6	0.44	1.14	2.03	3	Rhizosolenia sp.
	1	50	14.6	0.33	1.18	1.32	3	
	2.5	25	14.6	0.28	1.30	1.32	3	
	4	10	14.5	0.33	1.18	1.32	3	
	8	1	14.4	0.23	1.18	1.32	3	
9	0	100	15.8	0.48	0.95	1.97	3	Rhizosolenia sp.
	2	50	15.8	0.58	0.82	1.97	3	
	3.5	25	15.8	0.34	1.08	1.97	3	
	6	10	15.7	0.19	0.85	1.97	3	
	12	1	12.2	0.68	0.91	1.32	3	

TABLE 16

Environmental conditions and dominant species for investigations of the effect of light intensity on carbon-14 assimilation (experiments 10 - 21)

Expt. no.	Depth (m)	Light (% of surface intensity)	Temp. (°C)	Nitrate (μg-at NO_3-N.ℓ^{-1})	Phosphate (μg-at PO_4-P.ℓ^{-1})	Silicate (μg-at SiO_3-Si.ℓ^{-1})	Water Type	Dominant Species
10	3	50	10.4	21.13	2.67	22.88	1	Coscinodiscus sp.
	17.5	1	9.1	27.03	3.65	26.99	1	
11	6	50	10.2	22.46	2.56	16.32	1	Coscinodiscus sp.
	36	1	9.2	24.16	3.71	28.87	1	
12	2.5	50	11.4	3.52	1.38	8.33	2	Rhizosolenia sp.
	16	1	9.4	26.53	2.64	19.04	1	
13	1.5	50	12.2	15.07	2.16	24.18	2	Microflagellates
	11	1	9.9	24.58	2.68	27.89	1	
14	2	50	12.1	17.80	2.40	17.57	2	Microflagellates
	12	1	11.0	20.78	2.18	18.20	2	
15	1	50	13.9	5.24	1.91	20.71	2	Mircoflagellates
	6.5	1	13.1	8.32	2.56	23.22	2	
16	1	50	14.4	5.03	1.85	20.71	2	Microflagellates
	6.5	1	12.8	9.86	2.24	21.34	2	
17	2	50	12.7	3.86	1.42	1.20	2	Rhizosolenia sp.
	13.5	1	12.1	4.51	1.78	1.45	2	
18	1.5	50	13.2	2.77	1.34	1.08	2	Rhizosolenia sp.
	11	1	12.5	4.73	1.77	1.45	2	
19	1.5	50	13.8	1.94	1.03	14.31	2	Rhizosolenia sp.
	11	1	12.3	5.31	1.82	15.48	2	
20	1	50	14.3	1.20	1.26	1.02	3	Rhizosolenia sp.
	7	1	12.5	3.98	1.78	15.48	2	
21	1	50	12.3	0.71	1.22	1.14	3	Rhizosolenia sp.
	5.5	1	12.1	0.92	1.02	0.96	3	

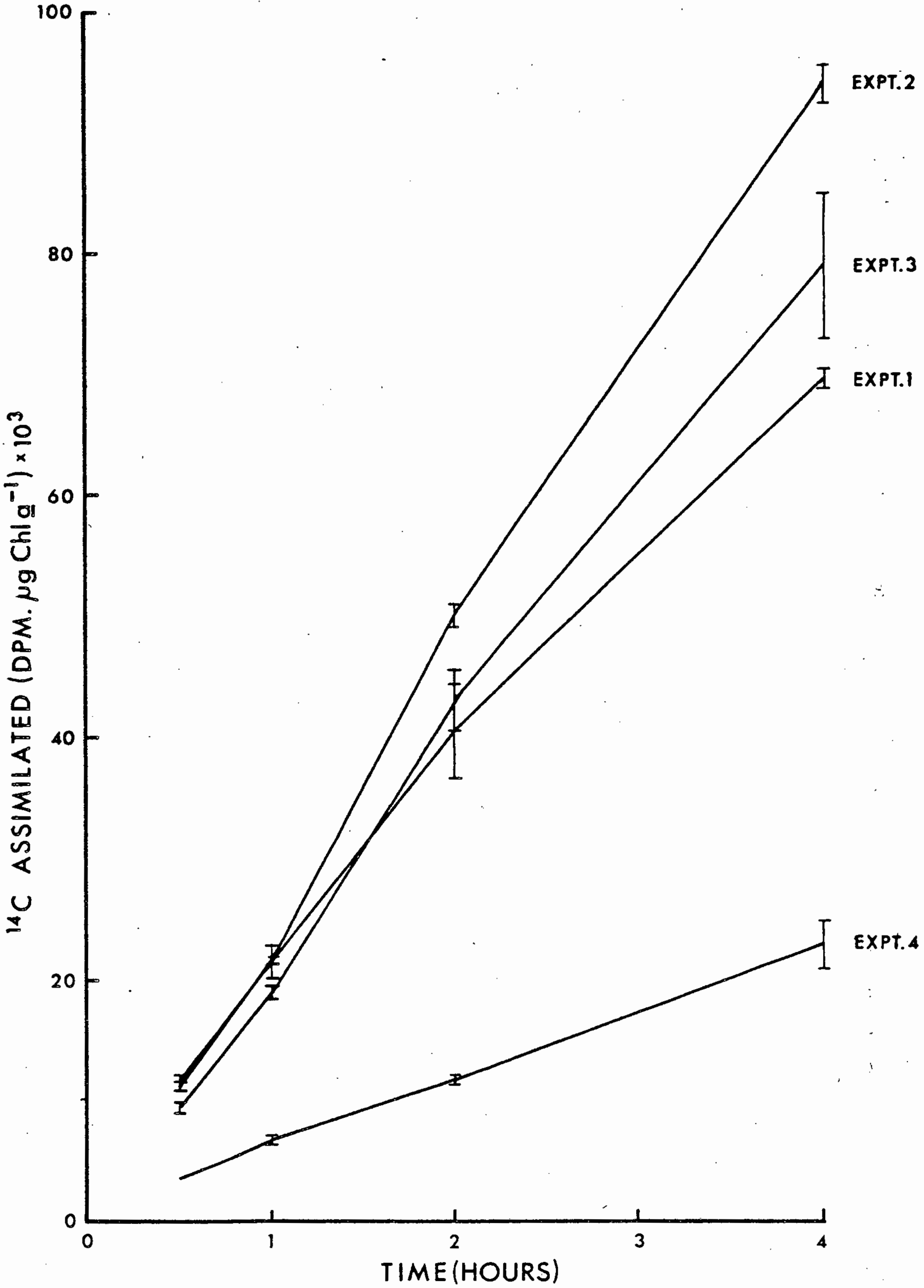


Fig. 8. Time-series assimilation of carbon-14 when 12 replicate 1ℓ samples of surface water were each incubated with 50µCi of ^{14}C-bicarbonate at 100% light intensities. Six replicate 0.5ℓ aliquots were removed for analysis after each incubation period. Vertical bars represent twice the standard error. Experiments 1, 2 and 3 were performed in Type 2 water and experiment 4 in Type 3 water.

to distinguish clearly between these two species.

Time-series experiments

While 4-hour incubation periods were used in the preliminary experiments described above it had to be established whether this was a suitable period. Time-series experiments were, therefore, performed at 100% light intensities on microflagellate communities. The results in Fig. 8 show that the rate of ^{14}C-bicarbonate assimilation increased approximately linearly between 0.5 and 4 hours. The assimilation rate of carbon-14 by the community in experiment 4 was much lower than in the other experiments; this may be due to the fact that the experiment was conducted in Type 3 water containing low concentrations of nitrate.

The distribution of carbon-14 between the various fractions showed no significant variation with increasing time of incubation in all experiments (Table 17). This finding and the linear increase in activity to 4 hours confirmed that a 4-hour incubation was suitable for carbon-14 assimilation studies.

Depth profiles of carbon-14 assimilation

Brown (1980) has used carbon-14 to measure primary productivity in a nearshore region off the Cape Peninsula and found that production was greater in the upper than in the lower photic zone. Light intensity decreases with increasing depth and simulated *in situ* depth profile experiments were performed to determine the pattern of carbon-14 assimilation in samples drawn from within the euphotic zone.

Total assimilation of carbon-14 by phytoplankton was greater in the upper layers of the euphotic zone than at the bottom, irrespective of whether there were adequate or low concentrations of nitrate available (Figs. 9 and 10). Very low rates of assimilation were measured at depths corresponding to the 1% light level. Maximum assimilation occurred at depths corresponding to the 50% or 25% light intensity level in experiments 5, 7 and 9, while in experiments 6 and 8 maximum assimilation was measured at the surface. In the former experiments photoinhibition may have reduced the rate at which carbon-14 was assimilated at the surface.

The total assimilation rate was paralleled closely by the incorporation of the label into the TCA-soluble fraction, where the percentage incorporated decreased with increasing depth. The pattern of incorporation into the TCA-insoluble fraction was the converse of that in the TCA-soluble fraction,

TABLE 17

Chlorophyll a concentrations and patterns of carbon-14 distribution for the time-series assimilation of carbon-14

Experiment no.	Water type	Chlorophyll a ($\mu g.\ell^{-1}$)	Incubation time (h)	Total activity (DPM. μg Chl a^{-1}) x 10^3	Percentage carbon-14 in		
					Ethanol-sol. fraction	TCA-sol. fraction	TCA-insol. fraction
1	2	1.24	0.5	11.5	28.8	34.8	36.2
			1	21.5	27.4	37.2	35.2
			2	40.6	26.4	35.2	38.2
			4	69.7	25.7	31.8	42.3
2	2	3.36	0.5	11.2	27.3	40.8	31.7
			1	21.7	26.8	40.8	32.2
			2	50.1	26.2	40.3	32.5
			4	93.9	26.0	39.1	34.7
3	2	19.46	0.5	9.5	31.1	41.3	27.3
			1	19.1	29.8	40.3	29.8
			2	43.2	27.7	39.5	32.6
			4	79.1	28.0	39.9	31.9
4	3	21.20	0.5	3.5	35.0	41.9	22.9
			1	6.8	34.0	41.2	24.6
			2	11.8	33.0	44.8	22.4
			4	22.9	34.9	41.9	23.1

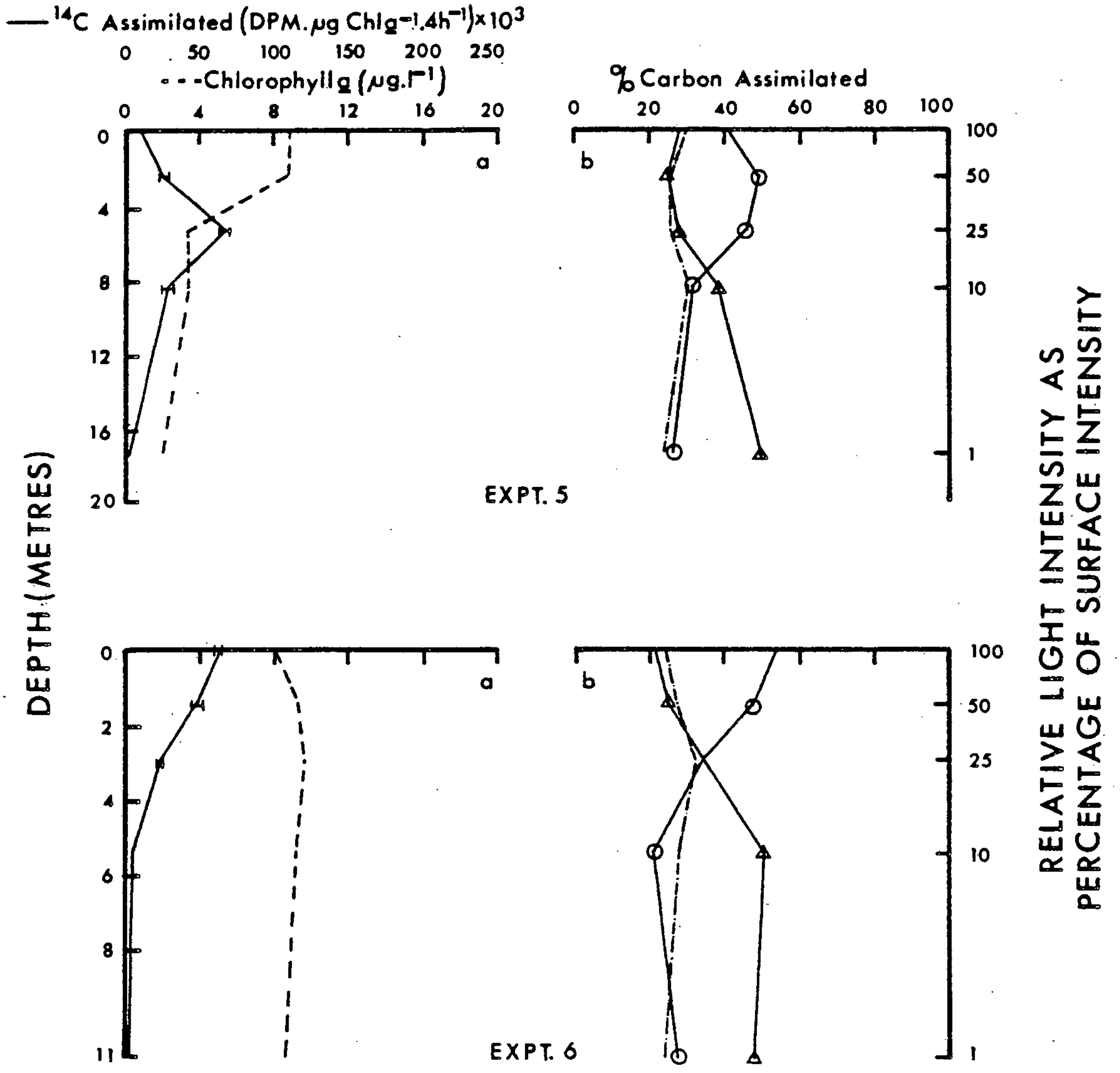


Fig. 9. (a) Depth profiles of chlorophyll a concentrations and assimilations of carbon-14 in Type 2 water. Assimilation results are means of two replicate incubations and horizontal bars indicate twice the standard error. (b) Relative incorporation into ethanol-soluble (–·–·–), TCA-soluble (○) and TCA-insoluble (△) fractions.

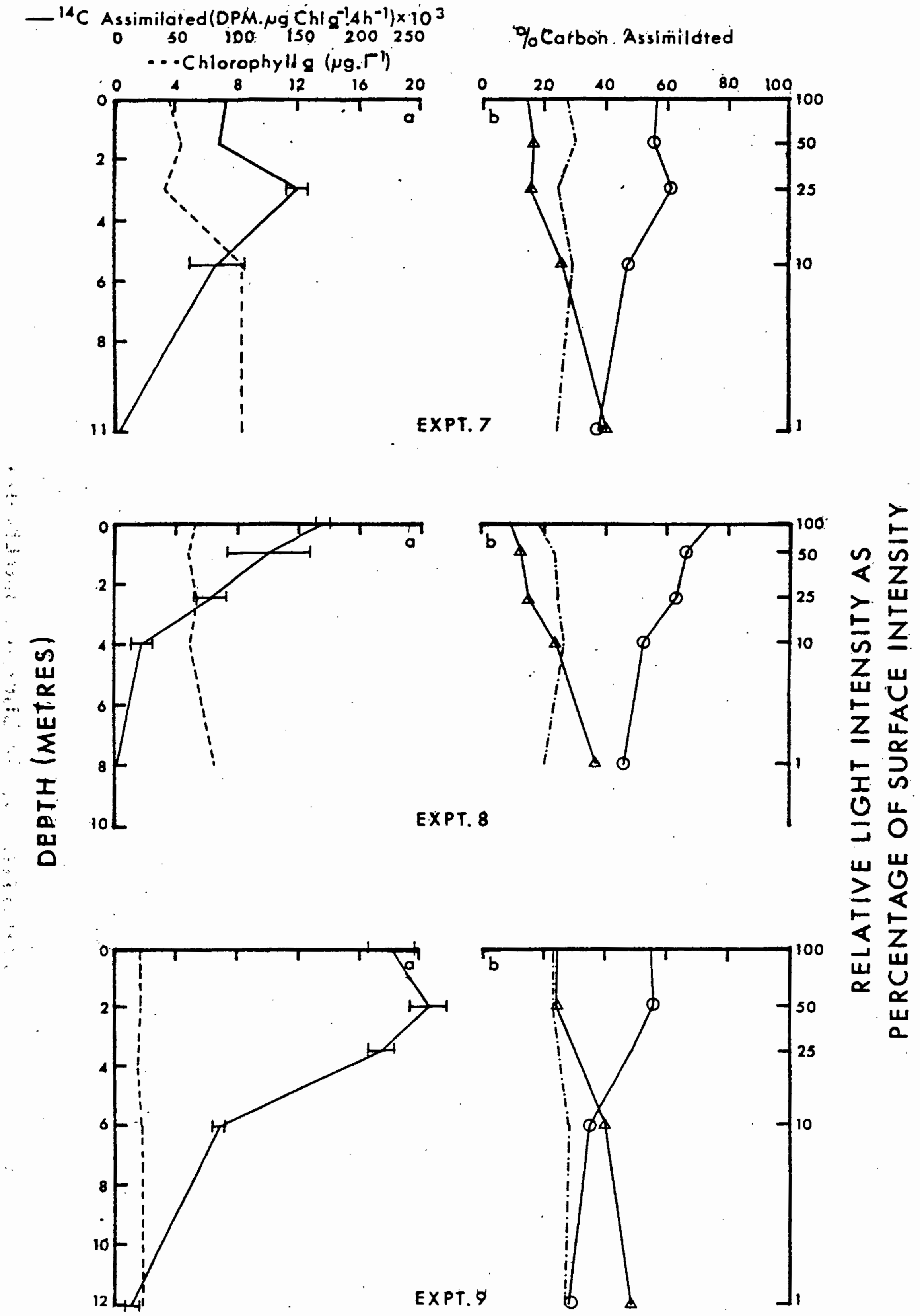


Fig. 10. (a) Depth profiles of chlorophyll a concentrations and assimilations of carbon-14 in Type 3 water. Assimilation results are means of two replicate incubations and horizontal bars indicate twice the standard error.
(b) Relative incorporation into ethanol-soluble (-·-·-·-), TCA-soluble (○) and TCA-insoluble (△) fractions.

TABLE 18

The pattern of carbon-14 assimilation when 1ℓ water samples, drawn from depths corresponding to the 50% and 1% light levels, were incubated at 50% and 1% light intensities for 4 hours. Results are the means of 4 replicate incubations ± standard errors of the mean in paranthesis

Water type	Expt. no.	Light (% of surface intensity)	Chlorophyll a ($\mu g.\ell^{-1}$)	Total assimilation (DPM.μg Chl a^{-1}) x 10^3	Percentage carbon-14 in		
					Ethanol-sol. fraction	TCA-sol. fraction	TCA-insol. fraction
1	10	50	1.49	94.4 (8.3)	24.7 (0.1)	36.9 (1.0)	38.2 (1.0)
		1	0.62	3.2 (0.8)	35.4 (5.6)	32.4 (3.1)	32.0 (8.0)
	11	50	0.50	150.5 (10.8)	23.2 (0.4)	39.6 (0.7)	36.9 (0.4)
		1	0.74	1.2 (0.2)	34.6 (1.0)	31.8 (0.8)	33.5 (0.6)
	12	1	1.00	2.3 (0.2)	39.5 (3.2)	30.1 (2.7)	30.3 (1.5)
	13	1	1.62	2.5 (0.1)	44.2 (2.2)	25.8 (0.1)	29.8 (2.0)
2	12	50	11.21	68.4 (1.6)	25.3 (0.3)	48.9 (0.3)	25.6 (0.1)
	13	50	5.23	140.5 (3.9)	29.2 (0.6)	40.7 (0.9)	29.9 (0.3)
	14	50	4.85	115.9 (5.2)	25.5 (0.3)	44.9 (0.6)	29.4 (0.2)
		1	6.47	0.8 (0.1)	30.4 (0.7)	28.3 (0.7)	41.1 (1.2)
	15	50	14.45	87.3 (1.5)	29.3 (0.4)	39.4 (0.7)	31.1 (0.3)
		1	13.45	1.0 (0.6)	28.3 (1.2)	31.0 (1.6)	40.5 (1.2)
	16	50	16.69	72.7 (2.9)	31.6 (0.9)	35.3 (1.3)	33.0 (0.4)
		1	10.64	0.7 (0.1)	29.4 (2.8)	30.3 (1.6)	40.2 (1.2)
	17	50	4.73	25.9 (0.5)	19.1 (0.3)	43.9 (0.8)	36.7 (0.5)
		1	15.32	0.8 (0.2)	18.8 (0.7)	40.9 (0.8)	40.1 (1.0)
	18	50	9.19	50.1 (0.6)	20.7 (0.2)	51.6 (0.4)	27.5 (0.2)
		1	12.92	0.9 (0.2)	25.6 (2.1)	37.8 (0.7)	36.4 (1.6)
	19	50	6.83	77.2 (4.6)	23.4 (1.0)	48.5 (2.9)	27.9 (1.9)
		1	6.46	1.2 (0.1)	24.0 (0.2)	39.2 (0.1)	36.6 (0.3)
	20	1	7.45	0.9 (0.1)	25.8 (5.2)	34.5 (5.6)	39.5 (5.2)
3	20	50	10.68	35.9 (1.8)	19.6 (0.3)	51.7 (1.7)	28.4 (1.5)
	21	50	27.40	41.0 (5.4)	23.3 (1.7)	60.1 (2.9)	16.4 (1.1)
		1	27.65	1.9 (0.1)	31.7 (1.5)	27.9 (3.2)	40.2 (1.7)

while there was no marked change in assimilation into the ethanol-soluble fraction down the water column.

The most significant observation in these experiments was the change in the distribution of carbon-14 between the TCA fractions with a change in depth. In the upper euphotic zone a greater proportion of the label was incorporated into the TCA-soluble fraction; 35 - 54% in Type 2 water ($\underline{n}$=6) and 48 - 74% in Type 3 water ($\underline{n}$=6). At the bottom of the euphotic zone (1% light depth) 36 - 50% was incorporated into the TCA-insoluble fraction ($\underline{n}$=5).

Effect of light intensity on carbon-14 assimilation

Due to marked differences observed in the pattern of carbon-14 incorporation between samples from the upper and lower layers of the euphotic zone, further experiments were performed to examine the effects of light intensity under conditions of varying nutrient availability. The 50% and 1% light levels were selected as suitable contrasting intensities and 12 experiments were conducted in various types of water.

A high rate of total carbon-14 assimilation at the 50% light level was found in Type 1 water dominated by Coscinodiscus species (experiments 10 and 11, Table 18) and in Type 2 water dominated by microflagellates (experiments 13 - 16, Table 18). In both Type 2 water (experiments 12 and 17 - 19, Table 18) and Type 3 water (experiments 20 and 21, Table 18) a variable rate of assimilation of carbon-14 was found in Rhizosolenia communities at the 50% light level. At the 1% light level a very marked decrease in the total assimilation of carbon-14 was observed in all experiments. The highest activity at this light level was 3.2 x 10^3 DPM.µg chlorophyll $\underline{a}^{-1}$, while the highest activity at the 50% light level was 150.5 x 10^3 DPM.µg chlorophyll $\underline{a}^{-1}$ after four hours.

The distribution of carbon-14 between the separated fractions generally followed the pattern observed in experiments 5 - 9. At the 50% light level a greater proportion of the label was incorporated into the TCA-soluble fraction, while a low light intensity (1%) favoured an increased incorporation of carbon-14 into the TCA-insoluble fraction. However, there were some differences between experiments depending on the type of water in which they were performed.

In Type 1 water (Table 18) there was an approximately equal distribution of carbon-14 between the two TCA fractions at both the 50% and 1%

light levels. But the percentage of incorporation varied between 37 and 40% in each fraction at the 50% light level and between 26 and 33% in each fraction at the 1% light level. The amount of carbon-14 in the ethanol-soluble fraction was, consequently, smaller at the 50% light level (23 - 25%) than at the 1% light level (35 - 44%).

A large uptake of carbon-14 into the TCA-soluble fraction at the 50% light level was a general feature in all the experiments in Type 2 water (Table 18). Percentage incorporation varied between 35 and 52%. The percentage of the label in the TCA-insoluble fraction was higher at the 1% light level (37 - 42%) than at the 50% light level (26 - 37%), although the level of incorporation did not always exceed that determined in the TCA-soluble fraction. Incorporation into the ethanol-soluble fraction did not show any notable differences between light levels or between water types and varied between 19 and 32%.

A particularly large uptake of carbon-14 into the TCA-soluble fraction (52 - 60%) was exhibited by phytoplankton in Type 3 water at the 50% light level (Table 18). Consequently, the amount detected in the TCA-insoluble fraction (16 - 28%) and in the ethanol-soluble fraction (20 - 23%) was small. At the 1% light level a greater percentage of the label was detected in the TCA-insoluble fraction (40%) than in the TCA-soluble (28%) and ethanol-soluble (32%) fractions.

DISCUSSION

In all experiments the assimilation of carbon-14 has been shown to vary significantly in its distribution between the TCA-soluble and TCA-insoluble fractions. Morris et al. (1974) demonstrated that most of the label in each fraction was incorporated into specific macromolecules, and thus these fractions in this discussion will be referred to in terms of the particular molecules. The TCA-soluble fraction can be regarded as the polysaccharide fraction and the TCA-insoluble fraction as protein. The ethanol-soluble fraction contains small molecular weight metabolites for protein and polysaccharide synthesis, plus some lipids and pigments.

Incubation period

The time-series experiments revealed that a 4-hour incubation period was suitable in these investigations. This period was used in local primary production measurements by Brown (1980) and has been employed by

Morris _et al_. (1974) and Morris & Skea (1978) in their assimilation studies. However, time-series experiments by Li _et al_. (1980)showed that the rate of carbon-14 assimilation was only linear for 2 hours and then decreased to 4 hours. While the distribution of the label between fractions was similar at all time intervals in the present investigations, Li _et al_. (1980) detected a shift of carbon-14 from the metabolite pool to macromolecules when the length of the incubation period was increased from 2 to 4 hours. The differences between these independent experiments may be due to species selection. Li _et al_. (1980) studied _Oscillatoria thiebautti_, a blue-green alga, while the experiments reported here were performed on microflagellate communities.

Effect of light intensity

It is apparent that a change in light intensity markedly affected the assimilation of carbon-14 and its distribution between fractions. In Type 2 and Type 3 water a decrease in light intensity decreased the rate of total assimilation and the incorporation into polysaccharide, but increased the incorporation into protein. In Type 1 water total assimilation decreased with a decrease in light intensity, but incorporation into the protein and polysaccharide fractions was approximately equal.

An increase in the relative rate of protein synthesis at low light intensities was also encountered in the Gulf of Maine (Morris & Skea, 1978) and in the Caribbean Sea (Li _et al_., 1980). But another proposal by Wallen & Geen (1971a,b) suggests that the increased incorporation of carbon-14 into protein may be due to a change in light quality i.e. enhanced protein metabolism is associated with blue-green light. This suggestion was postulated from the results of culture experiments and from incubations performed _in situ_ down the water column. The experiments in the present study were conducted in incubated neutral-density bottles where the effects of a change in light quality would be minimal, but intensity would be reduced. While the effects of light quality on carbon-14 assimilation cannot be discounted, the results presented in Figs. 9 and 10 and Table 18 indicate that a change in light intensity influenced the flow of carbon-14 into the various end-products of photosynthesis. Further evidence for the effects of light intensity is provided by the results of investigations in the Southern Ocean where reduced light intensity caused increased incorporation of carbon-14 into lipids (Smith & Morris, 1980a). This effect was most marked at very low temperatures and 90% of the assimilated carbon-14 was detected in the lipid fraction at a water temperature of-1.0°C and a light intensity 10% of incident (Smith & Morris, 1980b).

The following explanation of the observed effects of light is proposed: At optimum light intensities a certain amount of assimilated carbon and energy is channelled into protein for growth and cell maintenance, while excess is stored in the polysaccharide fraction. At low light intensities assimilation rates are reduced and less excess carbon and energy are available to produce polysaccharides. In Chapter 3 it was shown that the concentration of protein did not increase at depths where low light intensities prevailed (Figs. 4 and 6), therefore the greater incorporation of carbon-14 into protein indicates enhanced turnover of protein to maintain the living cell.

Effect of temperature and nutrients

Other environmental variables such as temperature and the availability of nutrients appear to affect the pattern of carbon-14 fixation. In Type 1 water, when the temperatures were low (10°C or less) and high concentrations of nutrients were available, the distribution of label between protein and polysaccharide was found to be approximately equal. Active growth must have recently commenced under these conditions with the cells producing similar amounts of the macromolecules to maintain rapid growth. At low light intensities, however, most of the carbon-14 was detected in the ethanol-soluble fraction. Since the nutrient supply was not limiting, it appears that a combination of low temperatures (9°C) and low light intensity reduced the rate at which the precursors were utilised to synthesize protein and polysaccharide.

Morris & Skea (1978) detected enhanced protein synthesis at low light intensities in communities from nutrient-rich water, but a less dramatic response was exhibited by communities from water containing low concentrations of inorganic nutrients. In the Caribbean Sea Li et al. (1980) observed a similar response and concluded that at a particular station the community was in a nutritionally poor state when nutrient concentrations were low. In the present study this phenomenon was not observed because the percentage incorporation of carbon-14 into protein at low light intensities is similar in both Type 2 and Type 3 water. It appears that phytoplankton cells in local waters containing low concentrations of nitrate have adequate intracellular reserves of nitrogen to maintain normal metabolic processes and are not in a nutritionally poor state.

The incorporation of carbon-14 into polysaccharide at high light intensities (100% and 50%) did not show differences between samples from Type 2 and Type 3 water. In Type 2 water the percentage of the label detected in the polysaccharide fraction varied between 35 and 54%, while the percentage in this fraction in Type 3 water varied between 52 and 74%. The enchanced synthesis of polysaccharides in low nitrate water complements the results of Chapter 3 in which high concentrations of carbohydrates were measured in Type 3 water. Assimilation studies by Hitchcock (1978) revealed that increased synthesis of ethanol-soluble compounds occurred in diatom populations under conditions of low nutrient availability at high light intensities. Both the findings of the present investigation and those of Hitchcock (1978) are at variance with the results of Morris *et al*. (1974), who observed increased fixation of carbon-14 into protein in a *Phaeodactylum tricornutum* culture grown under conditions of nitrogen deficiency. The differences between these three studies may be due to the conditions of growth in the natural environments differing from those of the culture environment, and/or due to species-specific differences in the labelling patterns of different communities.

Differences between species

The photosynthetic response of different species to changing environmental conditions is an important ecological consideration and some differences were detected between species in this study. In Type 2 water the percentage incorporation of carbon-14 into protein and into polysaccharides at low light intensities was approximately the same for both the diatom and microflagellate communities (Table 18). However, at high light intensities the diatoms assimilated a higher percentage of carbon-14 into polysaccharides (44 - 54%) compared to the microflagellates (35 - 45%). A slightly higher percentage of the label was detected in the protein fraction in microflagellates (29 - 33%) than in the diatoms (22 - 28%). It is apparent that diatoms have a greater capacity for synthesizing reserve polysaccharides than do microflagellates and this may be important for their survival in an upwelling environment that is characterised by a pulsed nutrient supply.

Conclusions

It appears that light is the most important environmental variable influencing the synthesis of macromolecules in local phytoplankton communities. Polysaccharides are synthesized at a faster rate than proteins at high light intensities, but there is an increase in the relative rate of protein synthesis at the expense of carbohydrate synthesis, at low light intensities. Low nitrate concentrations induce an increase in the synthesis of polysaccharides but the rate of protein synthesis appears to be unaffected by a decrease in nitrate supply.

In conclusion the following hypothesis is proposed to explain the effects of light and nitrate concentration on the synthesis of protein and carbohydrate in phytoplankton communities in an upwelling environment:

At high light intensities and high nitrate concentrations, a greater proportion of carbon-14 is assimilated into carbohydrate than into protein, reflecting a faster rate of polysaccharide synthesis. Low light intensity decreases the rate of synthesis of carbohydrate while protein synthesis is maintained. Decreased nitrate availability leads to continued protein synthesis at the expense of carbohydrate synthesis at low light intensity, but enhances the synthesis of carbohydrate at high light intensity.

CHAPTER 5

THE DYNAMICS OF A PHYTOPLANKTON BLOOM

The previous two chapters have described physiological changes in phytoplankton from different bodies of water. Investigations reported in this chapter were designed to study the dynamics of a phytoplankton bloom in a single body of water. The chemical and biological changes that occur in the local upwelling region are large and rapid (Andrews & Hutchings, 1980) and the blooms of phytoplankton that develop may be investigated over short periods of time. *In situ* studies of these blooms may be conducted with some confidence that the same water mass and the same populations are being investigated. By placing a drogue in the water at a particular depth such a water mass may be followed continuously over the course of several days.

Following water masses with the aid of drogues has been conducted with considerable success in biological investigations in other upwelling areas (Ryther *et al*., 1971; Herbland *et al*., 1973; Nelson & Goering, 1978). It is, however, pertinent to consider some of the limitations of drogues. The horizontal shear of water flow, the slippage of water past the drogue and vertical mixing are three important factors that have to be considered when assuming that a drogue is moving with a particular body of water (Hutchings, pers. comm.).

The horizontal shear of water masses is due to surface water moving faster than water below the surface as a result of the effects of wind. If a drogue is placed at a particular depth then the speed of the water mass above the drogue is usually faster than the the water mass in which the drogue is positioned, while the water mass below the drogue moves at a slower speed. It is presumed that the water masses in the vicinity of the drogue all move in the same direction.

Slippage of water past the drogue results when forces act on the drogue to create reduced pressure in front and a high pressure behind the drogue. A drogue can only move with a body of water if these pressures are set up around the drogue. Mixing of surface water to lower depths is a significant phenomenon in upwelling areas due to the effects of strong winds. Therefore a drogue, fixed at a particular depth, cannot account for this vertical mixing when modifications of the water mass may be occurring.

Despite these problems a drogue is a useful and practical means of following a water body for biological research. During December 1979 cold clear water was detected off the Cape Peninsula and a drogue was placed at a depth of 10 metres in a patch of water whose surface temperature was between 10° and 11°C. The drogue was tracked for 4 - 5 days (see Fig. 2, Chapter 2) and at regular intervals the ship was brought close to the drogue for observations and collection of samples. Results of temperature and salinity measurements at 10 metres, species composition and the mean drift of the drogue supported the assumption that the same mass of water was being followed during the course of the study.

The results of the investigations described in Chapters 3 and 4, and a diel study on the pattern of carbon-14 assimilation in natural populations by Morris & Skea (1978), suggest that the following hypothesis may be proposed for physiological changes in a developing phytoplankton bloom in an upwelling region :

During active growth the concentration of protein is greater than the concentration of carbohydrate when sufficient nutrients are available in the euphotic zone. Under these conditions carbohydrate synthesis exceeds protein synthesis when excess CO_2 is assimilated in the upper euphotic zone during the day. At night protein synthesis is conserved using carbohydrate as an energy reserve. When the supply of nitrate is depleted carbohydrate synthesis is enhanced in the euphotic zone, with the concentration of carbohydrate exceeding that of protein.

This hypothesis is tested in the study described in this Chapter.

METHODS

Observations were conducted at approximately 0800, 1300 and 1900 hours each day, except for the first day when no measurements were made at 0800 hours and on the second day when extra measurements were made at 2230 hours. Light and temperature measurements were made initially, and then samples were drawn from five depths in the euphotic zone and at 10 metre intervals beyond the euphotic zone to the bottom for analysis of salinity, nutrients, chlorophyll a and species composition. Samples for the anaylsis of protein and carbohydrate were only drawn from five depths within the euphotic zone. Further samples were drawn from the depth corresponding to the 50% light intensity for incubation with ^{14}C-bicarbonate. The sampling procedure and methods of analysis that were used have been outlined in Chapter 2 and

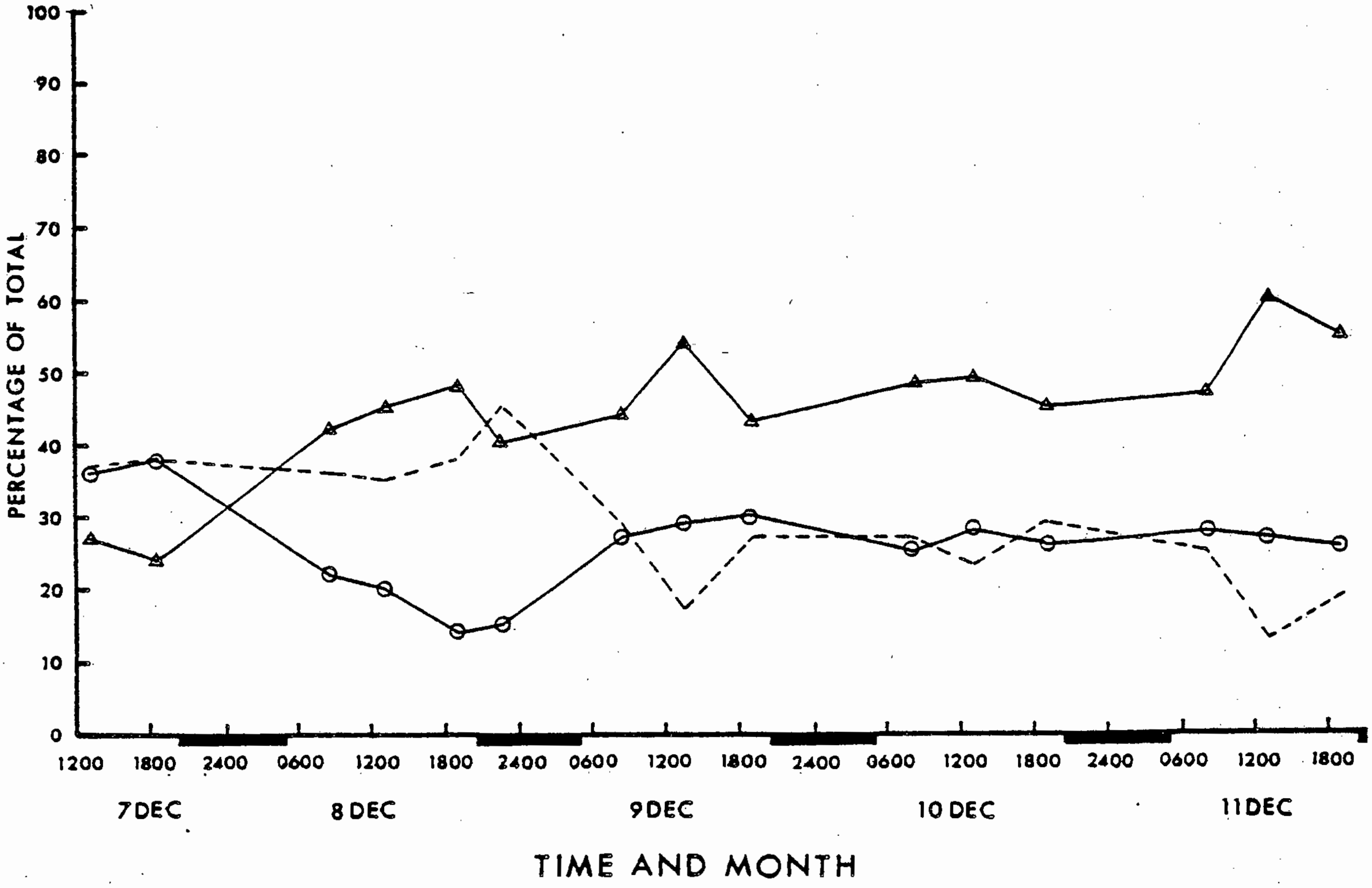


Fig. 11. Contribution of Chaetoceros compressus (△), Skeletonema costatum (○) and other species (----) to the total cell concentration. Total concentration is the number of cells in the water column (surface to bottom) estimated as the sum of the cell counts at each sample depth.

detailed in Appendix 2 (chlorophyll a), Appendix 4 (protein and carbohydrate) and Appendix 5 (carbon-14 assimilation). Salinity samples were analysed with an inductively-coupled salinometer.

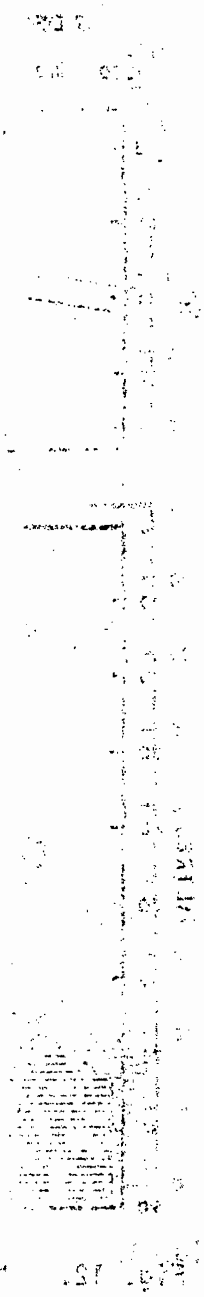
RESULTS

Physical characteristics and species composition

Southerly winds blew during the period of the study and varied in speed between 1.5 and 12.8 $m.s^{-1}$. Strong winds (>8 $m.s^{-1}$) blew mainly in the afternoons but only lasted for a few hours. At the 10 metre depth (position of the drogue) temperatures varied between 10.11 and 12.17°C while salinities varied between 34.75 and 34.84‰. These small variations indicated that the drogue was following a stable well mixed parcel of water. Further evidence for a single water mass was provided by the speed of the drogue and the species composition. The drogue moved a distance of 28.2 kilometres in 102 hours at a mean rate of 0.27 kilometres per hour. This is relatively slow for the movement of water in the local upwelling region (G. Nelson, pers. comm.). The diatoms, Chaetoceros compressus and Skeletonema costatum, were dominant throughout the study although other diatoms were present in fewer numbers. The contribution of these dominant species to the total cell concentration (55 - 87%) is shown in Fig. 11. A detailed study of all the species encountered in this investigation has been undertaken by Olivieri (in prep.).

The surface temperature was 10.8°C at the beginning of the study (1300 hours, 7 December) and increased to a maximum of 13°C at 1300 hours on 10 December (Fig. 12). As the surface waters warmed the depth of the 10°C isotherm increased from 13.5 metres to a maximum of 32 metres. A well defined thermocline was not detected during the study, therefore the 10°C isotherm was arbitarily taken as the lower limit of the mixed layer. The water below this isotherm was upwelling water, in accordance with the categorisation proposed by Andrews & Hutchings (1980) (Fig. 1, Chapter 1) for water masses in the local upwelling region.

The euphotic layer, defined as the depth of penetration of 1% of the incident radiation, was found to range between 9 and 65 metres. Fig. 12 shows that the euphotic zone extended below the lower limit of the mixed layer into the upwelling water during the initial stages of the study. But as the surface water warmed and the depth of the mixed layer deepened, the euphotic zone became much shallower and extended to less than half the



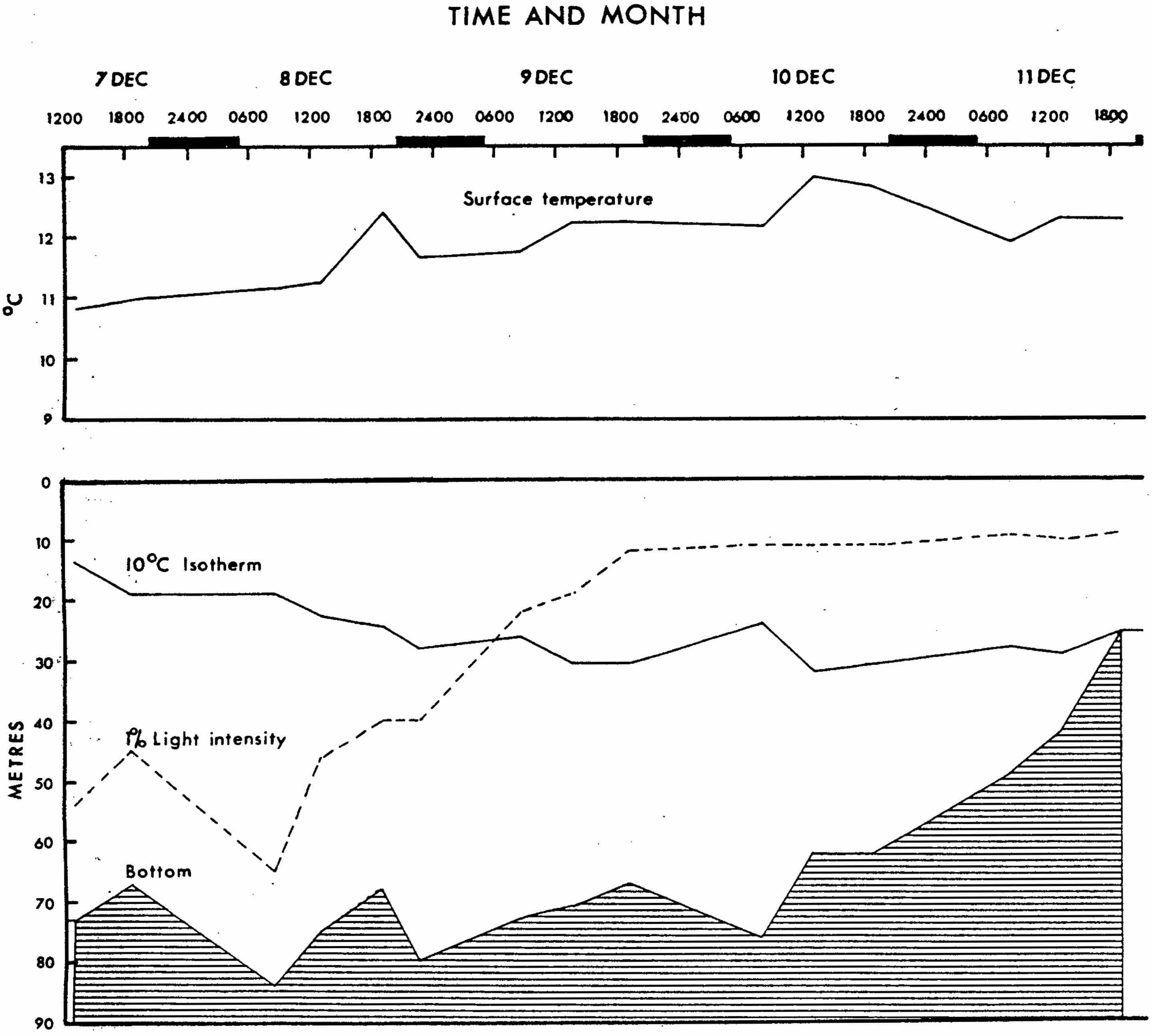


Fig. 12. Variations in surface temperature and depths of the 10°C isotherm and 1% light intensity.

depth of the mixed layer during the later stages of the study.

Chlorophyll a and nutrients

Intense biological activity occurs in the mixed layer (Andrews & Hutchings, 1980) and most of the phytoplankton standing stock (measured as mg chlorophyll a.m^{-2}) was found in this water (Fig. 13). The concentration of nutrients in the mixed water was lower than in the upwelled water, but did not show any large variation during the course of the study. The increase in total chlorophyll a concentration in the water column with a concomitant decrease in the total concentration of nutrients indicates that nutrients were utilised by phytoplankton in the water column.

Fig. 14 shows that nutrient utilisation occurred in the euphotic zone. As the chlorophyll a concentrations increased there was a rapid decrease in the concentration of nutrients. During the later stages of the study higher concentrations of nutrients were detected below the euphotic zone, while a comparison of the chlorophyll a concentrations in Figs. 13 and 14 reveals that a significant proportion of the phytoplankton crop was located between the euphotic zone and the lower limit of the mixed layer.

Variations in the concentrations of chlorophyll a and inorganic nutrients in the euphotic zone are shown in Fig. 15. Low chlorophyll a concentrations were measured at the beginning of the bloom and this increased rapidly to a maximum in 3 days (Fig. 15 and Table 19). Chlorophyll a concentrations increased in successive 24-hour periods from the commencement of growth to the peak of the phytoplankton bloom (Table 19). A simultaneous decrease in the concentration of nutrients occurred and it may be noted that the lowest concentrations of nitrate coincided with the highest concentration of chlorophyll a (Fig. 15). Nitrate concentrations decreased with successive 24-hour periods to the peak of the bloom (Table 19) and the overall decrease in nitrate concentration during the 3-day period was greater than that for silicate and phosphate.

On the last day of the study (11 December) surface temperatures (Fig. 12) and the concentration of chlorophyll a (Fig. 15) decreased, but an increase in the concentration of inorganic nitrate (Fig. 15) was observed. These changes indicated that the body of water being followed was mixed with other water in shallow depths as the drogue moved closer to the shore (Fig. 2, Chapter 2 and Fig. 12).

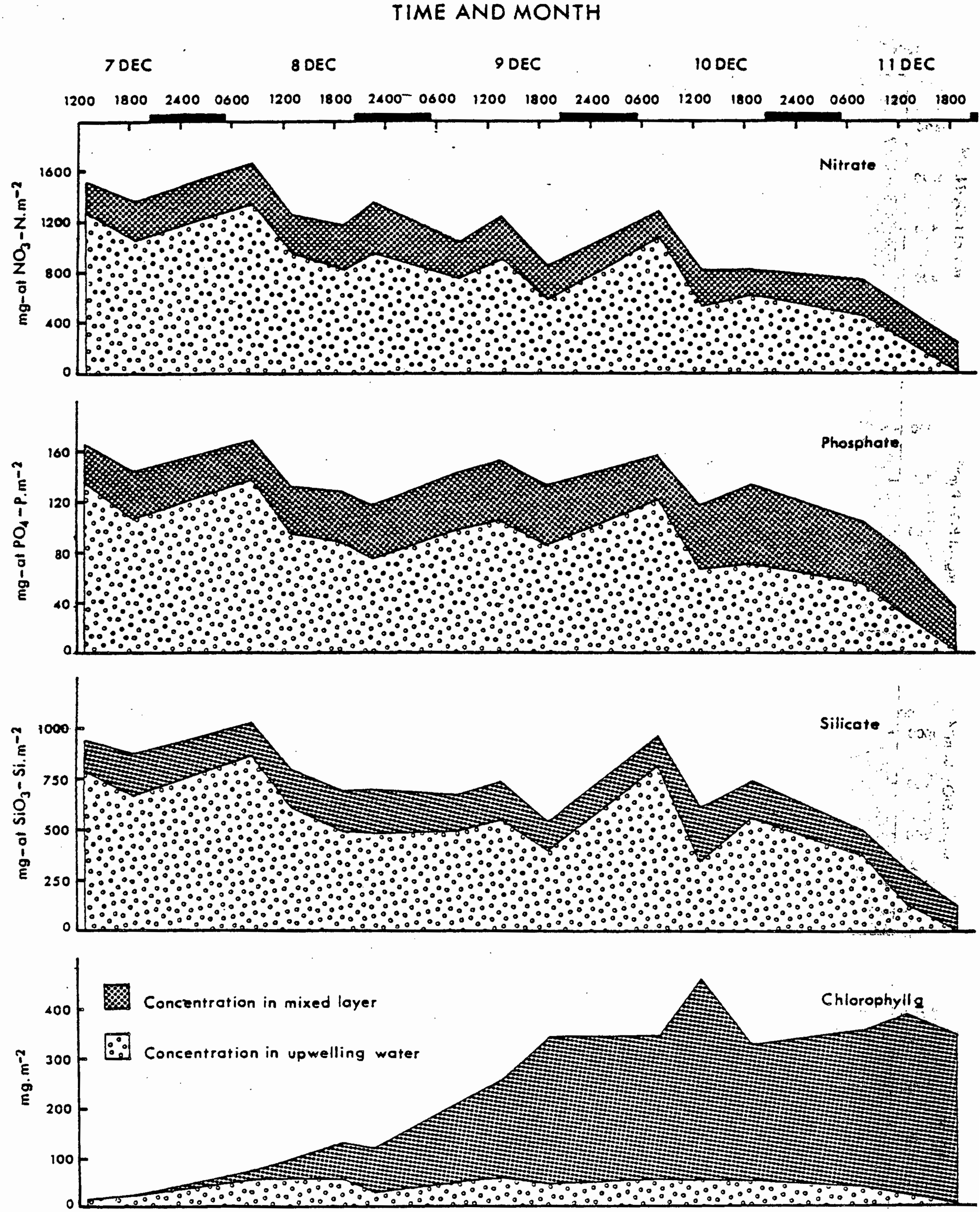


Fig. 13. The distribution of nutrients and chlorophyll a between the mixed layer and upwelling water. The upper line in each graph indicates the total concentration of each variable integrated between the surface and the bottom for a square metre water column.

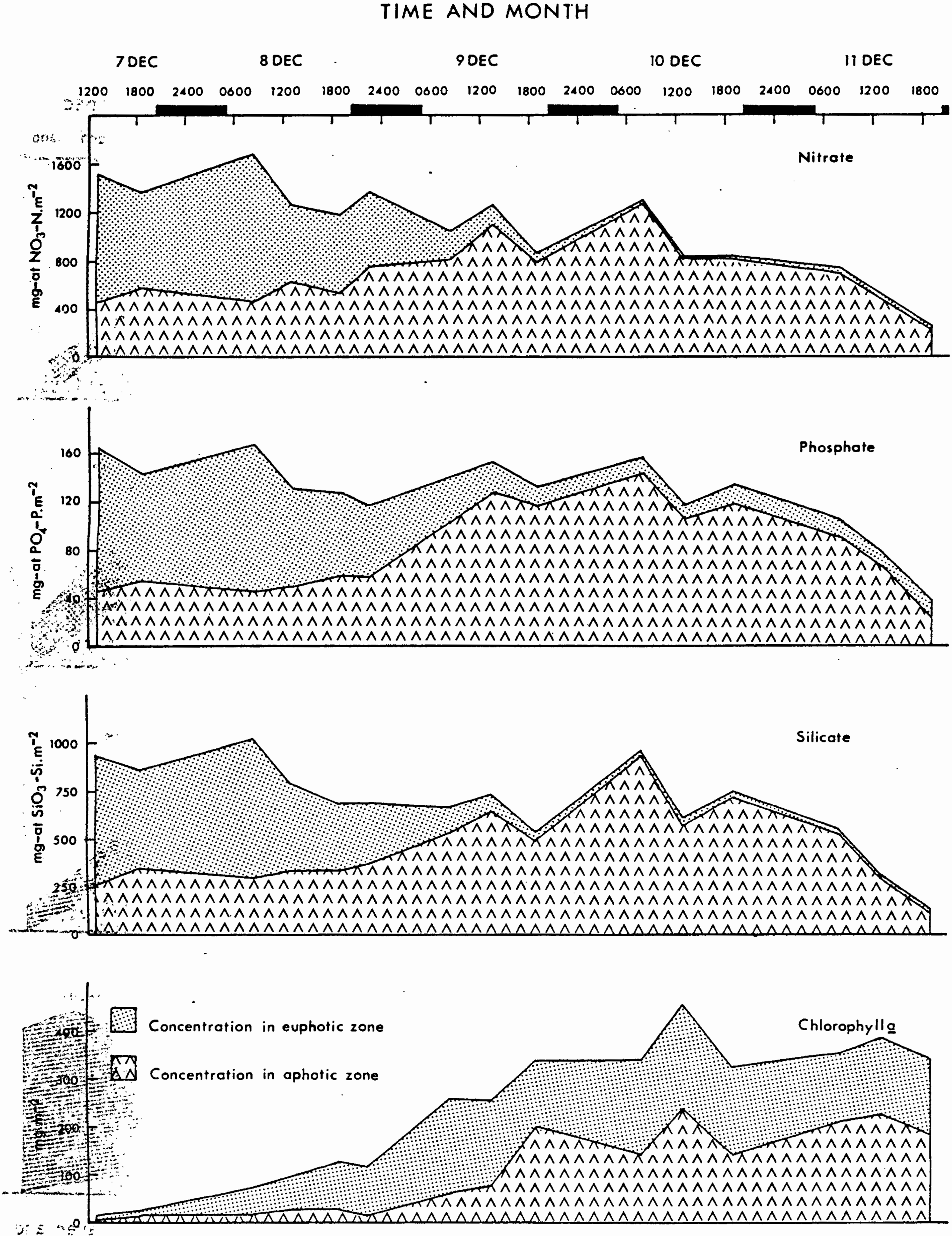


Fig. 14. The distribution of nutrients and chlorophyll a between the euphotic and aphotic zones. The upper line in each graph indicates total concentration of each variable integrated between the surface and the bottom for a square metre water column.

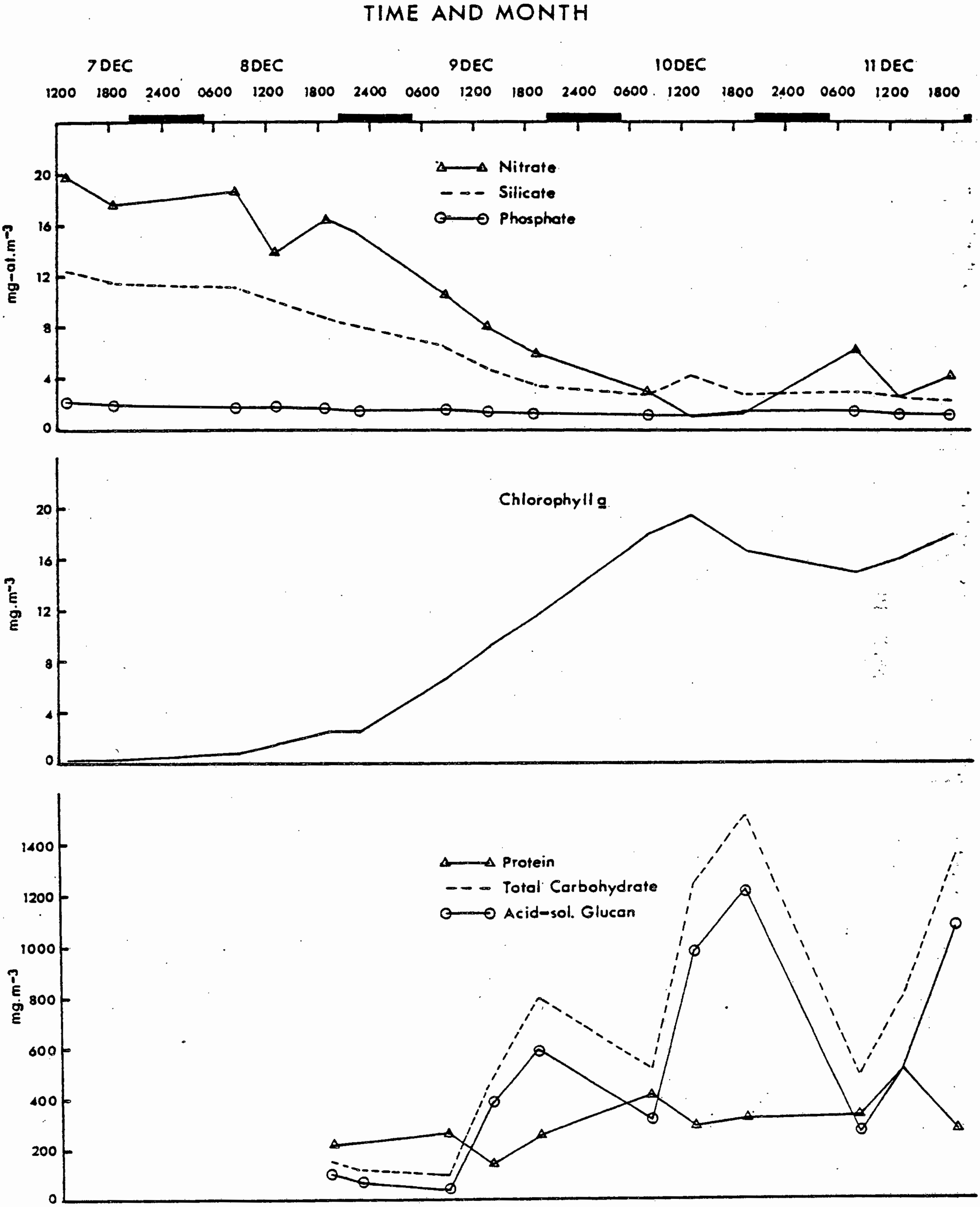


Fig. 15. Concentrations of nutrients, chlorophyll a, protein and carbohydrates in the euphotic zone. Results are the mean concentrations per cubic metre for the euphotic zone shaded area shown in Fig. 14.

TABLE 19

Increases in chlorophyll a concentration and decreases in nutrient concentrations for three 24 h periods from the commencement of growth to the peak of the bloom during December, 1979

Period (24h)	Chlorophyll a ($mg.m^{-3}.24h^{-1}$)	Nitrate ($mg\text{-}at.m^{-3}.24h^{-1}$)	Silicate ($mg\text{-}at.m^{-3}.24h^{-1}$)	Phosphate ($mg\text{-}at.m^{-3}.24h^{-1}$)
7 Dec 13h00 to 8 Dec 13h00	+1.3	-6.0	-2.5	-0.4
8 Dec 13h00 to 9 Dec 13h00	+7.6	-5.7	-5.2	-0.4
9 Dec 13h00 to 10 Dec 13h00	+10.3	-7.0	-2.0	-0.3
Total (3d)	($mg.m^{-3}.3d^{-1}$)	($mg\text{-}at.m^{-3}.3d^{-1}$)	($mg\text{-}at.m^{-3}.3d^{-1}$)	($mg\text{-}at.m^{-3}.3d^{-1}$)
7 Dec 13h00 to 10 Dec 13h00	+19.2	-18.7	-9.7	-1.1

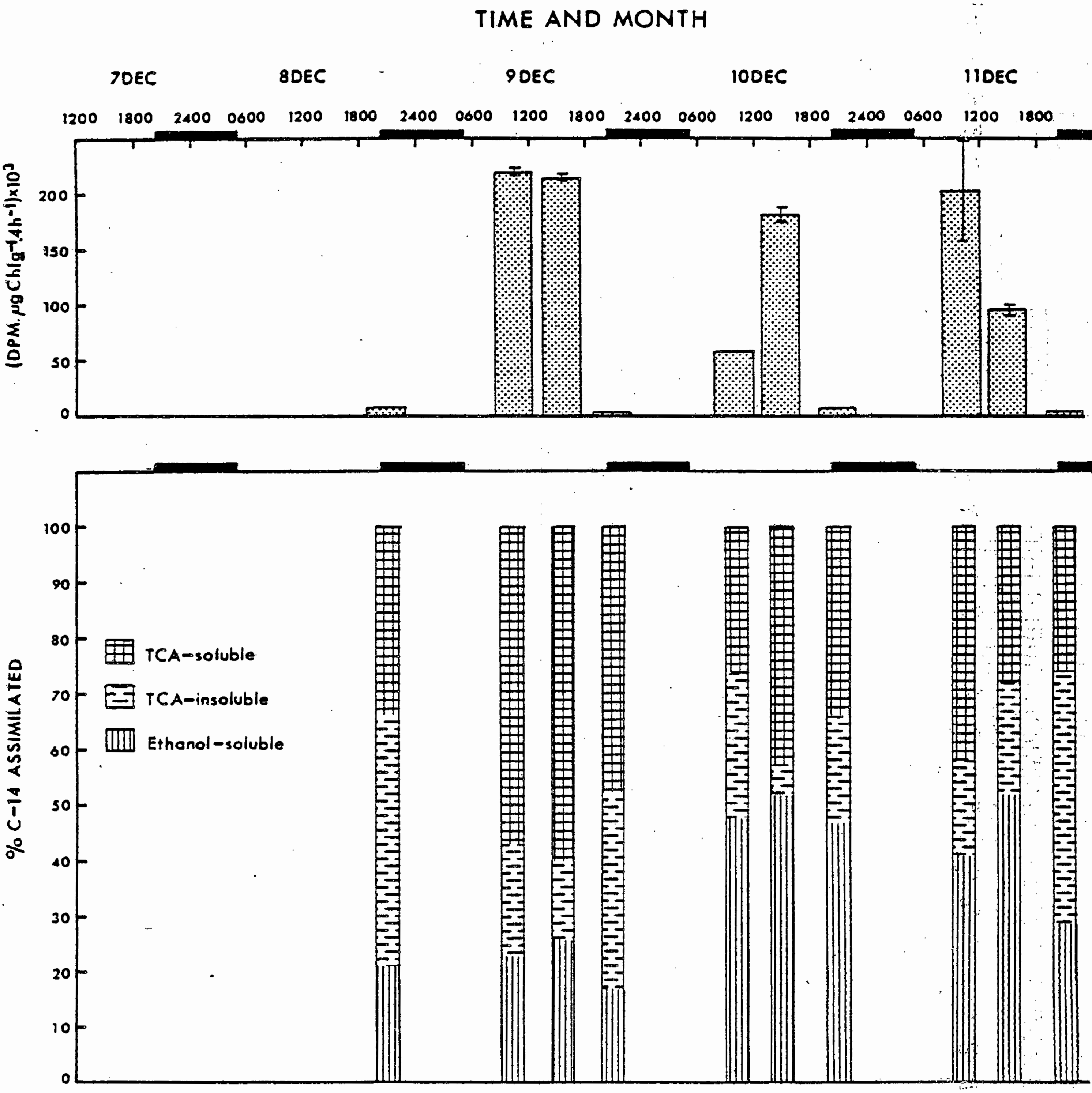


Fig. 16. Total carbon-14 assimilation and the percentage assimilated into ethanol-soluble, TCA-soluble and TCA-insoluble fractions for samples drawn from the 50% light depth and incubated at 50% light intensity for 4 hours. Results are the means of two replicate incubations. In the upper figure vertical bars indicate twice the standard error.

Protein and carbohydrate

Protein and carbohydrate concentrations varied considerably during the course of the study and did not display the same pattern as chlorophyll a. These measurements commenced on the second day once it had been established that significant increases in chlorophyll a were occurring. There was an overall increase in the concentration of protein as the bloom developed, with the highest concentration being measured just prior to the peak of the bloom (Fig. 15). The total carbohydrate and the acid-soluble carbohydrate (glucan) concentrations decreased at night and increased significantly during the day (Fig. 15). Protein concentrations were greater than the glucan concentration during the early development of the bloom and thereafter only in the early mornings. The highest total carbohydrate concentration (1500 $mg.m^{-3}$) coincided with the low concentration of inorganic nitrate at the peak of the bloom.

Carbon-14 assimilation patterns

The pattern of carbon-14 assimilation by the community at the 50% light intensity is presented in Fig. 16. Very slow assimilation rates were measured during all the evening/night incubation periods, while the fastest rates were recorded during the daylight hours on 9 December as the bloom developed. When the bloom had reached its peak (10 December) and on the last day of the study the assimilation rate was slower than on 9 December.

Distribution of the label between various fractions showed some significant variations over the course of the study. While the bloom was developing a high percentage of carbon-14 was incorporated into the TCA-soluble fraction during the morning (56.8%) and afternoon (59.7%) incubation periods. An increase in the incorporation of the label into the TCA-insoluble fraction and a decrease into the TCA-soluble fraction occurred during the evening/dark incubations. At the peak of the bloom higher percentages of carbon-14 were, however, detected in the ethanol-soluble fraction (46.8 - 52.4%) than in the TCA fractions. On 11 December continued high percentages of the label were detected in the ethanol-soluble fraction during the morning and afternoon incubations, but incorporation into this fraction decreased considerably during the evening/night incubation when most of the assimilated carbon-14 was channelled into the TCA-insoluble fraction.

DISCUSSION

Rate of growth

The results of this study show that the growth of phytoplankton in the Benguela nearshore region can be very rapid. The increase in the chlorophyll a concentration of 19.2 $mg.m^{-3}$ in three days indicates faster growth than the results of Ryther et al. (1971) who conducted a drogue study in the Peru coastal current. Chlorophyll a concentrations increased 11.9 $mg.m^{-3}$ in 2.5 days in the euphotic zone off Peru. Chlorophyll a values may also be used to estimate organic production (Herbland et al., 1973) and the results reported here yield a primary production estimate of 11.0g $C.m^{-2}$ in three days. In an upwelling region off Mauritania Herbland et al. (1973) estimated that primary production reached 13.5g $C.m^{-2}$ in 5.5 days.

The very rapid rates of phytoplankton growth in upwelling areas contrast with the slower growth rates that occur in non-upwelling areas such as the East coast of North America. In these temperate waters phytoplankton blooms only take place in spring and autumn (Morris & Skea, 1978; Hitchcock, 1978) and take weeks rather than days to develop. From the data of Hitchcock (1978) it was estimated that chlorophyll a concentrations took 2 weeks to increase 14.5 $mg.m^{-3}$ in Narragansett Bay, while concentrations of the pigment increased 7.5 $mg.m^{-3}$in 6 weeks in the Gulf of Maine (estimated from the data of Morris & Skea, 1978).

Influence of light on nutrient uptake

Nitrate was taken up at a fast rate in the euphotic zone (see Table 19), implying that light has an important influence in controlling the uptake of nutrients (Morris, 1974). No investigations on this aspect of phytoplankton physiology have been undertaken in local waters, but studies elsewhere have confirmed that light is important. Nelson & Conway (1979) concluded that the light regime in the waters off Baja California and North West Africa was more important than nutrient concentration in controlling the biological availability of inorganic nitrate, ammonium and silicate. MacIsaac and Dugdale (1972) found that light intensity was very often the limiting factor controlling nutrient uptake in eutrophic waters e.g. upwelling regions) with most of the inorganic nitrogen taken up in the form of nitrate and the maximum uptake occurring near the surface. Preliminary experiments by Reshkin & Knauer (1979) have also indicated that phosphate uptake by phytoplankton communities in Monterey Bay, California depends upon light intensity.

Reserve carbohydrate

An indication of the role of reserve carbohydrate in diatoms is provided by the pattern of variation in the concentration of the acid-soluble glucan. Increased glucan levels during daylight hours implied that surplus carbon compounds were stored as polysaccharides. But at night the decreasing glucan concentration reflects a breakdown of the polysaccharides to provide energy for the synthesis of cellular components during the hours of darkness. These polysaccharides may serve as a carbon buffer for diatom cells (Mague *et al*., 1978) increasing if excess carbon compounds are supplied by photosynthesis, and decreasing to maintain soluble compounds within the cells when the uptake of CO_2 ceases.

The greater assimilation of carbon-14 into the polysaccharide (TCA-soluble) fraction during the day, and negligible assimilation at night, provided evidence for the role of carbohydrate as an energy reserve, while the continued maintenance of protein synthesis during the hours of darkness was reflected by the increased assimilation of the label into the TCA-insoluble fraction. Results of a diel study by Morris & Skea (1978) indicates that there is a movement of carbon from polysaccharide to protein at night, and it is likely that a similar phenomenon occurs in local phytoplankton communities.

Growth patterns

The carbon-14 results imply that changes occurred in the growth pattern of the community as the bloom developed. During the early stages of growth a fast rate of total carbon-14 assimilation and the high level of incorporation into the polysaccharide fraction indicated that the cells were in an active phase of growth. At the peak of the bloom, however, the rate of total carbon-14 assimilation was slower and high percentages of the label were detected in the ethanol-soluble fraction. In Narragansett Bay Hitchcock (1978) has also shown that most of the assimilated label was incorporated into the ethanol-soluble fraction when a winter-spring bloom had reached its peak. It appears that the synthesis of polysaccharides from precursors in the metabolite pool proceeded at a slow rate. This indicates that the growth pattern had changed to a slow-growing phase as the bloom reached its peak. The larger reserve of glucan during this phase (Fig. 15) may be due to slow utilisation rather than to a fast rate of production.

Conclusions

By following a patch of upwelled water marked with a drogue for 5 days, it has been confirmed that Type 1, Type 2 and Type 3 water, discussed in previous chapters, are indeed successive stages in the development of a phytoplankton bloom in a plume of upwelled water. It might therefore be appropriate to rename these Stages 1, 2 and 3 respectively, of a maturing upwelling plume.

This study has confirmed that light and the concentration of inorganic nitrate are the important environmental variables influencing the growth of phytoplankton in the local upwelling region. Carbon-14 assimilation patterns and the measurement of biochemical constituents revealed that carbohydrate is an important reserve of energy in diatom communities, as found by Haug et al. (1973) in the Trondheimsfjord and Hitchcock (1978) in Narragansett Bay. Diatoms can divide very rapidly and doubling rates of <10 hours have been measured in Skeletonema and Chaetoceros species (Raymont, 1980). The significant increases in soluble glucan during the day and rapid utilisation at night are consistent with this short generation time of diatoms.

The results presented in Figs. 15 and 16 indicate that the hypothesis proposed has to be modified. While protein concentration was greater than carbohydrate concentration in the mornings during active growth, the carbohydrate concentration exceeded the protein concentration later in the day (Fig. 15). Thus a protein/carbohydrate ratio greater than unity can only be used as an indicator of active phytoplankton growth if samples are taken in the morning, but cannot be used in a diel study.

The suggestion that nitrate depletion leads to enhanced carbohydrate synthesis is incorrect. The results in Fig. 16 show that a greater proportion of carbon-14 was assimilated into the ethanol-soluble fraction than into the polysaccharide fraction under these conditions, indicating that the rate of carbohydrate synthesis declined at the peak of the bloom. This finding is at variance with the results in Chapter 4 where enchanced carbohydrate synthesis was measured in Rhizosolenia dominated communities in low nitrate water (Fig. 10 and Table 18). A possible explanation for these differences is that Chaetoceros and Skeletonema species appear to assimilate a large excess of CO_2 for glucan synthesis during active growth and consequently synthesize glucan at a slow rate in the slow-growing phase (Fig.16).

In contrast, Rhizosolenia species apparently synthesize most of their glucan reserves in the slow-growing phase (Type 3 water; Fig. 10 and Table 18).

A revised hypothesis for physiological changes in a Chaetoceros-Skeletonema bloom is proposed :

During active growth the concentration of protein is greater than the concentration of carbohydrate in the morning, but later in the day carbohydrate synthesis exceeds protein synthesis when excess CO_2 is assimilated in the upper euphotic zone. At night protein synthesis is conserved using the carbohydrate accumulated during the day as an energy reserve. When the supply of nitrate is depleted the bloom reaches a peak and growth changes to a slow-growing phase. In this phase the synthesis of carbohydrate declines as the energy reserves are utilised at a slow rate.

CHAPTER 6

PHYSIOLOGICAL CHANGES FOLLOWING UPWELLING : A SYNTHESIS

Upwelling ecosystems are generally characterised by the growth of large, long-chain diatoms (Ryther et al., 1971), and Margalef (1967) has described these systems as a progression from a low diversity, highly productive ecosystem maturing in time to one of high diversity and low production. The dominance of diatoms may be attributed to their fast uptake of nutrients and slow specific rate of respiration. These characteristics allow the cells to out-compete other species under conditions of pulsed nutrient supply, as found in upwelling regions (Parsons, 1979). Diatom cells take up nutrients rapidly when they are available, but survive on their reserves when nutrients are depleted. The Benguela upwelling system may be regarded as one of the major upwelling systems of the World's oceans. The waters in the vicinity of the Cape Peninsula therefore provided an ideal environment in which to investigate the physiological responses of natural diatom communities.

The biochemical composition of phytoplankton has mainly been studied in culture and Parsons et al. (1961), Antia et al. (1963), Myklestad & Haug (1972), Myklestad et al. (1972) and Myklestad (1974, 1977) have contributed significantly to the understanding of biochemical changes in relation to nutrient supply. When adequate nutrients are available and cells are growing exponentially large amounts of chlorophyll and protein are produced as opposed to small amounts of carbohydrate and fat. Nitrogen depletion leads to a marked increase in the carbohydrate content, increased production of fat but low production rates of chlorophyll and protein. The accumulation of carbohydrate was shown to be due to the synthesis of acid-soluble β-1,3 glucan, a general feature in diatoms. This important reserve of energy is likely to be associated with living phytoplankton because of its biochemical properties and solubility in sea water.

These observations have been used as a basis for interpreting the results of biochemical measurements on local communities. During active upwelling of cold water containing high concentrations of nutrients, low concentrations of chlorophyll a, ATP, protein and carbohydrate were recorded (Table 7, Chapter 3) indicating that a low standing stock was present and very little growth occurred. When upwelling lessened and temperatures increased to >10°C the concentrations of the biochemical variables increased and protein/carbohydrate ratios >1 indicated that the communities were in an active phase of growth. Continued growth led to a depletion in the supply of nutrients, particularly nitrate, and an increase in the concentration of the biochemical variables. When nitrate concentrations decreased to low levels very high concentrations of glucan were measured, resulting in the protein/carbohydrate ratio decreasing to values <1 (Table 7, Chapter 3). Although this indicated a change in nutrient status, the chlorophyll *a* and ATP concentrations remained high suggesting that a senescent phase had not been reached. It was concluded, therefore, that the communities had reached a slow-growing phase in this aged upwelled water.

Glucan was found to be the important biochemical variable for determining the physiological state and growth phases of the communities. The values of the protein/carbohydrate, glucan/ATP and glucan/chlorophyll ratios all varied in accordance with changes in the concentration of glucan, since this variable was a common factor in the three ratios. Decreases in the protein/carbohydrate ratio, due to increases in the concentration of glucan, have also been found to be important in assessing the physiological state of diatom communities in the Trondheimsfjord (Haug *et al.*, 1973) and in Narragansett Bay (Hitchcock, 1978).

The very significant positive correlation between chlorophyll *a* and ATP, protein and carbohydrate (Table 5, Chapter 3) confirmed that physiological changes were occurring in local phytoplankton communities. This finding complements those of Fiala and Jacques (1974), Hitchcock (1977) and Garfield *et al.* (1979) for other upwelling regions. Significant correlations between ATP and chlorophyll *a* were obtained by Fiala & Jacques (1974), who concluded that ATP was closely related to phytoplankton in the euphotic zone off North West Africa. Garfield *et al.* (1979) report high correlations between protein and chlorophyll *a* in the euphotic zone in the Peru upwelling system. Hitchcock (1977) suggested that a positive correlation between total carbohydrate and chlorophyll *a* implied that particulate carbohydrate is derived primarily from living phytoplankton in an upwelling region off North West Africa. However, chlorophyll *a* measurements include both active and inactive forms of chlorophyll, the latter form being associated with plant detritus.

To study the distribution of protein and carbohydrate in living phytoplankton and detrital matter, the linear regression coefficients of these biochemical variables on glucan were used to estimate the percentage of the variables in each fraction (Tables 10 and 11, Chapter 3). It was found that there was a smaller percentage of protein in living phytoplankton than in the bacteria/detritus fraction during both the active and slow-growing phases of growth. The amount of carbohydrate, on the other hand, was greater in living phytoplankton than bacteria/detritus in these two growth phases. While Lancelot-Van Beveren (1980) has estimated the biochemical composition of phytoplankton in the North Sea using linear regressions on chlorophyll a, this is the first report of the use of linear regressions on glucan for estimating the biochemical composition of phytoplankton. The fact that glucan is only present in living phytoplankton, and not associated with detrital matter, suggests that this new method should be used in preference to the method of Lancelot-Van Beveren (1980) for estimating the biochemical composition of living diatom communities. This method also allows the biochemical composition to be estimated at different phases of growth.

The changes in the protein/carbohydrate ratio have provided some valuable information about local phytoplankton populations, but the study reported in Chapter 5 reveals that the carbohydrate content of diatoms in an active phase of growth can be greater than the protein content. Protein levels were higher than the carbohydrate content in samples collected during the morning, but samples collected later in the day had a greater carbohydrate content (Fig. 15, Chapter 5). It appears that sufficient protein for cell maintenance is produced by communities in the morning and continued photosynthesis during the rest of the day produces a large reserve of carbohydrate in the form of soluble glucan. This reserve is utilised by the cells during the hours of darkness as reflected by the sharp decrease in glucan concentration from 1900 to 0800 hours (Fig. 15, Chapter 5).

These changes complement the results of other investigations. Handa (1969) found that the carbohydrate content of Skeletonema cells increased when exposed to light, but decreased in the dark. Foy & Smith (1980) showed that carbohydrates accumulated rapidly in Oscillatoria cultures during the light period followed by a decline in the dark. In contrast, protein concentrations increased, after a lag phase, in the light and continued to increase during the dark period. Assimilation of carbon-14 by natural communities in the Gulf of Maine (Morris & Skea, 1978) showed increased uptake into polysaccharides during the day and a decrease at night, while incorporation into protein continued during both the day and night.

The study reported in Chapter 5 is the first report of diel changes in the biochemical composition of a phytoplankton community in an upwelling ecosystem, and the growth and physiological responses were found to change in a matter of hours. It is concluded that the measurement of diel variations in the protein/carbohydrate ratio over several days in a single mass of water is more informative than comparing different bodies of water as described in Chapter 3.

All the measurements of biochemical composition were undertaken on samples collected from within the euphotic zone, but these gross measurements were not sensitive enough to detect the important effects of light. By measuring the patterns of carbon-14 assimilation into major end-products of phytoplankton photosynthesis some significant effects were observed. In cold freshly upwelled water containing high concentrations of nutrients the distribution of the label between protein and polysaccharide fractions was approximately equal at both high (50% of surface) and low (1% of surface) intensities of light. At 1% intensity, however, a high percentage of carbon-14 was measured in the ethanol-soluble fraction, containing mainly precursors, and it was concluded that low temperatures (9°C) and low light intensities had the combined effect of reducing the rate of protein and carbohydrate synthesis from their precursors (Table 18, Chapter 4). Morris _et_ _al_. (1974) have also detected a high percentage of carbon-14 in the ethanol-soluble fraction of batch cultures of _Phaeodactylum_ _tricornutum_ grown at low temperatures (7°C). In contrast, Smith & Morris (1980a,b) have shown that a large proportion (80%) of carbon-14 is incorporated into lipids in diatom communities in the Southern Ocean under conditions of very low temperatures (-1°C) and low light intensities.

High percentages of carbon-14 were observed in the polysaccharide fraction of phytoplankton samples from maturing upwelled water when incubations were performed at light intensities corresponding to 25%, 50% and 100% of incident. A similar phenomenon was observed in samples from aged upwelled water ($<2\ \mu$g-at NO_3-N.ℓ^{-1}), but the percentage incorporation was greater than in the samples from the maturing water. In both types of water a decrease in light intensity to low levels resulted in a decrease in the incorporation of the label into polysaccharide and an increased incorporation into protein (Figs. 9 and 10 and Table 18, Chapter 4).

These results show similarities and differences with previous work in the field. Morris _et_ _al_. (1974), Morris & Skea (1978) and Li _et_ _al_. (1980) found that cells subjected to low irradiance and nutrient limitation assimilated a greater proportion of carbon-14 into protein. In the present investigations low irradiances increased the flow of carbon-14 into protein, but low nitrate concentrations had the effect of increasing activity in the polysaccharide fraction at high light intensities. This increase in carbohydrate synthesis under these environmental conditions has not been reported before and it appears to be a species-specific effect. The phenomenon was observed in Rhizosolenia communities (Fig. 10 and Table 18, Chapter 4), but not in a Chaetoceros-Skeletonema bloom where a reduction in the synthesis of protein and carbohydrate was recorded in samples from water containing low concentrations of nitrate (Fig. 16, Chapter 5). Further differences between species are emphasized by the observations of Morris et al. (1974) who found an increased proportion of protein synthesis in a Phaeodactylum tricornutum culture when the medium was depleted of nitrogen. The combined effect of low nitrate concentration and low irradiance increased the flow of carbon-14 into the protein fraction, but increased activity in the protein fraction was also observed at low irradiance in phytoplankton from water that was not nitrate deficient. It was concluded, therefore, that low light intensity was more important than low nutrient concentrations in influencing the incorporation of carbon-14 into protein in local communities.

In addition to light intensity light quality may affect the pattern of carbon fixation. Wallen & Geen (1971b) concluded that the proportion of the label in ethanol-insoluble fractions (primarily protein) increased with increasing depth where blue light predominates. Light quality was not studied in the present investigations but this should perhaps receive attention in future research. The effects of blue-green light on algal production and physiology were discovered by Jeffrey & Vesk (1977) and Vesk & Jeffrey (1977) to increase the concentration of photosynthetic pigments and the carbon fixation ability of many algal species. The concentration or activity of the enzymes concerned with carbon fixation also increased and these effects were most marked in diatoms.

The carbon-14 assimilation pattern was found to be a more useful indicator of a change in the growth phase in the diel study than the measurements of biochemical composition (Fig. 16, Chapter 5). The high percentage of incorporation of carbon-14 into polysaccharide at 50% light intensity when sufficient nutrients were available indicated that the community was in an active phase of growth. At the peak of the bloom nitrate concentrations were very low and smaller percentages of the label were detected in the polysaccharide and protein fractions than in the ethanol-soluble fraction. This reduced rate of synthesis of macromolecules from precursors in the metabolite pool indicated that growth had changed to a slow-growing phase.

The results of the investigations reported in this dissertation have revealed that significant variations in the biochemical composition and patterns of carbon-14 assimilation occur in phytoplankton communities in the local upwelling region. These variations are induced by environmental changes which in turn are controlled by fluctuations in the wind regime (Andrews & Hutchings, 1980). By synthesizing the hypotheses proposed in Chapters 3, 4 and 5 the following idealized sequence of events for the pattern of growth of a diatom community in an upwelling environment is suggested :

Under the influence of strong offshore winds cold water wells up to the surface along the coast, bringing a high concentration of inorganic nutrients and some phytoplankton cells into the euphotic zone (Stage 1). This water is carried offshore and warms to temperatures >10°C by solar heating and by mixing with other water bodies. Growth of phytoplankton commences in this maturing water (Stage 2) with protein and polysaccharide being synthesized at a faster rate in the upper than lower euphotic zone. As nutrients are taken up during active growth, chlorophyll _a_ concentrations increase and when protein synthesis reaches its maximum rate, excess carbon and energy are stored as polysaccharides during the daylight hours in the upper euphotic zone. In the lower euphotic zone the uptake of carbon and energy is reduced and the rate of polysaccharide synthesis is slower while protein production is maintained. At night the synthesis of protein continues as the cells utilise the reserve of energy accumulated as glucan during the day. At daybreak photosynthesis commences again, replenishing polysaccharide reserves in cells in the upper euphotic zone and maintaining protein synthesis in the lower euphotic zone.

Active growth continues in this pattern as the density of the cells in the community increases rapidly and the water body moves further offshore. When nutrients, particularly nitrate, are depleted and the density of phytoplankton cells reduces the light intensity by self-shading, the growth of the community changes to a slow-growing phase as the bloom reaches its peak (Stage 3). During this phase protein production proceeds at a slow rate throughout the euphotic zone, thus conserving intracellular reserves of nitrogen, while enhanced reserves of polysaccharide accumulate in cells in the upper euphotic zone due to their slow utilisation. Slow growth continues under these conditions, which seldom lasts for longer than a few days, until further offshore winds induce another upwelling event. The supply of nutrients in the euphotic zone will then be replenished and active growth will proceed once more as the nutrients are rapidly taken up by the diatom community.

It is stressed that the events described above is an idealized scenario which may be interrupted at any stage by any of the following environmental events : 1) Further southerly to south-easterly winds may drive the upwelling plume of water further offshore until it meets warmer water at the Oceanic front, situated between 20 and 100 km offshore (Andrews & Hutchings, 1980). Here the aged upwelled water (Stage 3) sinks below the thermocline as it moves offshore, giving rise to subsurface chlorophyll maxima (Andrews & Hutchings, 1980). The phytoplankton cells descend below the euphotic zone and are likely to senesce and die, forming detritus beyond the geographic range of the present study. 2) Onshore winds may drive the upwelled water back towards the coast to form blooms occasionally observed close inshore, eventually leading to downwelling of plankton-rich water. 3) Frequent occurrence of heavy swells generated in sub-Antarctic waters (Field _et al_., 1980) causes deep mixing, breaking down the thermocline and disturbing the water-column stability that is required for the full development of upwelling to Stage 3. This may be one mechanism for re-seeding phytoplankton cells into the next upwelling event.

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APPENDIX 1

J. exp. mar. Biol. Ecol., 1980, Vol. 45, pp. 83–93

THE BIOCHEMICAL COMPOSITION OF PHYTOPLANKTON IN AN UPWELLING REGION OFF SOUTH AFRICA

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Abstract: Variations in the concentrations of chlorophyll *a*, ATP, protein, and carbohydrate in phytoplankton have been investigated in a nearshore upwelling region off the Cape Peninsula. During active upwelling temperatures <10 °C, high nutrient concentrations and low concentrations of the biochemical constituents were measured. When upwelling lessened and conditions stabilized temperatures increased and blooms of phytoplankton appeared. High concentrations of chlorophyll *a* and ATP and a high protein/carbohydrate ratio were then recorded. At very low nutrient levels chlorophyll *a* and ATP concentrations were still high but an increase in the acid-soluble carbohydrate content and a corresponding decrease in the protein/carbohydrate ratio was observed. It was concluded that the ratio of protein to carbohydrate was a suitable indicator of the physiological state of a phytoplankton community in the local upwelling region.

INTRODUCTION

There are rapid chemical and biological changes along the west coast of South Africa during the summer months. Under the influence of southeasterly winds intense upwelling takes place, with high levels of nutrients being brought into the surface euphotic layer. Once conditions stabilize dense blooms of phytoplankton may appear in these waters (Andrews & Hutchings, in press).

In coastal waters off the Cape Peninsula, monitoring of the marine environment has revealed, on the basis of temperature and the concentration of nutrients, three types of water (Table I). For Type 1 water temperatures were <10 °C, while the concentrations of phosphates, nitrates, and silicates were high. These measurements indicated that there was active upwelling of cold water. When upwelling lessened and conditions stabilized temperatures increased to >10 °C and high or adequate concentrations of nutrients for phytoplankton growth prevailed (Type 2 water). In Type 3 water nutrient concentrations were low with temperatures still >10 °C. These types followed sequentially after water had upwelled and merged with other water bodies.

As the water changed from one type to another variations in the physiological state of the phytoplankton community were expected. To examine these physiological changes appropriate factors needed to be studied in relation to the variations in temperature and nutrient concentration. Chlorophyll *a*, ATP, protein, and carbohydrate are all important biochemical constituents of phytoplankton cells (Stewart, 1974) and were selected as suitable factors for such a study. Chlorophyll *a* is unique to plant cells whereas ATP, not being associated with non-living material, is only present in living organisms (Holm-Hansen & Booth, 1966). Protein and carbohydrate are metabolites whose concentrations in algal cells have been found to vary in accordance with the physiological state of a phytoplankton community (Antia *et al.*, 1963; Haug *et al.*, 1973).

TABLE I

Water types in the local upwelling ecosystem; Type 1, recently upwelled water; Type 2, maturing upwelled water; Type 3, aged upwelled water.

	Type 1	Type 2	Type 3
Temperature, °C	<10	>10	>10
Nitrate, µg-at. NO_3-N/l	>15	>2	<2
Silicate, µg-at. SiO_3-Si/l	>15	>2	–
Phosphate, µg-at. PO_4-P/l	>1.5	–	–

This paper reports the results of a preliminary investigation undertaken during the summer of 1977–78 to establish whether chlorophyll *a*, ATP, protein, and carbohydrate were suitable indicators of physiological change in relation to changes in the environment in the Cape Peninsula upwelling region.

METHODS AND MATERIALS

A single station was established at the base of an upwelling plume in 40 m of water, 2 km off Oudekraal (33°58′S : 18°20.6′E). Daily sampling was conducted over 3- to 5-day periods to total 20 days during the months of November, December, and February. This plan of sampling resulted in different bodies of water being monitored as they moved past the Oudekraal station. On each day samples were drawn only from within the euphotic zone at intervals corresponding to 100, 50, 25, 10, and 1% light levels. The 1% light level was taken as the lower limit of the euphotic zone.

The analysis for nutrients was performed on a Technicon Autoanalyser using the methods of Armstrong *et al.* (1967), and Strickland & Parsons (1972) for phosphate and nitrate (including nitrite) and Grasshoff (1965) for silicate. Temperatures were recorded with a bathythermograph and a hand held thermometer.

For the analysis of chlorophyll one l of water was filtered through a Whatman GF/F glass filter, 4.7 cm in diameter, pre-covered with 100 mg of magnesium carbonate. Chlorophyll *a* was determined by the spectrophotometric method of Strickland & Parsons (1972) correcting for phaeopigments. For ATP analysis 0.5 l was filtered through a Whatman GF/F glass-fibre filter and the filters were immediately frozen in liquid nitrogen. Filters were held in liquid nitrogen until analysed. The method of Holm-Hansen & Booth (1966) was used to assay for ATP using a Packard Prias liquid scintillation counter. Duplicate analyses were made on the extract from each filter.

Plankton samples for protein and carbohydrate analysis were collected by cascading 500–2000 l of water through 200-μm and 37-μm plankton nets. Plankton collected on the 37-μm net was temporarily stored in a refrigerated dark container and transferred to the laboratory within 8 h. The samples were centrifuged, briefly washed, re-centrifuged, frozen, and freeze-dried. The freeze-dried samples were stored at 5 °C until analysed.

Protein was extracted and assayed as described by Mayzaud & Martin (1975) using the Folin–Ciocalteu method of Lowry *et al.* (1951). Bovine serum albumin was used as standard. For total carbohydrate analysis 1 ml of 80% sulphuric acid was added to 1 mg of dry sample (Myklestad & Haug, 1972). After 20 h at 20 °C the mixture was diluted and carbohydrate determined by the phenol-sulphuric acid method of Dubois *et al.* (1956) using glucose as standard.

Carbohydrates were fractionated into acid- and alkali-soluble fractions according to the method of Haug *et al.* (1973). About 10 mg of dry plankton were extracted twice with 0.1 N H_2SO_4 (10 ml) for 2 h at room temperature and the extracts were combined. The residue was then extracted twice with 0.1 N NaOH (10 ml) for 2 h and the extracts combined. The phenol-sulphuric acid method, using glucose as standard, was used to determine the amount of carbohydrate in each extract. The amount of insoluble carbohydrate was estimated as the difference between total and soluble carbohydrate. Results for protein and carbohydrate analysis are the means of duplicate analyses expressed as percentage of dry matter.

RESULTS

During the period of study samples were drawn from 96 various depths. Of this total 30% were drawn from Type 1 water, 51% from Type 2, and 19% from Type 3. Therefore, on most days water containing high or adequate nutrients for phytoplankton growth was monitored at the Oudekraal station.

To investigate possible relationships between the various factors the product moment correlation coefficient (*r*) was calculated between all pairs of measured factors (Tables II and III). The negative correlations between physiological factors

TABLE II

Correlation coefficients between environmental and physiological factors ($P < 0.01$ except $^*P > 0.05$).

	Chlorophyll *a*	ATP	Protein	Carbohydrate
Temperature	0.325	0.490	0.459	0.320
Phosphate	−0.440	−0.556	−0.512	−0.423
Nitrate	−0.526	−0.648	−0.441	−0.195*
Silicate	−0.579	−0.668	−0.337	−0.089*

and nutrients were expected. Nutrient concentrations were high when chlorophyll *a* and ATP concentrations were low, but low when the population had increased. Chlorophyll *a* and ATP showed higher correlations with silicate and nitrate than with phosphate, whereas protein and carbohydrate showed a higher correlation with phosphate. A very significant correlation was obtained between chlorophyll *a* and ATP.

TABLE III

Correlation coefficients between physiological factors ($P < 0.01$ except $^*P > 0.05$).

	Chlorophyll *a*	ATP	Protein
ATP	0.815	-	-
Protein	0.332	0.545	-
Carbohydrate	0.047*	0.219*	0.572

Some examples of the results obtained for the three types of water are presented in Figs. 1, 2, and 3. Fig. 1 shows the vertical distribution of measured factors in Type 1 water. The temperatures were all <10 °C and the concentrations of nutrients were all very high. Phosphates were between 4 and 6 µg-at. PO_4-P/l and nitrates and silicates were in the region of 20 µg-at. NO_3-N or SiO_3-Si/l. Under these conditions the chlorophyll *a*, ATP, protein, and carbohydrate concentrations were very low.

Type 2 water is illustrated in Fig. 2 where temperatures were between 10 and 11 °C in the upper 10 m. Nutrient concentrations were lower than those recorded for Type 1 water but down to 10 m nitrate and silicate concentrations were still between 2 and 12 µg-at. NO_3-N or SiO_3-Si/l with phosphates between 1 and 2 µg-at. PO_4-P/l. At the bottom of the euphotic zone Type 1 water still prevailed and chlorophyll *a* and ATP concentrations were very low at this depth. In the upper layers of the euphotic zone chlorophyll *a* concentrations were >10 µg/l and the ATP concentration >0.5 µg/l. On this occasion the highest concentrations of these factors were measured at the surface. It appears that light inhibition had not occurred and nutrients were assimilated faster in the upper layers of the euphotic zone. The protein to carbohydrate ratio was 3.6 at 5 m and between 2 and 3 deeper in the water column.

In Fig. 3, which illustrates Type 3 water, the temperatures are shown to be in the region of 13 °C. Nutrient concentrations were very low with phosphates and nitrates being < 1 µg-at. PO_4-P or NO_3-N/l and with no silicate being detected at all. The chlorophyll *a* and ATP concentrations remained high but the carbohydrate content was much greater than the protein content resulting in a ratio of protein to carbohydrate of <1.

A comparison between the protein and carbohydrate results for Type 2 and Type 3 water showed that the protein content was similar for the two water types, but the carbohydrate content of Type 3 water was about two to three times higher than that of Type 2 water. Fractional analysis of the carbohydrates for Type 3 water is

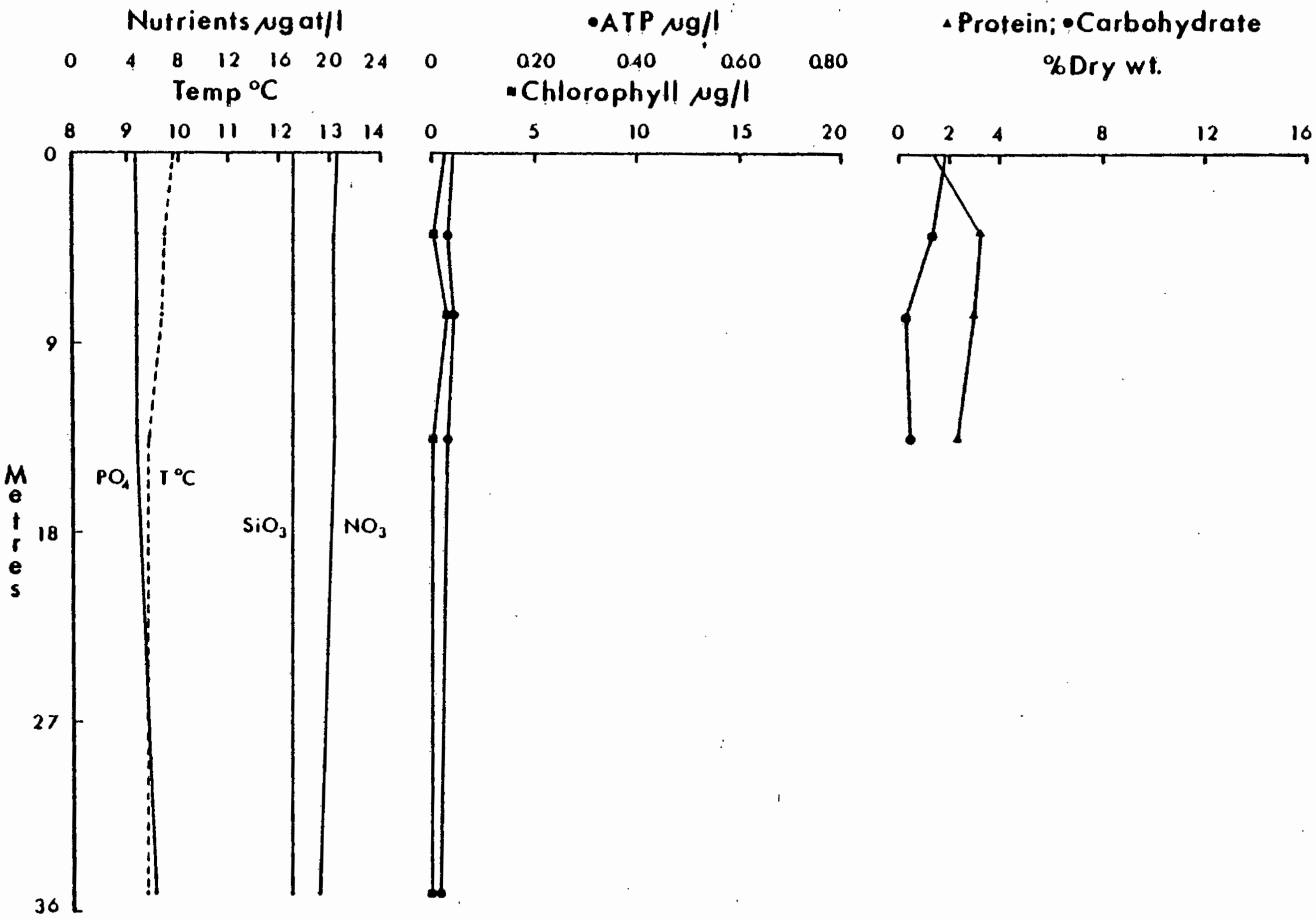

Fig. 1. Vertical distribution of environmental and physiological factors in Type I water.

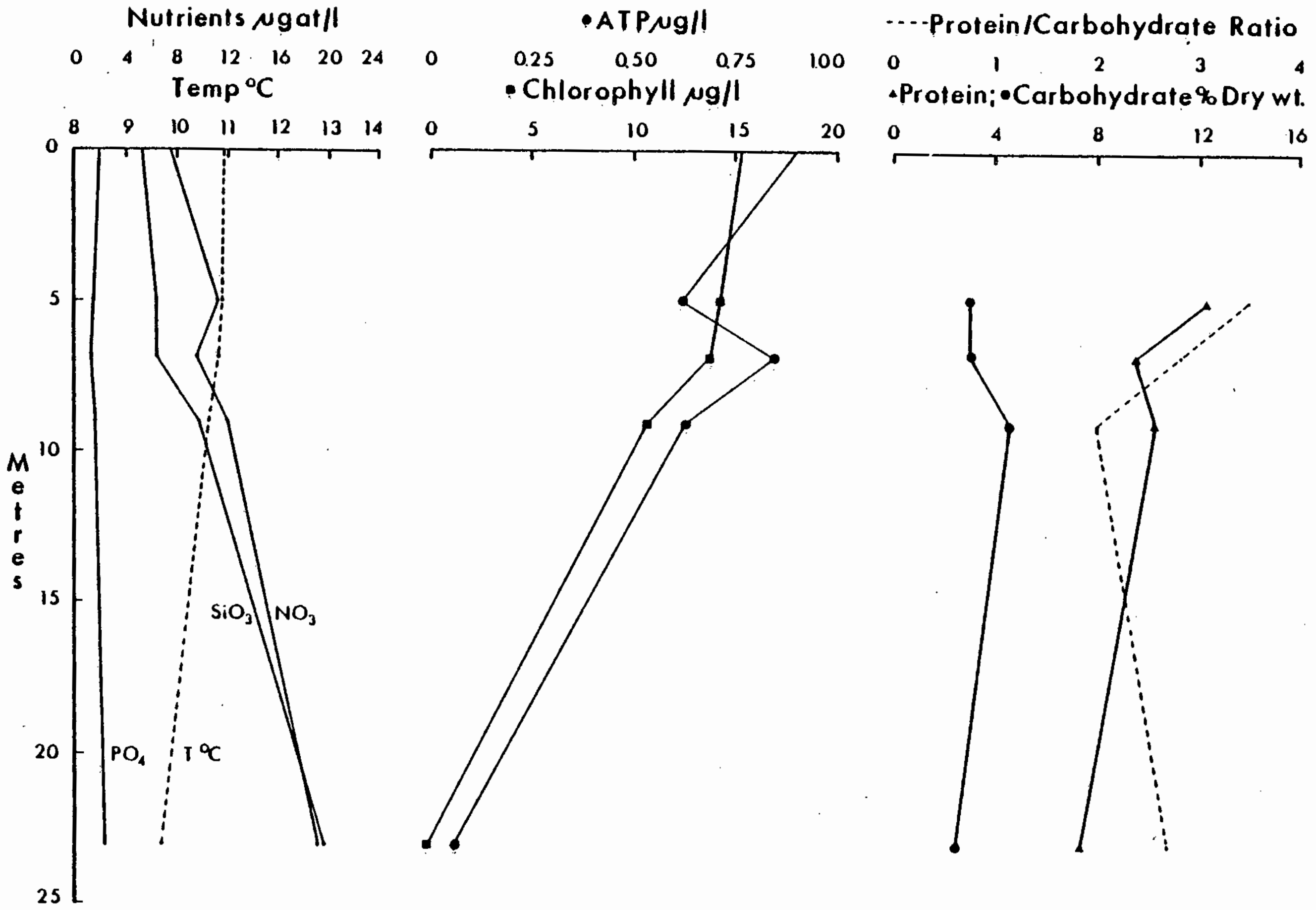

Fig. 2. Vertical distribution of environmental and physiological factors in Type 2 water.

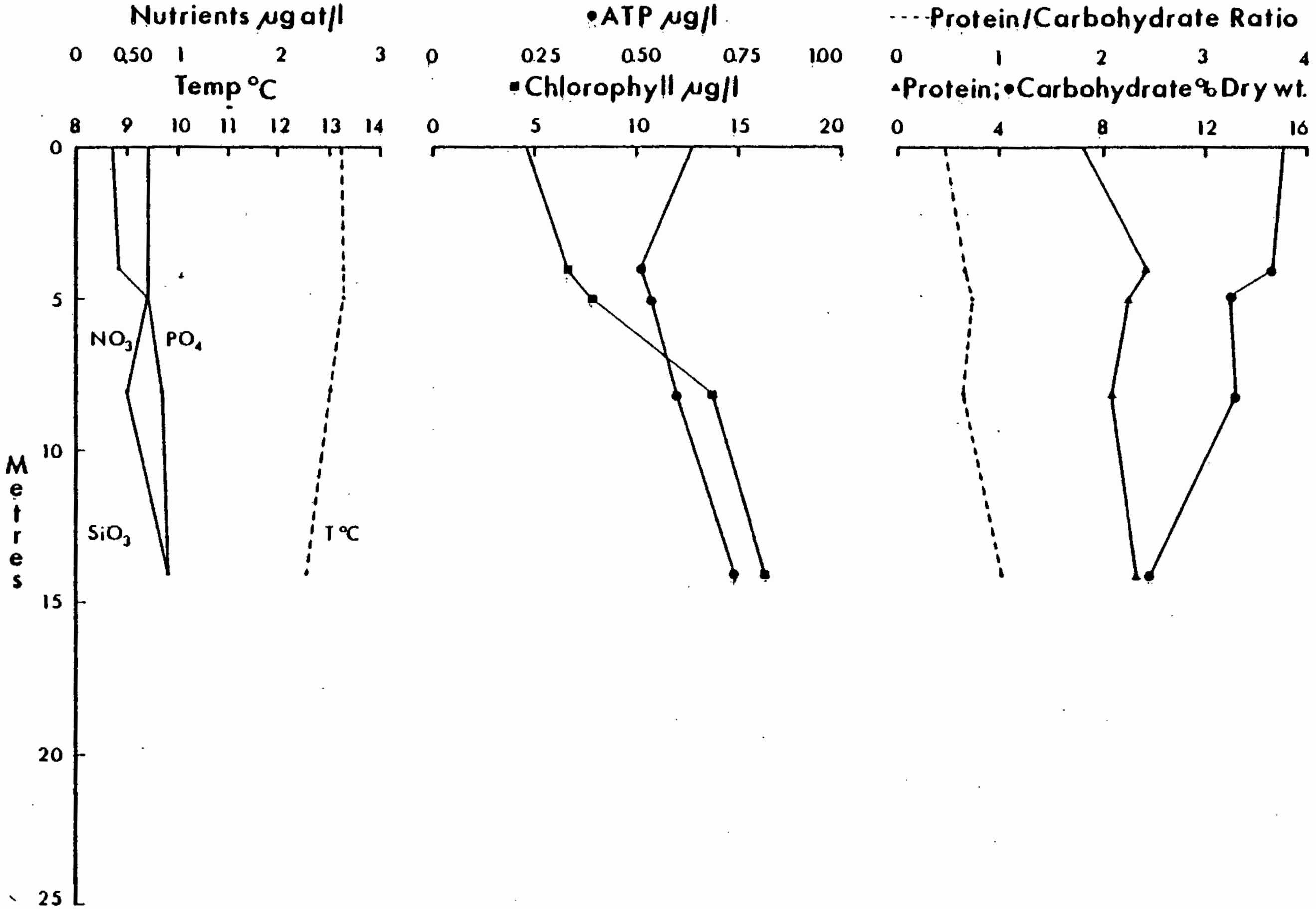

Fig. 3. Vertical distribution of environmental and physiological factors in Type 3 water.

TABLE IV

Carbohydrate composition of phytoplankton as percentage of dry matter for Type 3 water.

Depth (m)	Total carbohydrate	Acid-soluble fraction	Alkali-soluble fraction	Insoluble fraction
0	17.05	9.18	3.15	4.72
4	16.64	9.25	3.76	3.63
5	13.29	7.60	3.58	2.11
8	13.67	7.05	3.56	3.06
14	10.03	6.04	3.28	0.71

presented in Table IV and it may be observed that the acid-soluble fraction contributed over 50% to the total carbohydrate concentration. The significance of this will be discussed below.

DISCUSSION

This investigation has revealed that the changes in the nutrient and chlorophyll *a* concentrations were similar to the results from other upwelling areas (Strickland *et al.*, 1969; Estrada, 1974; Coste & Slawyk, 1974). Correlations between environmental and physiological factors indicated a relation between changes in the environment and changes in the concentration of physiological factors. Figs. 1, 2, and 3 illustrate the nature of these changes. The concentrations of chlorophyll *a* and ATP in Type 3 water were, however, at variance with the results from culture experiments where chlorophyll *a* and ATP were measured (Sakshaug, 1977; Sakshaug & Holm-Hansen, 1977), and field studies on phytoplankton pigments (Jensen & Sakshaug, 1973). These studies indicated that chlorophyll *a* and ATP concentrations decreased at low nutrient concentrations, but high concentrations of these factors were measured by the author in low nutrient waters on several occasions. A possible explanation of the author's findings is that the local phytoplankton populations had not been exposed to the low nutrient conditions for a long enough period for a significant decline in the concentrations of chlorophyll *a* and ATP to occur, even though the protein/carbohydrate ratio indicated a change in nutritional status.

The significant correlation between chlorophyll *a* and ATP compares favourably with the findings of Fiala & Jacques (1974) off North West Africa. Encountering a wide variety of ecological situations, Fiala & Jacques found a significant correlation of 0.638 between chlorophyll *a* and ATP. They concluded that ATP was closely related to phytoplankton except near the limits of the euphotic zone.

The negative correlations between chlorophyll *a* and nitrate and silicate, and between ATP and nitrate and silicate indicated that the phytoplankton communities at Oudekraal were composed predominantly of diatoms. Analysis for phytoplankton species composition, presented in Table V, confirmed that diatoms dominated the phytoplankton populations in all three types of water. *Thalassiosira aestivalis* Gran and Angst and *Skeletonema costatum* (Grev.) Cleve were the dominant species during November and December 1977.

The carbohydrate constituent was the most sensitive of the physiological factors to respond to a change from Type 2 to Type 3 water. As a result the ratio of protein/carbohydrate varied from a value >1, when nutrient availability was adequate, to <1 when nutrient concentrations were low.

TABLE V

Species composition and counts for selected days (Oudekraal, 1977).

Date	Depth (m)	Counts (cells/l)	Dominant species	Percentage of total counts	Water type
16 Nov.	0	690 000	*Thalassiosira aestivalis*	80.0	1
	7	365 000	at all depths	74.6	1
	11	500 000		78.5	1
	18	195 000		85.9	1
	36	42 500		76.5	1
17 Nov.	0	6 183 980	*Thalassiosira aestivalis*	93.5	2
	5	3 471 932	at all depths	92.9	2
	7	5 543 339		90.4	2
	9	2 542 643		91.7	2
	23	153 155		96.6	2
7 Dec.	0	817 500	*Thalassiosira aestivalis*	68.2	3
			Skeletonema costatum	18.0	
	2	2 825 000	*T. aestivalis*	35.3	3
			S. costatum	45.4	
	4.5	5 835 000	*T. aestivalis*	19.0	3
			S. costatum	32.9	
	10	1 847 500	*T. aestivalis*	46.8	3
			S. costatum	37.7	

Variations in the protein and carbohydrate composition of diatoms due to nutrient depletion have been indicated by other studies. Antia *et al.* (1963) found a marked increase in the carbohydrate content of phytoplankton grown in a large plastic sphere when the amount of available nitrogen was depleted, while the amount of protein as a percentage of algal weight decreased. Myklestad & Haug (1972), working with a diatom culture, showed that the proportion of acid-soluble carbohydrate was relatively low when adequate nutrients were available, but this fraction increased rapidly with the depletion of nitrate from the growth medium. This led to pronounced variations in the ratio of protein to carbohydrate. A study on the natural phytoplankton population in the Trondheimsfjord by Haug *et al.* (1973) produced similar results. The development of a spring bloom was characterized by a rapid decrease in the protein/carbohydrate ratio, with the concentration of nitrate in the surface layers rapidly decreasing during the same period.

The results of this study indicated that the physiological factors selected for study were sensitive to changes in the environment. While the chlorophyll *a* and ATP concentrations were low in Type 1 water and high in Type 2 water a similar significant difference between Type 2 and Type 3 waters was not observed. The ratio of protein to carbohydrate, however, did show significant differences between Type 2 and Type 3 waters. This ratio is, therefore, being used in further investigations of the growth patterns of phytoplankton in the Cape Peninsula upwelling region.

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APPENDIX 2

Fish. Bull. S. Afr. 14 (in press) 1981

ANALYSIS OF CHLOROPHYLL *a* IN PARTICULATE MATTER

M.J. SWART and R.G. BARLOW

A procedure to determine total chlorophyll *a* and active chlorophyll *a* in particulate matter is described. Pigments were extracted in 90 per cent acetone and total chlorophyll *a* determined from absorbance measurements at 665 nm. Active chlorophyll *a* was extracted into hexane by partitioning pigments between aqueous acetone and hexane according to the method of Whitney and Darley (1979). Absorbance measurements at 663 nm were made on the hexane extract before and after acidification with 5N hydrochloric acid and the decrease in absorbance was used to determine the amount of active chlorophyll *a*. Calibration curves, prepared with pure chlorophyll *a* standards, were used in the calculation of chlorophyll *a* concentration in preference to the equation proposed by Strickland and Parsons (1972).

Die metode vir die bepaling van totale chlorofil *a* en aktiewe chlorofil *a* in partikulêre materiaal word hier beskryf. Die pigmente is ge-ekstraleer in 90 persent asetoon. Totale chlorofil *a* is bepaal deur middel van spektrofotometriese absorpsie by 665 nm. Aktiewe chlorofil *a* is ge-ekstraleer in heksaan deur middel van pigment skeiding tussen die waterige asetoon oplossing en heksaan soos beskryf deur Whitney en Darley (1979). Absorpsie bepalings is gedoen op die heksaan ekstraksie by 663 nm voor en na aansuring met 5N soutsuur. Die daling in absorpsie lesing is gebruik om die hoeveelheid aktiewe chlorofil *a* te bepaal. Kalibrasiekurwes is vooraf opgestel met suiwer chlorofil *a* standaarde. Die kurwes is dan gebruik by die berekening van die chlorofil *a* konsentrasie. Voorkeur is aan hierdie berekening gegee bo die formule voorgeskryf deur Strickland en Parsons (1972).

The chlorophyll *a* method, described by Strickland and Parsons (1972), is widely used for determining phytoplankton biomass in natural environments, despite the interference of inactive chlorophyll products which absorb at the same wavelengths as chlorophyll *a*. A method has been developed to correct for the presence of phaeophytin *a* and phaeophorbide *a* (Lorenzen 1967), but interference from chlorophyllide *a*, which is measured as chlorophyll *a*, has been reported by Jeffrey (1974). Quantitative thin-layer chromatography was used by Jeffrey (op. cit.) for accurate measurements of chlorophyll a in coastal waters, but the method being too laborious for routine use, she suggested the introduction of a phase separation technique to improve current methodology.

Parsons (1963) developed a method for the microdetermination of chlorophyll c, in which interfering chlorophylls and carotenoids were removed by partitioning the pigments between aqueous acetone and hexane. Whitney and Darley (1979) reported an adaptation of this method which allows more accurate determination of chlorophyll a. The hexane hyperphase contains the carotenes, chlorophyll a and b, and phaeophytin a and b. The aqueous hypophase contains chlorophyll c, some of the carotenoids and the chlorophyll degradation products which lack the phytol chain (chlorophyllide a and b, and phaeophorbide a and b). Therefore, partitioning with hexane removes two classes of inactive products from the active chlorophylls. Active chlorophyll a can then be determined in the hexane layer, in the presence of phaeophytin a, by a slight modification of the method of Lorenzen (1967).

In this study, the method of Whitney and Darley (1979) has been used to determine active chlorophyll a, but a new technique has been developed for measuring total chlorophyll a. This report deals with the techniques used to determine active and total chlorophyll a in the same sample, with the objective of using the results to assess the physiological state of local phytoplankton communities.

METHODS

Reagents

90%(v/v) Acetone	- prepared from Analytical grade, BDH Chemicals Ltd.
Hexane	- Analytical grade (67°-70°C Boiling range), BDH Chemicals Ltd.
Anhydrous sodium sulphate	- Analytical grade, BDH Chemicals Ltd.
0.0085N Sodium chloride	- prepared from Analytical grade, BDH Chemicals Ltd.
5N Hydrochloric acid	- prepared from Analytical grade, BDH Chemicals Ltd.
Standard	- 99% chlorophyll a, Sigma Chemical Co.

Procedure

A total of 1-2ℓ of seawater was filtered through a 4.7 cm Whatman GF/F glass-fibre filter, precovered with 2 mℓ of 10%(w/v) $MgCO_3$. Immediately after filtering, the filter was folded, frozen in liquid nitrogen, wrapped in aluminium foil, labelled, and stored in liquid nitrogen until analysed.

The frozen filter was allowed to thaw at room temperature, then placed in a centrifuge tube with 12 mℓ of 90-per-cent acetone and, in the dark, ultrasonically agitated at 45kHz for 20 minutes. The filter was centrifuged at 3 800 x g (15°C) for 15 minutes and approximately 10 mℓ of the supernatant drawn off for assay.

For total chlorophyll a determination, a 3 mℓ aliquot of the acetone extract was decanted into a 1-cm cuvette and the absorbances measured against a 90-per-cent acetone blank at 750 nm and 665 nm in a spectrophotometer. The absorbance at 750 nm was subtracted from the absorbance at 665 nm to correct for turbidity interference. The total chlorophyll a in 10 mℓ of extract was determined from the calibration curve and corrected by multiplying by 1.2 to give the quantity in the original 12 mℓ extract. The concentration per litre of seawater was then calculated.

In order to determine the active chlorophyll a, 9 mℓ of the acetone extract was first transferred to a 50-mℓ separation funnel by draining the tube straight into it. After adding 3.5 mℓ of 0.0085N sodium chloride and 13.5 mℓ of hexane and gently mixing for one minute, the phases were allowed to separate and stabilize for 3-5 minutes. The lower, aqueous phase and the interface layer were drained from the funnel, and 10 mℓ of the hexane phase was collected and dried with 0.5 g of anhydrous sodium sulphate. The absorbance of the hexane extract (in a 1-cm cuvette) was read against a hexane blank at 750 nm and 663 nm in a spectrophotometer. In order to convert the active chlorophyll a to phaeophytin a, 5 mℓ of the hexane extract was acidified with 40 μℓ of 5N hydrochloric acid and the solution well mixed. The absorbances were again read at 750 nm and 663 nm.

All absorbance readings at 663 nm were corrected for turbidity interference, measured at 750 nm, and the difference in absorbance readings between non-acidified and acidified solutions was calculated. Active chlorophyll a was determined from the calibration curve and multiplied by 1.33 to give the quantity in the original extract. The factor of 1.33 was applied because only 9 mℓ of the original 12 mℓ acetone extract was used in the partition step. The concentration of active chlorophyll a per litre of seawater was then calculated.

Standard solutions were prepared with 5, 10, 15 and 20 μg chlorophyll a.10mℓ$^{-1}$ 90-per-cent acetone. Absorbances were measured at 750 nm and 665 nm and a standard curve prepared for determining total chlorophyll a concentration. The standard solutions were then extracted into hexane and the absorbances measured at 750 nm and 663 nm, before and after acidification with 5 N hydrochloric acid. A standard curve relating the decrease in absorbance to

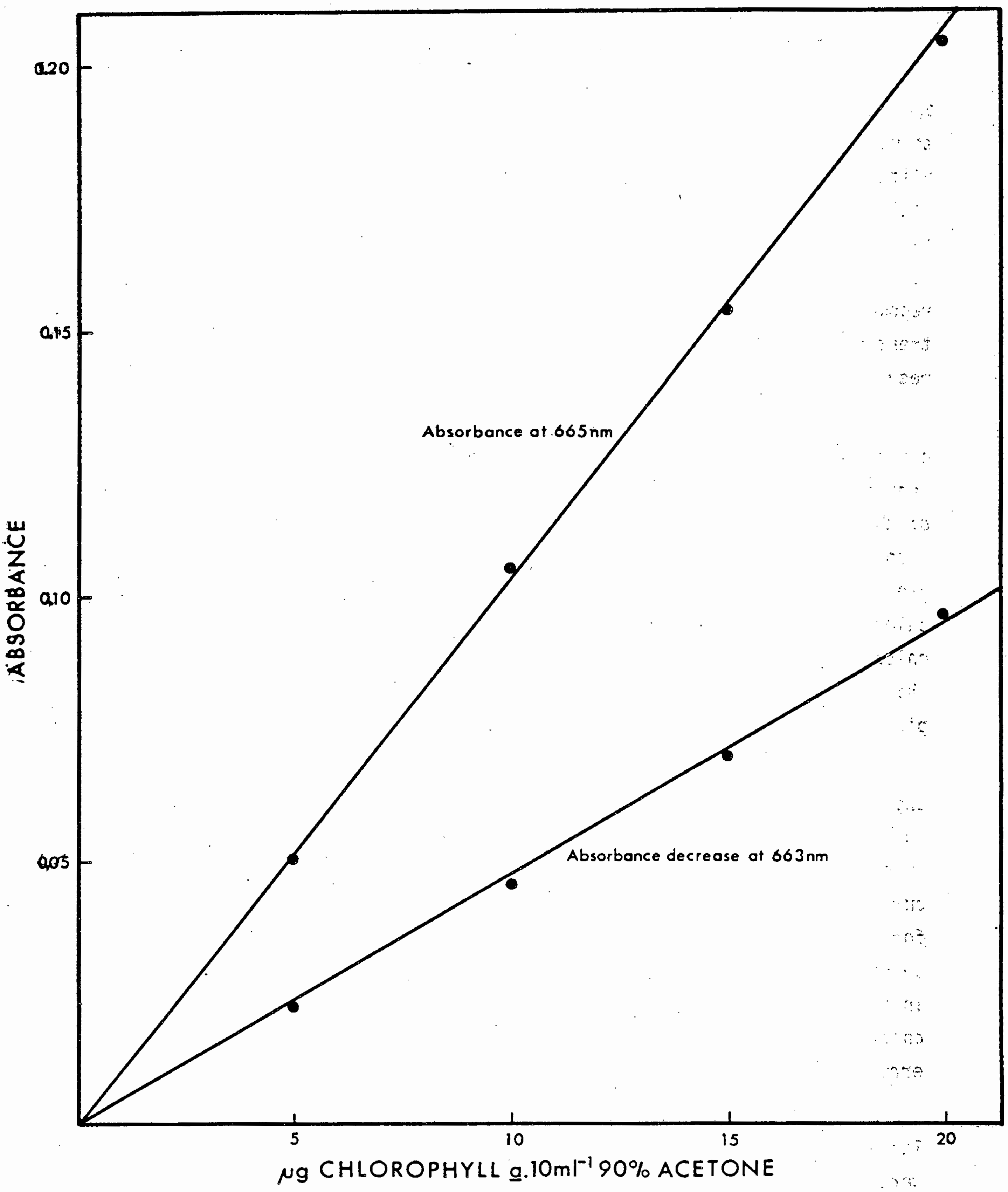

Fig. 1. Calibration curves for total and active chlorophyll a determination.

chlorophyll a concentration was prepared for determining the concentration of active chlorophyll a.

RESULTS AND DISCUSSION

Strickland and Parsons (1972) recommended that samples for chlorophyll analysis should be extracted immediately after filtering, but this was found to be impractical on the small research vessel used for sample collection. Filters were stored at -20°C in initial studies, but further tests revealed that the storage of filters in liquid nitrogen gave the highest results. The extraction of chlorophyll can be aided by initially grinding the phytoplankton cells in the dark for 20 hours (Strickland and Parsons op.cit.) However, the recommendation of the SCOR/Unesco Working Group 17 (1966) that ultrasonic treatment be employed was incorporated, and this technique reduced the time required for extracting pigments to 20 minutes.

The calibration curves for determining total and active chlorophyll a are illustrated in Figure 1. A special scan of the standard solution indicated that, in acetone, the pigment absorbed maximally in the red region of the visible spectrum at 665 nm, but at 663 nm when the pigment was extracted into hexane. The acidification step used in this method was similar to that used by Lorenzen (1967) with a reduction in absorbance being recorded when chlorophyll a was converted to the phaeophytin form. The phaeo-forms of chlorophyll a do not show a reduction in absorbancy when treated with acid and this enables chlorophyll a to be determined in samples containing phaeo-pigments.

Trichromatic equations were recommended by Strickland and Parsons (1972) and the SCOR/Unesco Working Group 17 (1966) for calculating chlorophyll a concentrations. However, it is felt that, with the availability of pure chlorophyll a, more accurate estimates can be made by preparing calibration curves, and this opinion is held by others (C.S. Yentsch, Bigelow Laboratory for Ocean Sciences, U.S.A., personal communication). Under the conditions described, the methods can be expected to be accurate within 3-per-cent. This figure was obtained by plotting the results and comparing the amount of chlorophyll actually present with that indicated by the plot. The percentage errors are shown in Table I.

Some chlorophyll a determinations from field samples are presented in Table II. The results show that all the active chlorophyll a concentrations were lower than total chlorophyll a. This was expected,because inactive forms of the pigment would be produced by rapidly growing phytoplankton

TABLE I : Accuracy of the chlorophyll *a* methods

Assay	Absorbance	Taken ($\mu g.10m\ell^{-1}$)	Found ($\mu g.10m\ell^{-1}$)	Error (%)
Total chlorophyll *a*	0.051	5.00	5.00	0
	0.105	10.00	10.25	2.5
	0.154	15.00	15.00	0
	0.204	20.00	19.85	0.7
Active chlorophyll *a*	0.023	5.00	4.87	2.6
	0.046	10.00	9.68	3.2
	0.071	15.00	15.00	0
	0.097	20.00	20.46	2.3

TABLE II : Chlorophyll *a* concentrations, Robben Island (12 km north west of Cape Town) October 1979

Station	Depth (m)	Total chlorophyll *a* ($\mu g.\ell^{-1}$)	Active chlorophyll *a* ($\mu g.\ell^{-1}$)
1	0	8.97	3.14
	2.5	8.84	4.57
	5.5	3.36	2.29
	8.5	3.36	2.51
2	0	8.09	4.29
	1.5	9.34	4.29
	3	9.45	5.72
	5.5	9.22	5.14
	11	8.47	4.86

communities in euphotic waters (Whitney and Darley 1979). In further investigations it is proposed to assess the value of the active chlorophyll a/ total chlorophyll a ratio as an indicator of the physiological response of phytoplankton to changes in the environment.

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APPENDIX 3

Fish. Bull. S. Afr. 14 (in press) 1981

ANALYSIS OF ADENOSINE TRIPHOSPHATE IN PARTICULATE MATTER

R.G. BARLOW

A simple method is described for determining ATP using firefly luciferin-luciferase and a liquid scintillation counter. The procedure may be used to determine ATP in particulate matter in the range 2×10^{-7} to 10×10^{-10} mole. It was found that samples preserved in liquid nitrogen yielded the highest recoveries of ATP.

'n Eenvoudige metode word beskryf vir die bepaling van ATP met vuurvleig lusifirien-lusiferasie deur middel van 'n Beta sintilasieteller. Die metode kan gebruik work vir die bepaling van ATP in partikulêre materie in die bestek van 2×10^{-7} tot 10×10^{-10} grammolekuul. Daar is bevind dat monsters wat in vloeibare stikstof bewaar was 'n hoër opbrengs van ATP getoon het.

In studies of phytoplankton production and food-chain dynamics, a measure of the living biomass of particulate matter suspended in the sea is often desired. Such measurements can be made microscopically, but this is time consuming and it is easier and quicker to measure some chemical constituent that is present in all living cells though not associated with detrital material. Adenosine triphosphate (ATP) is a cellular constituent that fulfils these requirements.

ATP measurements have been used locally to quantify living biomass in physiological studies of local phytoplankton communities. Holm-Hansen and Booth (1966) developed the ATP method, but the techniques reported by Strickland and Parsons (1972) form the basis of the method used here.

The method involves the oxidation of a substrate, luciferin, by an enzyme, luciferase, and the emission of light in the process. ATP is required as an energy source in the reaction and the quantity of luminescence is proportional to the amount of ATP present. Strickland and Parsons (1972) described a laboratory-built apparatus that measured the emitted light but, in this study, use was made of a commercial liquid scintillation counter to measure the luminescence. Other adaptations of the method for application to local conditions were also made and are reported.

METHODS

Reagents

Tris buffer	-	0.02M;pH7.75; prepared from AnalaR Tris (hydroxymethyl) methylamine, BDH Chemicals Ltd.
Enzyme mixture	-	Firefly lantern extract, FLE-50, Sigma Chemical Co.
Standard	-	Sigma grade disodium adenosine triphosphate, Sigma Chemical Co.

Procedure

Half a litre of seawater was filtered through a 4.7 cm Whatman GF/F glass-fibre filter. Immediately after filtering, the filter was folded, frozen in liquid nitrogen, wrapped in aluminium foil, labelled, and stored in liquid nitrogen until analysed.

An amount of 5 mℓ of boiling Tris buffer was pipetted into a 150-mℓ beaker (covered with a watch glass) on a boiling water bath, and ATP was extracted by placing the frozen filter in this boiling solution for five minutes. The extract was transferred to a clean dry tube and the filter extracted with a further 3 mℓ of boiling buffer for two minutes. The extracts were combined, cooled, made up to 8 mℓ with distilled water, frozen in liquid nitrogen, and stored at -20°C until analysed.

The enzyme mixture was prepared by extracting 50 mg of firefly lantern extract with 5 mℓ of Tris buffer for 2-3 hours at room temperature. The mixture was centrifuged at 300-400 x g (room temperature) for two minutes and the supernatant decanted into a clean dry tube. This extract was allowed to stand for a further hour before diluting to 10 mℓ with Tris buffer. The diluted preparation was used within three hours.

Standards were freshly prepared for each analysis with 0.1; 0.05; 0.01; 0.005; 0.0005 μg ATP.$m\ell^{-1}$ Tris buffer, including a blank (1 mℓ Tris buffer) for background counts. Aliquots (1 mℓ) of blanks, standards and sample extracts were added to a series of 8 mℓ glass scintillation vials in counting racks suitable for a Packard Prias liquid scintillation counter. An empty vial was placed in the first position of each rack and the counter set in the single count automatic mode, out of coincidence, with counting windows appropriately set for optimum counting efficiency. The counter was programmed to count each vial for six seconds.

TABLE I : ATP concentrations in particulate matter from 20ℓ of surface water; Sea Point, August 1977.

Sample number	ATP($\mu g.\ell^{-1}$)	
	Freshly extracted	Frozen before extraction
1	0.199	
2	0.103	
3	0.122	
4	0.127	
5	0.166	
6	0.100	
7		0.235
8		0.219
9		0.281
10		0.227
11		0.268
12		0.180

As soon as the printout of counts from the empty vial began, 0.2 mℓ of enzyme was injected into the next vial through a small hole drilled in the cap. The contents of the vial were swirled briefly and the vial inserted into its appropriate position in the counting rack. Counting commenced 27 seconds after the addition of the enzyme. The count was an integral measurement of the area under the luminescent decay curve between 27 and 33 seconds after the start of the reaction. Subsequent samples were reacted and counted at a similar time interval. Duplicate analyses on the extract from each filter were performed.

The background count of the blank determination was subtracted from the counts of all samples and standards to obtain net counts. Net counts and standard concentrations were transformed to logarithmic functions, and equations of the form $\ell n \underline{y} = a + b \ell n \underline{x}$, relating counts to standards, were formulated for each set of standards. The concentration of ATP in each sample was calculated from either of these equations.

RESULTS AND DISCUSSION

ATP must be extracted immediately after filtering to prevent the degradation of ATP, but extraction at sea was found to be totally impractical on the small research vessel used for collection of samples. Therefore, samples were frozen in liquid nitrogen immediately after filtering and held in this liquid at -190°C until extracted. The frozen filters were then extracted in boiling buffer in a shore-based laboratory. The results presented in Table I show that frozen samples gave a higher ATP yield than freshly extracted samples.

In the original method (Holm-Hansen and Booth 1966), the amount of emitted light was determined by tracing the decay curves with a pen recorder and then measuring the area under the curves. The utilization of liquid scintillation counter improved the procedure by recording the area under the decay curves as an integral count of the emitted luminescence. Furthermore, the pen recording technique involved adjustment of a range-selector sensitivity switch each time a sample was assayed. With the scintillation counter, counting windows were preset to optimize counts within the range of the standards.

It was necessary to calculate two standard equations for each set of standards. The standard concentration range from 0.0005 to 0.1 μg ATP.mℓ$^{-1}$ Tris buffer was found to be too wide to be adequately expressed by one equation and an inflection point at a concentration of 0.01 μg ATP.mℓ$^{-1}$ buffer was estimated. An equation covering the concentration range from 0.0005 to 0.01

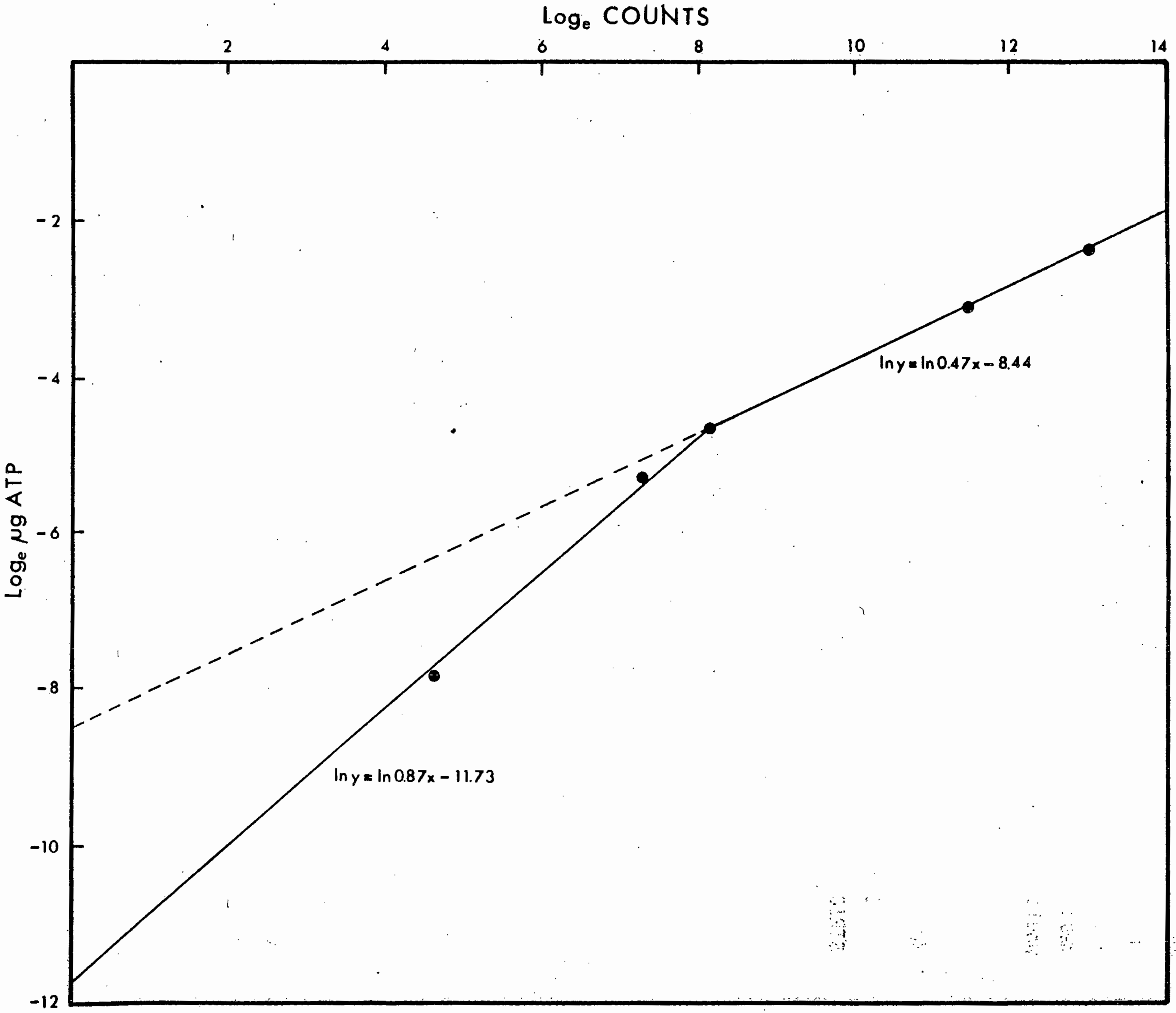

Fig. 1. Standard equations for ATP determination.

μg ATP.mℓ^{-1} buffer was therefore formulated and another for the range from 0.01 to 0.1 μg ATP.mℓ^{-1} buffer. Figure 1 illustrates these standard equations.

ATP measurement in the ocean estimates total living biomass which includes phytoplankton, zooplankton and bacteria. Therefore, in surface waters, ATP needs to be related to other parameters, such as chlorophyll a, when it is used in phytoplankton studies. Probably the most useful application of ATP measurements would be estimating bacterial biomass in deep water, where there would be no contribution from living plankton.

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APPENDIX 4

Fish. Bull. S. Afr. 14 (in press) 1981

ANALYSIS OF PROTEIN AND CARBOHYDRATE IN PARTICULATE MATTER

R.G. BARLOW and M.J. SWART

Modifications to published methods for determining protein and carbohydrate in particulate matter are described. Samples were collected on glass-fibre filters and preserved by freeze-drying. For protein anaylsis, dry filters were extracted twice with 0.1N sodium hydroxide at 80°C for 10 minutes and the protein precipitated with 10 per cent trichloroacetic acid in the presence of 1 mg of sodium deoxycholate. The precipitated pellet was then reacted with Folin-Ciocalteu reagent according to the procedure of Lowry et al. (1951). Carbohydrates were fractionated into an acid-soluble fraction, an alkali-soluble fraction and an insoluble fraction. The carbohydrate content in each fraction was determined by the phenol-sulphuric acid method of Dubois et al. (1956) and total carbohydrate calculated as the sum of the three fractions. The accuracy of the methods and the reasons for introducing the various modifications are discussed.

Die aanpassings in metodes vir die bepaling van proteiën en koolhidrate in partikulêre materie word hier beskryf. Monsters is op glasvesel filtreerpapier versamel en bewaar deur middel van vriesdroging. Vir proteiën analises is die gedroogde filter twee keer agtereenvolgens ge-ekstraheer met 0.1N natriumhidroksied teen 80°C vir 10 minute. Die proteiën neerslag is gevorm met 10 persent trichloorasynsuur in 'n 1 mg natriumdeoxychloor medium. Die neerslag word dan met Folin-Ciocalteu reagens gereageer volgens die metode beskryf deur Lowry et al. (1951). Koolhidrate is afgebreek tot suur-oplosbare, alkali-oplosbare en onoplosbare fraksies. Die koolhidrate inhoud in elke fraksie is bepaal deur middel van die phenol-swawelsuur metode van Dubois et al. (1956). Die totale konsentrasie van koolhidrate is bereken as die som van die fraksies. Die akkuraatheid van die metodes en die redes vir die aanpassings gemaak, word hieronder bespreek.

In primary production studies in the Cape Peninsula upwelling region the physiological response of phytoplankton communities to changes in the environment has been monitored. Analysis of samples for their protein and carbohydrate content revealed that the protein/carbohydrate ratio was a useful parameter for monitoring this response, particularly with respect to changes in extracellular nutrient concentration. The analytical procedures were based on published methods, but this paper reports certain modifications that were introduced to adapt the methods for local conditions.

The extraction of protein was based on the procedure described by Mayzaud and Martin (1975), but a lower concentration of the extracting solution and a shorter extracting time were employed. The precipitation of protein and the colour reaction using the Folin-Ciocalteu phenol reagent follows the method of Bensadoun and Wienstein (1976). However, the final concentration of the precipitating reagents had to be increased twofold to ensure complete precipitation of the protein.

Carbohydrates were fractionated into an acid-soluble fraction (soluble polysaccharides), an alkali-soluble fraction (short and medium length saccharides) and an insoluble fraction (insoluble polysaccharides) according to the method of Haug _et al_. (1973). Modifications to the length of extracting time and the determination of total carbohydrate were introduced to adapt the method for samples collected on glass-fibre filters. The carbohydrate content in each fraction was determined by the phenol-sulphuric acid method of Dubois _et al_. (1956).

Details of the methods are described together with a discussion of the reasons for introducing the various modifications.

METHODS

The collection of samples at sea was performed by filtering 1-2ℓ of seawater through a 4.7 cm Whatman GF/F glass-fibre filter. Immediately after filtering, the filter was folded, frozen in liquid nitrogen, wrapped in an aluminium jacket (15 x 50 mm), labelled, and freeze-dried.

Protein determination

Reagents - 0.1N sodium hydroxide
2%(w/v) sodium deoxycholate
20%(w/v) trichloroacetic acid (TCA)
A- 2%(w/v) sodium carbonate in 0.1N sodium hydroxide
B-0.5%(w/v) cupric sulphate in 1%(w/v) sodium citrate
C-1mℓ of B mixed with 50 mℓ of A just before use
Folin-Ciocalteu reagent- diluted 1:1 with water just before use
Standard - Bovine serum albumin
All reagents were prepared with analytical grade chemicals,
BDH Chemicals Ltd.

Procedure - Just prior to the extraction step, the contents of the burette used to measure the volume of the 0.1N sodium hydroxide extracting solution was run back into the reservoir bottle and the solution was then well mixed. Each dry filter was ultrasonically agitated at 45 kHz for 10 minutes in 10 mℓ of extracting solution before being heated at 80°C for 10 minutes to extract the proteins. After cooling, the filter was centrifuged at 3 800 x g (15°C) for 20 minutes, the supernatant removed and the filter dried by pressing a filter stick, attached to a vacuum line, down onto the filter and sucking dry. The filter was then re-extracted with 10 mℓ of 0.1N sodium hydroxide at 80°C for 10 minutes, cooled and centrifuged. The second supernatant was removed and the filter discarded.

Duplicate 1 mℓ aliquots of both supernatants were diluted to 2 mℓ with distilled water, 0.05 mℓ of sodium deoxycholate was added and, after mixing, the solutions were allowed to stand at room temperature for 15 minutes. To each solution, 2 mℓ of TCA was then added and the solutions centrifuged at 3 800 x g (15°C), for 45 minutes. The supernatants were gently removed from the precipitated pellets by suction through a Pasteur pipette attached to a vacuum line. Care was taken not to remove a fine precipitate that collected at the surface.

The precipitated pellets were dissolved in 3 mℓ of reagent C and then reacted with 0.3 mℓ of Folin-Ciocalteu reagent. After mixing, the colour of the reaction mixtures were allowed to develop for 45 minutes in the dark. Absorbances were read against a blank in a 1-cm cuvette at 660 nm in a spectrophotometer. The amount of protein was determined from a calibration curve and total protein calculated as the sum of the protein content in each extract.

Standard solutions were prepared with 5, 10, 20, 30, 40 and 50 µg of bovine serum albumin per 1 mℓ of 0.1N sodium hydroxide, whereas blank solutions contained 1 mℓ of sodium hydroxide only. In preparing calibration curves, standards and blanks were treated with the precipitating and colour reagents as described above.

Carbohydrate determination

Reagents -
0.1N sulphuric acid
29N sulphuric acid
Sulphuric acid, specific gravity 1.84
0.1N sodium hydroxide
5%(w/v) phenol
Standard - D-glucose
All reagents were prepared with analytical grade chemicals,
BDH Chemicals Ltd.

Procedure - Each dry filter was ultrasonically agitated at 45 kHz for 10 minutes in 10 mℓ of 0.1N sulphuric acid and allowed to stand at room temperature for four hours. After centrifuging at 3800 x g (15°C) for 20 minutes, the supernatant was transferred to a clean tube and the filter dried by pressing a filter stick (pore size 15-40 µm), attached to a vacuum line, down onto the filter and sucking dry. The filter stick was stored in a saturated solution of sodium tetraborate to prevent clogging of the porous glass.

The dried filter was resuspended in 10 mℓ of 0.1N sodium hydroxide and the mixture allowed to stand at room temperature for a further four hours. After centrifuging, the supernatant was removed and the filter dried once more.

To the dry filter was added 1 mℓ of 29 N sulphuric acid, and extraction for insoluble carbohydrate allowed to proceed at room temperature for 20 hours. The mixture was diluted to 5 mℓ with distilled water (in an ice bath), centrifuged, and the supernatant removed.

While thoroughly mixing, 1 mℓ of 5-per-cent phenol was added to duplicate 1 mℓ aliquots of each supernatant. If required, an appropriate dilution was made to dilute the concentration of carbohydrates into the range of the calibration curve. Suplhuric acid (5 mℓ) was rapidly added to the centre of the assay solution to ensure a fast exothermic reation. Caution was exercised in handling the tubes to prevent spillage of the hot solutions. After mixing, the solutions were cooled and the absorbances read against a blank in a 1-cm cuvette at 490 nm in a spectrophotometer. The concentration of carbohydrate in each fraction was determined from a calibration curve, and total carbohydrate calculated as the sum of the three fractions.

Standard solutions were prepared with 10, 20, 30, 40 and 50 µg of glucose per 1 mℓ of distilled water, whereas blank solutions contained 1 mℓ of distilled water only. In preparing calibration curves, standards and blanks were treated with phenol and sulphuric acid as described above.

RESULTS AND DISCUSSION

Mayzaud and Martin (1975) and Haug et al. (1973) collected samples by towing a net horizontally through the surface layers of coastal waters. Weighed portions of freeze-dried particulate matter were then analysed for their protein or carbohydrate content. In the present study, samples were collected from discrete depths in the water column, and it was therefore more convenient to filter water samples through glass-fibre filters to concentrate the plankton. This sampling procedure necessitated a few changes in the analytical techniques.

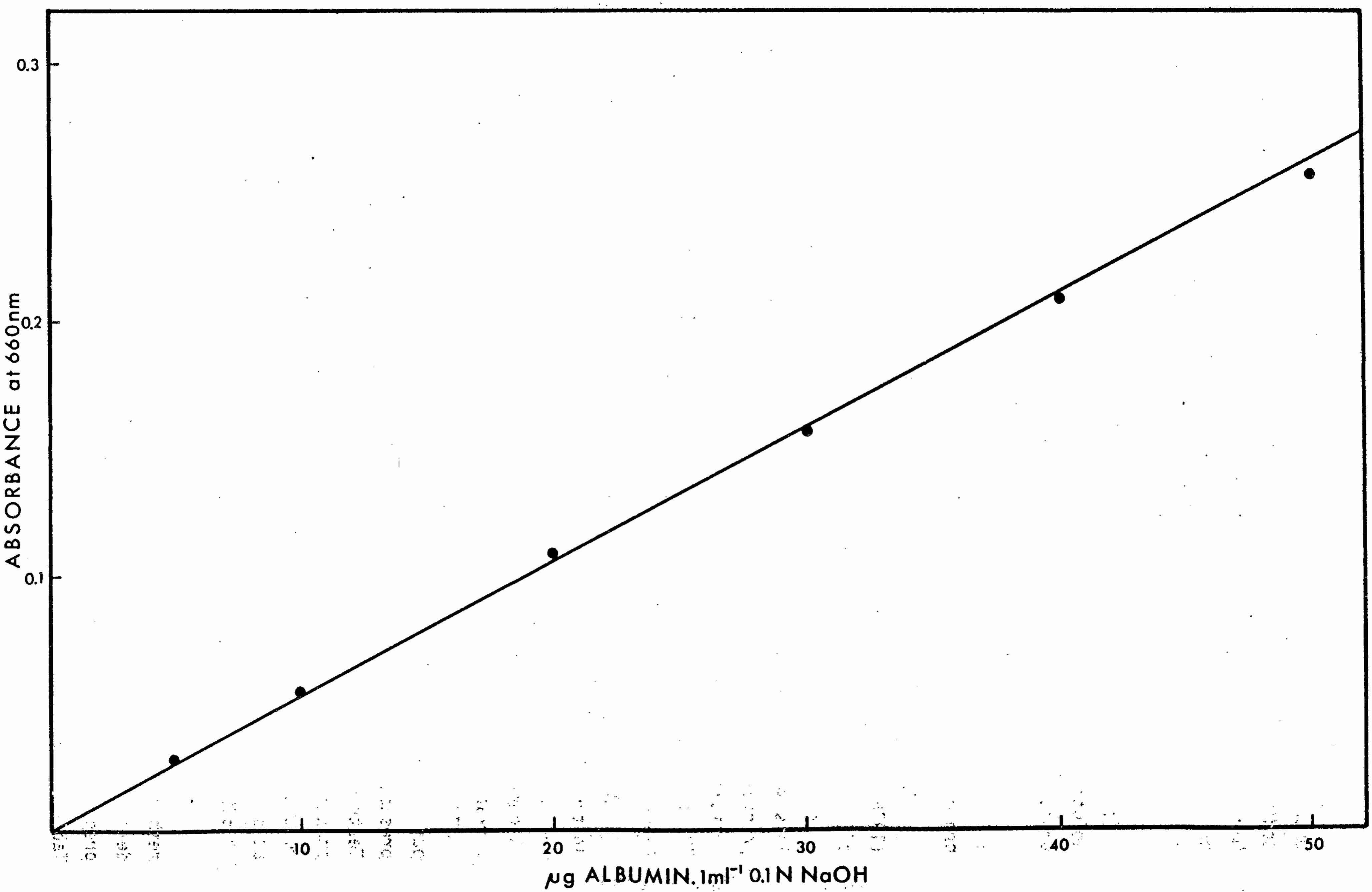

Fig. 1. Calibration curve for protein determination.

In preliminary tests on the protein method, various strengths of the extraction solution and different extractive periods were examined. The 1N sodium hydroxide solution and 30-minute extraction time used by Mayzaud and Martin (1975) were found to be unsatisfactory because they gave low yields of protein. Extraction in 0.1N sodium hydroxide at 80°C for 10 minutes gave the best results for samples with a low protein content. However, extraction from these low-protein samples for longer than 10 minutes gave a lower yield. This may have been the result of protein being degraded in the alkaline solution during longer extraction periods. For samples with high levels of protein, 10 minutes was found to be insufficient to extract all the protein, and longer extraction times had to be employed. To compromise between these extremes, all samples were extracted twice for 10 minutes in order to determine the total protein content.

In order to precipitate proteins out of solution, Bensadoun and Weinstein (1976) used a final TCA concentration of 6-per-cent for a concentration of 500 µg of sodium deoxycholate per 4 mℓ of reaction mixture. In the present study in standards prepared in 0.1N sodium hydroxide, it was found that these concentrations were too low to precipitate out all the protein. Various strengths of the precipitating reagents were therefore tested for efficiency of protein precipitation. The addition of 1 mg of sodium deoxycholate and 2 mℓ of 20-per-cent TCA to 2mℓ of standard solution was found to give the highest protein recovery. Higher concentrations did not improve the yield of protein.

A wavelength of 750 nm was used to read the absorbances of the assay mixture in the original protein method developed by Lowry *et al.* (1951). This wavelength was optimum for the range 5 - 25 µg of protein. Lowry *et al.* (*op. cit.*) suggested that, for stronger solutions, absorbance readings could be kept in a workable range by reading near a wavelength of 500 nm. Bensadoun and Weinstein (1976) used a wavelength of 660 nm, approximately midway between 500 and 750 nm, but as they gave no specific reasons for using the shorter wavelength it was presumed that 660 nm was selected for a particular range of protein concentrations. Tests during this study revealed that, at 750 nm, the absorbance readings of standard solutions were 10-per-cent higher than the readings determined at 660 nm. However, the calibration curve prepared at 750 nm was not quite linear, an observation noted by Lowry *et al.* (*op.cit.*). Linearity was obtained for curves prepared at 660 nm and it was decided to use this wavelength in the assay procedure.

A calibration curve for protein analysis is illustrated in Figure 1. The method described can be expected to be accurate to within 4-per-cent. This was determined by plotting the results and comparing the amount of protein actually present with that indicated by the plot. The percentage errors are shown in Table I.

TABLE I : Accuracy of the protein and carbohydrate methods

Assay	Absorbance	Taken ($\mu g.m\ell^{-1}$)	Found ($\mu g.m\ell^{-1}$)	Error (%)
Protein	0.028	5.0	5.2	4.1
	0.054	10.0	10.3	3.0
	0.108	20.0	20.7	3.5
	0.157	30.0	30.0	0
	0.208	40.0	39.7	0.8
	0.255	50.0	48.8	2.4
Carbohydrate	0.104	10.0	10.2	2.0
	0.205	20.0	20.0	0
	0.307	30.0	30.0	0
	0.405	40.0	39.5	1.2
	0.508	50.0	49.5	1.0

TABLE II : Differences between standard curves for carbohydrate determination

Solvent	Slope of curve	Percentage error of distilled water curve relative to other solvents
Distilled water	0.0089	
0.1N sulphuric acid	0.0090	1.1
0.1N sodium hydroxide	0.0088	1.1
5.8N sulphuric acid	0.0091	2.2

In the fractionation of carbohydrates, Haug et al. (1973) extracted 20 mg of dry material twice with dilute acid for two hours and then extracted the residue twice with dilute akali for a further two hours. The carbohydrate content of the extracts was then determined. With this technique, it was found in this study that at least 2 mℓ of the acid extract was retained by the glass-fibre filters after centrifugation. This retained portion contaminated the alkali extract. To overcome this, filters were extracted only once with dilute acid or alkali for four hours. The result was the same in terms of yield of carbohydrate as when they were extracted twice for two hours. It was essential, however, to dry the filters after each extraction step to prevent contamination of the next extraction.

Haug et al. (1973) determined total carbohydrate by hydrolysing a dry sample with 1 mℓ of 29N suplhuric acid for 20 hours, then diluting the mixture and determining the amount of carbohydrate in the solution. The amount of insoluble carbohydrate was estimated as the difference between total and soluble carbohydrate. Hitchcock (1977) found, however, that the hydrolysis of total particulate matter yielded an underestimate of the carbohydrate concentration. In preliminary tests, a comparison was made between the two methods of carbohydrate determination i.e. hydrolysis according to the method of Haug et al. (1973) and the procedure described here. Higher yields of total carbohydrate were obtained by summation of the carbohydrate content in each fraction than by hydrolysis of the total sample, confirming the findings of Hitchcock (op.cit.). Hydrolysis in strong acid may result in the decomposition of furfural that produces the colour with phenol and sulphuric acid in the assay mixture.

In order to prepare calibration curves for the carbohydrate determination, standards were dissolved in distilled water, 0.1N suplhuric acid, 0.1N sodium hydroxide and 5.8 N suplhuric acid. The latter solution was prepared by diluting 29N sulphuric acid five-fold in accordance with the determination of insoluble carbohydrate. The slopes of the curves obtained with these four solutions are shown in Table II, the error differences between the distilled water curve and the curves prepared with the other solvents being 2-per-cent. This difference was not considered to be significant enough to warrant preparing three calibration curves, and therefore the distilled water curve was used for determining carbohydrate in all three fractions. It would appear that inorganic substances such as dilute sulphuric acid and sodium hydroxide do not interfere with the phenol-sulphuric acid method of sugar analysis. Similar observations were noted by Handa (1966), when the method was examined for its applicability to estimate dissolved carbohydrate in seawater.

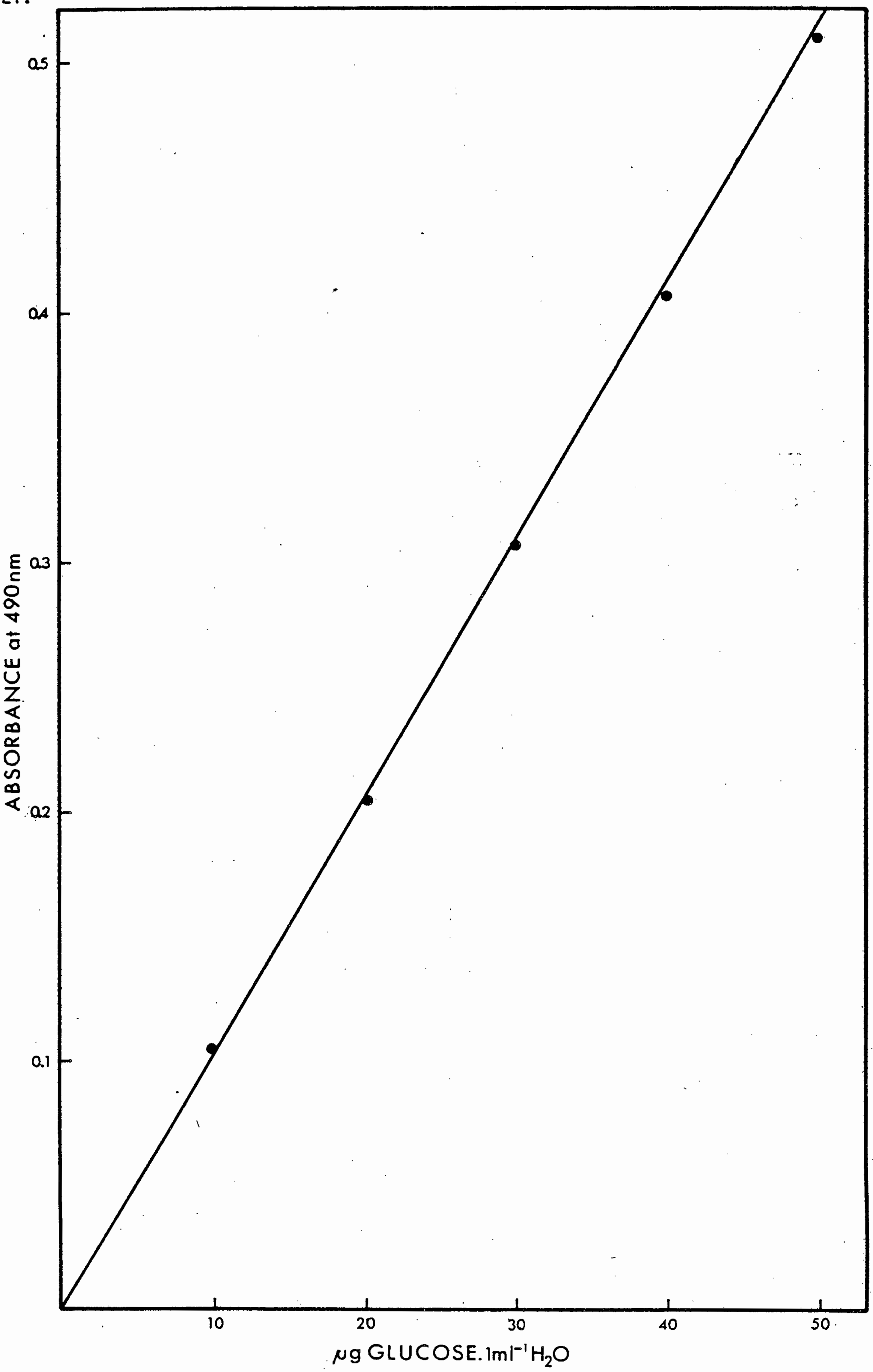

Fig. 2. Calibration curve for carbohydrate determination.

TABLE III : Protein and total carbohydrate results, Oudekraal (10 km south of Cape Town) 1978.

Station	Depth (m)	Protein ($\mu g.\ell^{-1}$)	Carbohydrate ($\mu g.\ell^{-1}$)	Protein/Carbohydrate
1	0	172.0	117.7	1.46
	6.5	151.5	93.2	1.62
	10	89.0	61.5	1.44
	16	116.0	60.0	1.93
	37	93.0	14.5	6.41
2	0	335.5	1183.0	0.30
	2.5	353.0	1108.5	0.32
	4	306.5	1145.6	0.27
	5	354.5	612.0	0.58
	9.5	436.0	734.0	0.59

A calibration curve for carbohydrate analysis is illustrated in Figure 2. The method can be expected to be accurate to within 2 per cent which is the accuracy recorded by Dubois et al. (1956). This figure was calculated in a manner similar to that described for the protein method and the percentage errors are shown in Table I.

Some results of protein and carbohydrate determinations, using the methods described here, are presented in Table III. It may be noted that there is a difference in the concentrations of protein and carbohydrate between the two stations with a resultant difference in the ratio of protein to carbohydrate. Changes in this ratio are being studied as an indicator of the physiological state of natural phytoplankton communities with respect to nutrient availability.

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APPENDIX 5

Fish. Bull. S. Afr. 14 (in press) 1981

MEASUREMENT OF CARBON-14 ASSIMILATION PATTERNS IN PHYTOPLANKTON

R.G. BARLOW

A method,based on the procedure of Morris *et al*. (1974), is described for determining the assimilation of carbon-14 into the products of photosynthesis. Simulated *in situ* incubations were performed in a deck incubator followed by collection of the phytoplankton on glass-fibre filters and preservation by freeze-drying. Dry samples were then fractionated into an ethanol-soluble fraction (amino acids, monosaccharides, some lipids and pigments), a TCA-soluble fraction (polysaccharides) and a TCA-insoluble fraction (protein). The amount of carbon-14 assimilated into each fraction was monitored on a liquid scintillation counter. Reasons for modifying the original procedure are discussed.

ŉ Methode, gebaseer op die prosedure van Morris *et al*.(1974), word beskryf vir die bepaling van die assimilasie van koolstof-14 in die produkte van fotosintese. Nageboötste *in situ* inkubasies is uitgevoer in ŉ dek inkubator wat opgevolg is deur versameling van die fitoplankton op glasvesel filtreerpapier en preservering deur middel van vriesdroging. Vir analisie is die droe monster afgebreek in ŉ etanol-oplosbare fraksie (amino-sure, monosakkariede, sommige vette en pigmente), ŉ TCA-oplosbare fraksie (polisakkariede) en ŉ TCA-onoplosbare fraksie (proteien). Die hoeveelheid koolstof-14 opgeneem in elke fraksie is gemonitor met behulp van die Beta sintillasieteller. Redes vir die aanpassing van die oorspronklike metode word bespreek.

Steemann-Nielsen (1952) introduced the carbon-14 method of measuring primary productivity in the ocean and it has made a significant contribution to understanding the ecology of marine phytoplankton. The extent to which it has improved the understanding of the physiological state of natural communities is more difficult to assess. One reason for this is that the overall rate of photosynthesis by phytoplankton can remain approximately constant over wide differences in the physiological state of the organisms. A possible way of modifying the carbon-14 method into a mechanism indicating the physiological state is to measure the pattern of photosynthesis as well as the overall rate.

Morris et al. (1974) introduced an appropriate modification by measuring the incorporation of carbon-14 into a number of different products during photosynthesis of a culture of Phaeodactylum tricornutum. The method involves an extraction of phytoplankton samples into ethanol-soluble and ethanol-insoluble compounds, with the latter group being further separated into compounds soluble and insoluble in trichloroacetic acid (TCA). Evidence was presented that carbon-14 incorporated into the TCA-soluble fraction was fixed into polysaccharide and that in the TCA-insoluble fraction into protein. The ethanol-soluble fraction would include low molecular weight metabolites such as amino acids and monosaccharides as well as some lipids and pigments.

In investigations into primary production conducted in the Cape Peninsula upwelling region, the carbon-14 method has been used to measure the gross production of phytoplankton. Physiological responses of the communities to changing environmental conditions were also monitored, the 'modified carbon-14 method' proving useful in these studies. A few modifications to the method of Morris et al. (1974) were introduced to adapt it for local field work and the technique adopted is presented here.

METHODS

Reagents

Sodium bicarbonate -^{14}C	- Radiochemical Centre, Amersham.
Absolute ethanol	- BDH Chemicals Ltd.
5%(w/v) Trichloroacetic acid -TCA	- BDH Chemicals Ltd.

Procedure

A total of 5 mCi of sodium bicarbonate -^{14}C was diluted to an appropriate volume with distilled water (pH 9.0). Aliquots (1mℓ) of this stock solution, containing 50 μCi sodium bicarbonate -^{14}C, were transferred to glass ampoules in which they were sealed and sterilized in an autoclave. Neutral-density bottles were prepared by coating clear glass bottles with varying concentrations of black polyurethane paint in polyurethane varnish. This reduced the amount of light penetrating the bottles to the required intensity, as tested with a Biospherical quantum meter.

Replicate 1ℓ water samples were drawn from appropriate depths in the euphotic zone into the corresponding neutral-density bottles. Simulated in situ incubations were performed by spiking each bottle with 50 μCi of sodium bicarbonate -^{14}C, then placing the bottles in a deck incubator flushed with running seawater for four hours. At the end of the incubation period, the contents of each bottle were filtered through a 4.7 cm Whatman GF/F glass-fibre filter, washed with filtered seawater, immediately frozen in liquid nitrogen, wrapped in aluminium foil, labelled, and freeze-dried. The samples were then stored at 5°C until fractionated.

Each dry filter was successively treated with 5 mℓ cold absolute ethanol for 5-10 minutes and 6 mℓ boiling ethanol for five minutes. The cooled solution was filtered through a 2.1 cm Whatman GF/C glass-fibre filter and both filters were washed with 3 mℓ cold ethanol before drying by suction. The combined extracts and washings constituted the ethanol-soluble fraction.

The two filters were treated with 6 mℓ 5% TCA in a boiling water bath for 30 minutes and, after cooling, the TCA solution was filtered through a 2.1 cm Whatman GF/C glass-fibre filter. The three filters were washed with 5 mℓ cold 5% TCA and again dried by suction. The combined extract and washings made up the TCA-soluble fraction, whereas the residue on the filters was designated the TCA-insoluble fraction.

A volume of 2 mℓ of each of the liquid fractions and the three filters containing the residue were assayed for radioactivity in Picofluor-30 scintillation cocktail on a Packard Prias liquid scintillation counter. All counts were converted to disintegrations per minute (DPM) after determining counting efficiency from efficiency correlation curves. Further blank corrections were made for radioactive bicarbonate retained by the glass-fibre filters during filtering. In calculating the proportion of carbon incorporated into each fraction, the total radioactivity was determined as the sum of the three fractions.

In order to prepare efficiency correlation curves, a freeze-dried sample of phytoplankton, not incubated with ^{14}C-bicarbonate, was fractionated into ethanol-soluble, TCA-soluble and TCA-insoluble fractions as described. Aliquots (in triplicate) of the ethanol- and TCA-soluble fractions, ranging in volume from 0.25 to 2.5 mℓ, and triplicate TCA-insoluble fractions (extracted filters), were added to 50.1 μl of ^{14}C-toluene standard (specific activity of 5x10^{4} DPM) in Picofluor-30 scintillation cocktail and counted. Nine replicate 50.1 μl aliquots of ^{14}C-toluene in scintillation cocktail were counted to determine maximum counting efficiency.

RESULTS AND DISCUSSION

Efficiency correlation curves were prepared by plotting the mean count of the triplicate ^{14}C-toluene standard analyses, expressed as a percentage of the activity of the standard, against the external standard ratio (See Figure 1). The curves for the ethanol- and TCA-soluble fractions were computed by a parabolic least squares fit and expressed by the following equations :

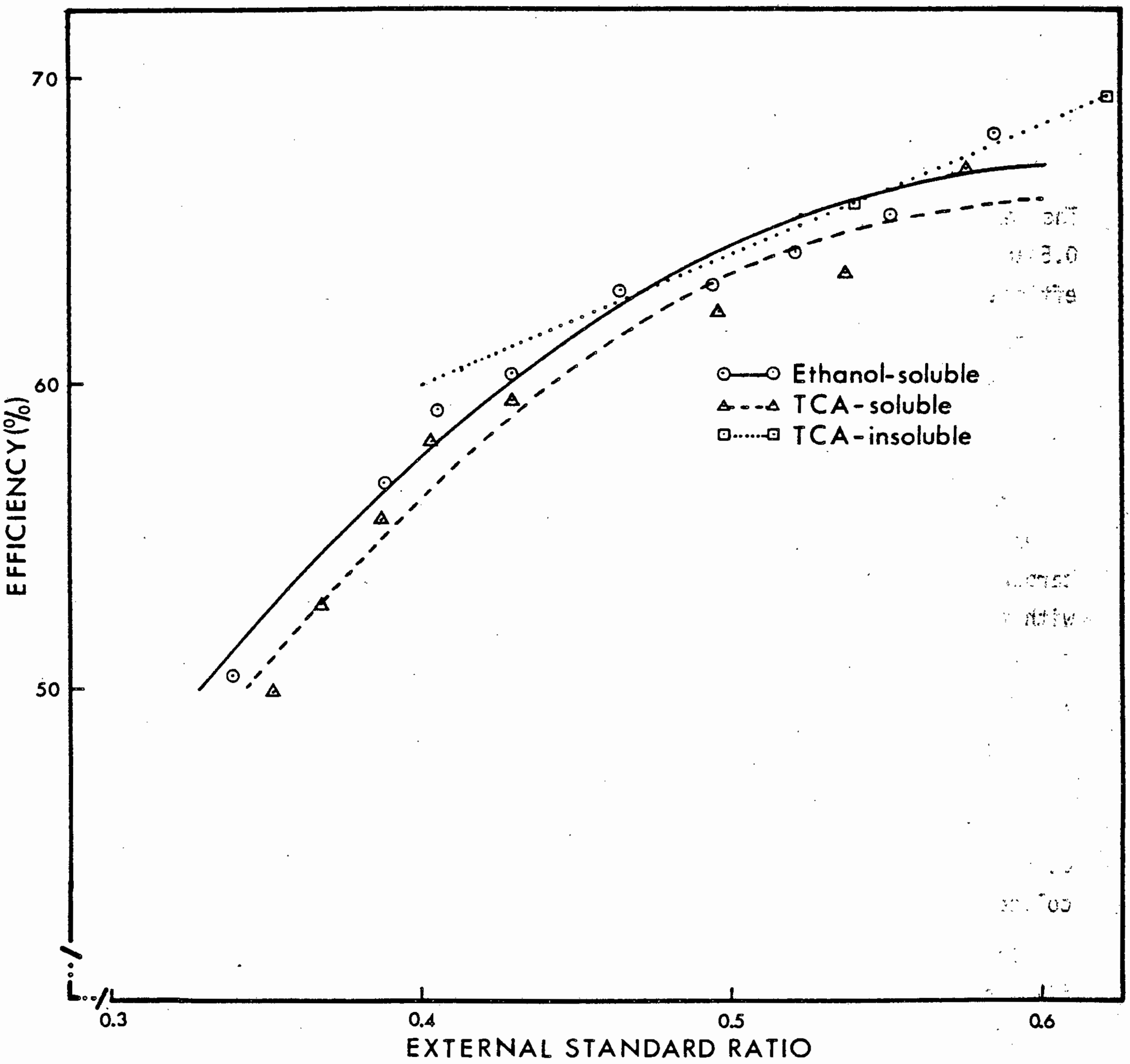

Fig. 1. Efficiency correlation curves for determining counting efficiency in three fractions.

ethanol-soluble $y = -209.9x^2+257.6x-11.9$

TCA-soluble $y = -248.8x^2+296.2x-22.4$

where y = percentage efficiency

and x = external standard ratio

For the TCA-insoluble fraction it was not possible to prepare a range of concentrations because the insoluble material remained on the filters. Therefore, only the maximum efficiency and the mean efficiency of the triplicate counts on whole fractions could be plotted to give a straight line graph expressed by the equation

$$y = 41.86x+43.3.$$

The external standard ratios of TCA-insoluble fractions were usually between 0.5 and 0.6 and the graph was considered suitable for calculating counting efficiencies in this narrow range.

In preliminary experiments to determine the quantity of carbon-14 to be used in incubations, samples were spiked with radioactive bicarbonate in a range from 10 μCi to 100 μCi. Incubation with 100 μCi gave the highest recovered activity, but 50 μCi gave adequate activities in each fraction and was used to spike samples in all subsequent experiments. The cost of each experiment was therefore reduced. Further experiments, comparing the total carbon-14 uptake determined by summation of the activities in each fraction with that determined directly, gave recovery values between 93 and 110 per cent.

After filtering, Morris et al. (1974) stored their samples in 80 per cent ethanol at -14°C until fractionating the cell constituents. Under local conditions, it was found to be easier to freeze and store the filters in liquid nitrogen at sea and then freeze-dry the samples in a shore-based laboratory. Storing the dry samples at 5°C in a refrigerator enabled the fractionation to be undertaken at a convenient time several weeks after the samples had been collected.

In the method of Morris et al. (1974), samples were collected on small filters and, thus, small volumes of extracting solvents were used. In the present study, larger filters were used because, firstly, it was easier to filter through large filters at sea and, secondly, a greater degree of control could be maintained during freezing of the samples. The dangers of liquid nitrogen burns were thus minimized. In order to accomodate the larger filters, volumes of the extracting solvents had to be increased proportionately.

Prior to counting the radioactivity in their fractions, Morris et al. (1974) had to evaporate the solutions to dryness before adding their scintillation cocktail. However, it was found that the evaporation step could be avoided by using Picofluor-30 cocktail which could absorb as much as 30 per cent by volume of ethanol or TCA. It was also suitable for use with the glass-fibre filters. Counting efficiencies for all fractions varied between 50 and 70 per cent, which was thought to be satisfactory if it is taken into consideration that toluene-^{14}C standards gave a maximum efficiency of 70 per cent with the Picofluor cocktail on the Prias liquid scintillation counter.

A certain amount of radioactivity was absorbed onto the glass-fibre filters during the filtration of the water samples and, even though the quantity was reduced by washing with filtered seawater, a correction still had to be made. Filtered seawater, spiked with 50 μCi of bicarbonate-^{14}C, was passed through a Whatman GF/F filter and the blank extracted into the various fractions as described. The background activity for each fraction was then determined.

The assimilation of carbon into the major end-products of phytoplankton photosynthesis is influenced by changes in the marine environment, particularly light intensity and nutrient availability. The simple and convenient method described in this report may be used to investigate these important physiological responses.

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