Carbon metabolism and growth of marine unicellular cyanobacteria belonging to the genus *Synechococcus*

by

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Ruder heads stand amazed at these prodigeous pieces of nature, whales, elephants, dromedaries, and camels: these, I confess, are the colossus and majestick pieces of her hand: but in these narrow engines there is more curious mathematicks: and the civility of these little citizens more easily sets forth the wisdom of their Maker.

Sir Thomas Browne, 1642

To Chris, for help, encouragement
and for always being there.

ABSTRACT

An investigation was made into the carbon metabolism of the marine unicellular cyanobacterium, *Synechococcus* sp., strain WH7803, in laboratory cultures and natural populations.

Synechococcus WH7803 grown in continuous culture was used to study the effect of different irradiances (10 - 90$\mu Em^{-2}s^{-1}$) on the growth and photosynthetic characteristics of the organism. Growth saturated at relatively low irradiances (@55$\mu Em^{-2}s^{-1}$). Parameters were measured for photosynthetic relationships, pigment concentrations and total quantities of DNA, RNA and protein present in the cells. Although the photosynthetic maximum was highest in cultures grown at high irradiances, alpha (initial slope) was steeper in low light-grown cultures indicating a higher photosynthetic efficiency in these cultures. The assays for DNA, RNA and protein were used to establish a general pattern of response under the different light conditions.

Respiratory rates of batch cultures were measured at different irradiances and the heterotrophic potential of these cultures was tested using an oxygen electrode. No significant alteration in the respiratory rate was observed for a range of organic compounds added to growing cultures in the dark. Trials with two inhibitors to remove heterotrophic microbial activity from natural populations proved insufficiently sensitive for further use.

Three studies of natural populations from the North Sea and Celtic Sea were carried out to determine the abundance and distribution of *Synechococcus* species, and to measure their rates of photosynthesis and other physiological/ ecological properties compared to other phytoplankton. As with laboratory cultures the data indicate that natural populations utilise low irradiances efficiently; however *Synechococcus* sp., have been found to be equally abundant and productive in the surface mixed layer where they experience high irradiances (1700$\mu Em^{-2}s^{-1}$). Cellular fractionation of ^{14}C labelled samples was used to determine the physiological consequences of growth at high irradiances. In laboratory cultures the percentage ^{14}C incorporated into protein remains high (@50%), at high and low irradiances, whereas incorporation into the polysaccharide and nucleic acids was typically higher at high irradiances. In natural assemblages the percentage incorporation into protein remained high (@55%) and into polysaccharide and nucleic acids relatively low at @17%. In natural assemblages significantly more label was detected in the lipid fraction.

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GENERAL INTRODUCTION

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1.1 Marine cyanobacteria and *Synechococcus* species.

Cyanobacteria are of widespread distribution in a variety of terrestrial, freshwater and marine habitats, but until recently they were thought to be an insignificant part of the oceanic phytoplankton community. Despite the ancient marine history of cyanobacteria (Brock, 1973) they were believed to be represented by only a few genera of which the filamentous *Trichodesmium* was the genus of most significance. *Trichodesmium* forms blooms in the tropical and subtropical seas and is of particular interest due to its ability to fix nitrogen (Fogg, 1973; Fogg et al., 1973). *Synechococcus* species remained unrecognised until 1979 when both Johnson and Sieburth (1979) and Waterbury et al., (1979) reported the discovery of planktonic chroococcoid cyanobacteria of cell dimensions approximately 0.5-1.0 × 1.0-2.0μm, occurring widespread in all the oceans and seas examined. They belong to the picoplankton size class which also includes bacteria, small eukaryotic algae, some fungi, small protozoa and gametes and spores of larger plants and animals. Picoplankton were defined by Sieburth et al., (1978) as organisms which can be selectively filtered with 0.1μm and 1.0μm Nuclepore filters, yielding particles in the 0.2 - 2.0μm size range.

Synechococcus species had been previously overlooked because of their small size; however they were observed when epifluorescent microscopy and staining techniques were introduced into oceanography.

When phycoerythrin-rich *Synechococcus* species are illuminated using green light of approximately 560nm, they fluoresce with an orange fluorescence which is easily distinguishable from the red fluorescence of the chlorophyll dominant eukaryotes (Figure 1.1). In addition to red-pigmented phycoerythrin-rich strains, green coloured phycocyanin-rich marine *Synechococcus* strains have been isolated, but these have only been found in high numbers in coastal regions. This study involved the investigation of marine phycoerythrin-rich *Synechococcus* strains. A batch culture of *Synechococcus* WH7803, which was used in laboratory investigations is shown in Figure 1.2.

Synechococcus species occur abundantly within the euphotic zone and have been isolated from both coastal and oceanic waters of the worlds oceans. The name *Synechococcus* has been applied to unicellular cyanobacteria observed in the field and to all the marine isolates using the broad definition of Rippka et al., (1979). This taxonomic group has cylindrical to ovoid cells that reproduce by transverse binary fission in a single plane and lack sheaths.

Marine *Synechococcus* species are predominantly coccoid in shape and range in size from 0.6-1.6μm diameter. *Synechococcus* cells of variable size have been isolated and visualised using electron microscopy (Johnson and Sieburth, 1979; Kursar et al., 1981; Joint and Pipe, 1984). Samples from the Celtic Sea had maximum cell dimensions 0.5-1.0μm (Joint and Pipe, 1984); small eukaryotic algae with diameters 0.85-2.0μm were also present. There is a tendency towards increased cell size with depth (Murphy and Haugen, 1985; Li and Wood, 1988) which has been associated with an increase in the size of the photosynthetic unit in reduced irradiances.

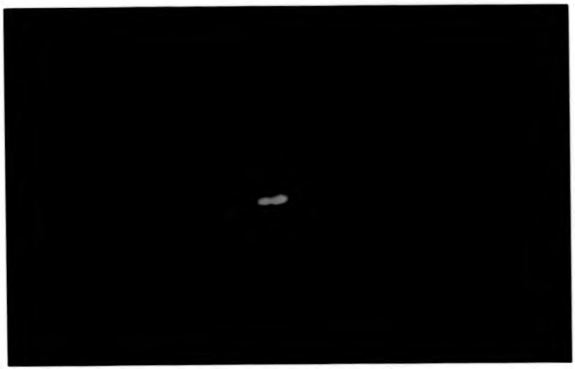

Fig. 1.1. Microscope view of *Synechococcus* WH7803

Fig. 1.2. Batch culture showing phycoerythrin-rich *Synechococcus* WH7803, maintained at a low irradiance of approximately 10μEm^{-2}s^{-1}.

An increase in cell size in surface waters of the Sargasso Sea has been connected with a nanomolar increase in nitrate concentrations (Glover et al., 1988a). In culture, cells are coccoid to rod-shaped 0.6-0.8 x 0.6-1.6μm and divide by binary transverse fission in a single plane (Waterbury et al., 1979) (Figure 1.3(a)). Strains maintained in continuous illumination have a tendency towards more rod shaped cells whereas if maintained in a light:dark (14:10) cycle they are more coccoid and have cell volumes about 30% less as determined by carbon per cell (Cuhel and Waterbury personal communication; cited by Waterbury et al., 1986). The cells maintained in a light:dark cycle more closely resemble natural assemblages.

1.2 Properties of marine *Synechococcus*

Ultrastructurally, *Synechococcus* strains have the expected prokaryotic features. The cell envelope is made up of the cytoplasmic membrane and a gram negative cell wall consisting of a peptidoglycan layer and an outer membrane (Waterbury et al., 1986) (Figure 1.3(b)). Variation in the ultrastructure of the cytoplasm, particularly thylakoid arrangement, and the cell wall of chroococcoid cyanobacteria has been illustrated by several authors (Johnson and Sieburth, 1979; Joint and Pipe, 1984; Takahashi et al., 1985; Marchant et al., 1987). Kursar et al., (1981) found that *Synechococcus* WH7803 (DC-2) had a cell wall sheath complex and some strains produced external spinae (Perkins et al., 1981). These hollow spinae are attached to the outermost layer of the cell wall and can be up to 2.7μm long, however, other than to increase the cells surface area, their function is unknown.

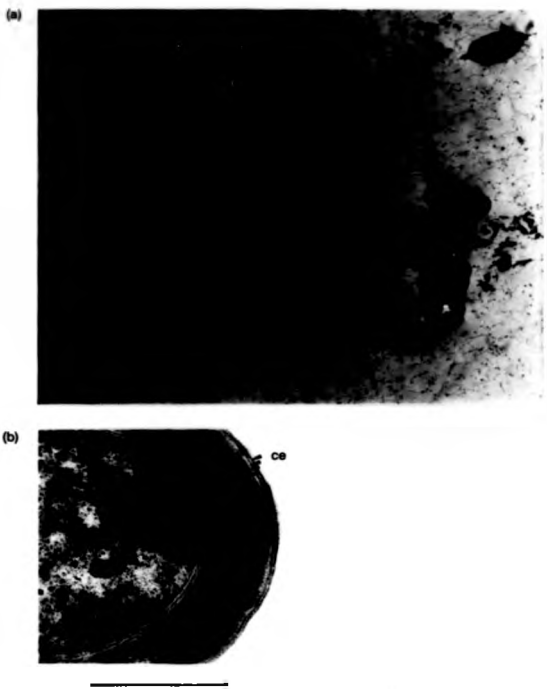

Fig. 1.3. Transmission electron micrographs of (a) individual and dividing cells of *Synechococcus* WH7803 (bar = 1μm) and (b) transverse section showing, thylakoids (t) and the multilayered cell envelope (ce) (bar = 0.25μm).
(By kind permission of N. Allen)

Thylakoids are located peripherally just internal to the cytoplasmic membrane separated by a distance of 40-50nm, the space necessary to house the phycobilisomes that are attached to the outer surface of the thylakoid (Waterbury et al., 1986). Phycobilisomes contain the accessory light-harvesting pigments (phycobiliproteins) and exist as aggregates of these pigments (Gantt, 1981; Cohen-Bazire and Bryant, 1982) (see section 1.4), however chlorophyll *a* is considered their primary photosynthetic pigment (Siegelman, personal communication; cited in Waterbury et al., 1979). Oceanic isolates of *Synechococcus* are reddish orange due to a predominance of the phycobiliprotein phycoerythrin. The phycobiliprotein content and composition of cyanobacteria can be affected by numerous environmental factors (Cohen-Bazire and Bryant, 1982); but Waterbury et al., (1986) found that marine *Synechococcus* species do not show complementary adaptation, that is the relative rates of synthesis of phycoerythrin and phycocyanin are constant for any given strain and unaffected by light quality.

Polyphosphate inclusions, glycogen granules and carboxysomes are frequently observed in transmission electron micrographs, but the storage products cyanophycin (Newman et al., 1987) and poly β-hydroxybutryate are absent (Waterbury et al., 1986). Polyhedral bodies have frequently been observed in transmission electron micrographs of *Synechococcus* (Johnson and Sieburth, 1979, 1982; Joint and Pipe, 1984; Takahashi and Hori, 1984) and are assumed to be carboxysomes. Codd and Marsden (1984) suggested that carboxysomes may protect the carboxylase function of ribulose-1,5-bisphosphate carboxylase from the competitive inhibition by oxygen and thereby reduce photorespiration. Low numbers of these carboxysomes were

observed in exponentially growing *Synechococcus* cultures (Joint, 1986) compared with *Synechococcus* species from natural assemblages. There was a high potential for photorespiration in laboratory cultures (Glover and Morris, 1981; Morris and Glover, 1981), but there is no available data on photorespiration of *Synechococcus* in natural assemblages. The presence of numerous carboxysomes suggests that photorespiration may not be important in natural communities (Joint, 1986).

All strains in the open ocean so far examined have an obligate requirement for elevated concentrations of sodium, magnesium, calcium and chloride indicating that they are intrinsically marine species. A few of the strains now in axenic culture also require growth factors (Waterbury et al., 1986); a vitamin supplement containing vitamin B_{12}, biotin and thiamine (Guillard, 1975) was found to be sufficient. *Synechococcus* can utilise nitrate and ammonia as their sole nitrogen source for growth and approximately half the strains looked at can utilise urea alone; however the *Synechococcus* strains so far examined do not fix nitrogen (Waterbury et al., 1985; El Hag, 1986).

Only a minority of cyanobacterial species studied are capable of dark aerobic growth on organic substrates (Rippka, 1972; Stanier, 1973). Waterbury et al., (1986) reported that all of the *Synechococcus* strains so far examined were obligate phototrophs. In tracer experiments it was found that acetate and adenine were assimilated, but without contributing significantly to cell carbon, whereas glucose and thymidine were not (Cuhel and Waterbury, 1984). These findings suggest that *Synechococcus* WH7803 is not capable of heterotrophic growth.

Waterbury et al., (1985) isolated a cyanobacterium of the genus

Synechococcus capable of swimming motility at 5-30$\mu m\ s^{-1}$. Variation in cell speed was a result of the age of the cell rather than variation in the irradiance. No plausible mechanism for their motility was found, however interpretation of additional data suggested that the most probable source of energy for *Synechococcus* motility was the sodium motive force (Willey et al., 1987).

The marine cyanobacteria have been assigned to the genus *Synechococcus* using the definition of Rippka et al., (1979). Although the different strains have similar morphology they show considerable genetic heterogeneity, as reflected by the span of DNA base composition 39-71%. The DNA base composition (the %mol G+C content) is constant for a given organism. Rippka and Cohen-Bazire (1983) proposed three major sub-groups based on the clustering of base compositions, but oceanic isolates fall between the three created clusters and this classification system is not much used. An estimate of the relatedness of several marine *Synechococcus* species was determined using DNA hybridisation (Douglas and Carr, 1988). The freshwater species *Anacystis nidulans* was entirely different from the marine species examined; however certain strains within the marine species showed distinct clustering trends and it was suggested that this procedure may be a taxonomic basis on which to define different groups of this genus.

1.3 Distribution and abundance of marine *Synechococcus*.

Synechococcus species have been observed in water collected from the euphotic zone over a wide geographic area at concentrations ranging from a few cells l^{-1} to 10^9 cells l^{-1} (Table 1.1). Most studies to date have used epifluorescence microscopy

Table 1.1. The abundance of phycoerythrin-rich *Synechococcus* from various geographical locations

location	latitude	depth (m)	cells l^{-1}	reference
Sargasso Sea	29°N	100	$1.9x10^6$	Johnson & Sieburth (1979)
Sargasso Sea	22°N	100	$2.2x10^6$	Johnson & Sieburth (1979)
Barbados Deep	12°N	50	$6.6x10^6$	Johnson & Sieburth (1979)
Grenada Deep	12°N	50	$1.4x10^7$	Johnson & Sieburth (1979)
Off Peru	13°S	0	$8.6x10^7$	Waterbury et al., (1979)
Off Peru	13°S	10	$2.6x10^7$	Waterbury et al., (1979)
Arabian Sea	21°N	10	$1.25x10^8$	Waterbury et al., (1979)
Arabian Sea	24°N	0	$5.3x10^7$	Waterbury et al., (1979)
S.California coastal	25 to 45°N	1	$1x10^6$ to $7.0x10^7$	Krempin & Sullivan (1981)
Chesapeake Bay	38°N	0	$1.3x10^8$	Exton et al., (1983)
Costa Rica Dome	9°N	0 to 50	$5x10^8$ to $1.5x10^9$	Li et al., (1983)
Biostat	9°N	0 to 75	$1x10^7$	Li et al., (1983)
Azores	36°N	65 to 89	$4x10^6$ to $1.7x10^7$	Platt et al., (1983)
Northwest Atlantic	41 to 43°N	–	$3.7x10^6$ to $4.9x10^7$	Douglas (1984)
Baltic Sea	54 to 60°N	1	0 to $2.3x10^7$	Schmaljohann (1984)
Lake Ontario	43 to 44°N	1	$8.5x10^8$	Caron et al., (1985)
Gulf of Maine	39 to 44°N	1 to 38	$1.3x10^6$ to $1.31x10^8$	Glover et al., (1985a)
Great Barrier Reef	14°S	0 to 10	$1x10^8$ to $2x10^8$	Moriarty et al., (1985)
Sargasso Sea	34 to 40°N	2 to 75	$5x10^6$ to $2.2x10^8$	Murphy & Haugen (1985)
Gulf of Maine	39 to 44°N	1 to 40	$1x10^6$ to $3.4x10^8$	Murphy & Haugen (1985)
North Atlantic	36 to 43°N	1 to 100	$2x10^6$ to $1.8x10^8$	Murphy & Haugen (1985)
Lago di Faro	38°N	1 to 20	$1.4x10^7$ to $1.8x10^7$	Platt et al., (1985)
Canadian Arctic	65°N	15	$2.1x10^6$	Smith et al., (1985)
Menai Straits	53°N	0	$1.5x10^6$	El Hag & Fogg (1986)
Irish Sea	53°N	0	$8.1x10^7$	El Hag & Fogg (1986)
Celtic Sea	50°N	30	$1.1x10^8$	Joint & Pomroy (1986)
Porcupine Sea Bight	50°N	1 to 15	$1.5x10^8$	Joint & Pomroy (1986)
North Pacific	35°N	74	$5.0x10^6$	Iturriaga & Marra (1986)
Indian Ocean	20 to 40°S	–	$3.1x10^7$ to $5.9x10^7$	Zaika (1986)
Mediterranean	30 to 45°N	–	$3.7x10^6$ to $2.93x10^7$	Zaika (1986)
Strait of Messina	38°N	1 to 10	$1.7x10^7$ to $2.7x10^7$	Magazzu et al., (1987)
Southern Ocean	47 to 56°S	1 to 5	$1x10^4$ to $1.6x10^7$	Marchant et al., (1987)
Southern Ocean	63 to 44°S	1 to 5	$5.8x10^3$ to $3.4x10^7$	Marchant et al., (1987)
Southern Ocean	48 to 60°S	1 to 5	$1.2x10^4$ to $3.6x10^6$	Marchant et al., (1987)
Southern Ocean	57 to 48°S	1 to 5	$4x10^4$ to $8.6x10^6$	Marchant et al., (1987)
Sargasso Sea	26 to 34°N	50	$1.4x10^7$	Iturriaga & Mitchell (1988)
Baltic Sea	59°N	0 to 10	$2.1x10^6$ to $2.43x10^6$	Kuosa (1988)
East Greenland	80°N	0 to 80	$4x10^4$	Gradinger & Lenz (1989)
East Greenland	80°N	80 to 120	6.2	Gradinger & Lenz (1989)
East Greenland	80°N	>120	$1.29x10^{-2}$	Gradinger & Lenz (1989)

– no depth given

to visualise and enumerate *Synechococcus* cells; however flow cytometry is emerging as the ideal enumeration method for small organisms because it quickly divides organisms into their natural size and pigment groups (Yentsch et al., 1983; Yentsch and Phinney, 1985; Olson et al., 1988).

Murphy and Haugen (1985) found that cell concentration gradually decreased as temperature decreased and Marchant et al., (1987) found a similar correlation between cell number and water temperature in a transect between Australia and Antarctica. Decreasing cell concentration with increasing water temperature was described by Waterbury et al., 1986 during a transect from Punta Arenas to Recife. The two former studies were carried out during the spring in the respective hemispheres whilst Waterbury's study was during the autumn, so the different observations may reflect the effects of nutrient availability and water temperature on growth and grazing. In tropical waters picoplankton show little seasonal variation in numbers and in polar waters where water temperature remains below 5°C throughout the year, *Synechococcus* species are generally not found; however Smith et al., (1985) reported 10^6 cells l^{-1} in the Canadian Arctic. Gradinger and Lenz (1989) also found varying numbers in the Arctic; lowest numbers were observed in the cold Polar Water (above 80m), and the highest numbers in the warmer Atlantic Intermediate Water below 120m. These observations suggested that the picocyanobacteria could serve as a tracer of warm water masses in the polar regions.

In temperate seas there appears to be a characteristic seasonal cycle with high cell numbers from May to October and low numbers during the winter and early spring (Krempin and Sullivan,

1981; El Hag and Fogg, 1986; Schmaljohann, 1984). Immunofluorescence techniques showed that phycoerythrin-containing *Synechococcus* strains in a coastal bay became significant in the summer and autumn (Campbell et al., 1983). Caron et al., (1985) reported cyanobacteria of similar morphology and abundance to those previously found in oceanic systems, in Lake Ontario. As with marine *Synechococcus* species they were strongly correlated with temperature and displayed a seasonal distribution (maximum, 6.5×10^8 cells l^{-1}).

Short term weather patterns such as storms or days of rain can dramatically effect *Synechococcus* abundance by altering nutrient concentrations (Glover et al., 1988a) and/or disturbing the grazers of *Synechococcus* species; however the role that nutrients and other environmental factors play in the control of the abundance of cyanobacteria has not been elucidated.

Cyanobacteria usually attain maximum rates of growth at relatively low irradiances (Richardson et al., 1983) and *Synechococcus* species might be expected to be restricted to regions fairly low down the water column, where light and mixing are reduced and the cells are less likely to be advected into the surface water where irradiance is high. It is generally accepted that picoplankton are adapted to growth at low light, and maximum cell numbers are frequently found towards the bottom of the euphotic zone (Takahashi and Hori, 1984; Iturriaga and Mitchell, 1986; Murphy and Haugen, 1985; Zevenboom, 1986; Li and Wood, 1988) however vertical distributions of phytoplankton may be influenced by the hydrographic regime of the particular water mass studied.

One factor contributing to the vertical distribution is the depth to which photosynthetically active light penetrates the water

column with 1% transmittance, the approximate lower limit for this group of cyanobacteria. Where concentrations are high below this level it is likely to be as a consequence of mixing.

In some of these and other investigations evidence of eukaryotic algae <2μm has been obtained (Johnson and Sieburth, 1982; Joint and Pomroy, 1983; Takahashi and Bienfang, 1983; Takahashi and Hori, 1984; Glover et al., 1985a, 1985b; Murphy and Haugen, 1985). The data available indicate that whereas cyanobacteria are most numerous at a depth corresponding to 1% of the incident irradiance at the surface, eukaryotes have a maximum at the 0.5% light level (Murphy and Haugen, 1985). Eukaryotes should have a competitive advantage over *Synechococcus* species at the lower sites of the euphotic zone because of differences in the composition of light harvesting pigments and the change in spectral composition of the light with depth (Glover et al, 1986b). Murphy and Haugen (1985) also found that the number of eukaryotic cells in the North Atlantic were generally an order of magnitude less than the numbers of cyanobacteria.

A distinct chlorophyll maximum is usually associated with the pycnocline and both Bienfang and Szyper (1981) and Takahashi and Bienfang (1983) have found 80% of the chlorophyll associated with cells <5μm and >3μm respectively. In oligotrophic waters with a permanent pycnocline the evidence suggests that picoplankton is most abundant in the subsurface chlorophyll maximum. Iturriaga and Marra, (1988) observed maximum cell abundances in the upper 50m north of a front (24°N) in the Sargasso Sea. South of the front maximum numbers were observed near 100m depth, supporting previous observations that abundance maxima follow the deepening of the euphotic zone. They

suggested that factors other than temperature alone, such as nutrient supply via vertical mixing may affect oceanic distribution of cyanobacteria.

In temperate waters a thermocline develops seasonally. Photosynthetic picoplankton are low in numbers in deep waters but if the water column is mixed their distribution tends to be uniform in the euphotic zone. Often the maximum concentration of *Synechococcus* species occurs at or near the surface (Waterbury et al., 1979; Krempin and Sullivan, 1981; Caron et al., 1985; Joint, 1986; Kuosa, 1988), and recent laboratory studies (Kana and Glibert, 1987a) have demonstrated the growth of *Synechococcus* WH7803 at irradiances up to $2000\mu Em^{-2}s^{-1}$. In stratified waters they attain maximum population densities towards the thermocline near the bottom of the euphotic zone (Murphy and Haugen, 1985; El Hag and Fogg 1986). This is not due to sedimentation as their small size makes this negligible and must be because they find optimum conditions for growth at this depth.

Patchiness in the distribution of cyanobacteria observed in the summer may be related to eddies (Fogg et al., 1985). Coastal, estuarine and upwelling areas have on the whole higher cyanobacterial numbers than oceanic areas, however because other phytoplankton species are also more abundant under these nutrient rich conditions cyanobacteria contribute less to the overall primary production.

1.4 The underwater light climate and photosynthetic pigments.

Primary production in marine cyanobacteria is limited to the depth of the euphotic zone of the sea, the extent of which varies with different environmental fluctuations and turbulent motions.

Light in the oceans is attenuated rapidly with depth and the photic zone where phytoplankton proliferate is greatly restricted in the vertical dimension (Jerlov, 1976; Kirk, 1983). The photic zone is usually considered to encompass depths to which greater than 1% of surface irradiance penetrates, although algae sometimes exhibit low rates of photosynthesis and growth just below this zone. Even the clearest of open ocean waters seldom have photic zones in excess of 200m; estuarine and coastal waters have photic zones ranging from <1 to 50m (Yentsch, 1980; Kirk, 1983). Dissolved substances and suspended particulate material including phytoplankton cells all contribute to light extinction, making the marine environment one in which energy to drive photosynthesis is at a premium (Yentsch, 1980). Relatively little of the incidant irradiation falling on the sea surface is reflected unless the sun is at a low altitude, therefore the ultimate fate of solar energy is to be absorbed. Apart from the rapid absorption of infra-red and ultraviolet radiation within the upper two metres of the sea, various wavelengths of the visible spectrum penetrate the water to different depths. Clear ocean water is most transparent to blue light 470-490nm, whereas coastal waters are most transparent in the yellow-green part of the light spectrum 500-600nm. Absorption of light in water is approximately logarithmic, however the depth to which light penetrates is dependent upon the dissolved and particulate matter present (including living organisms). Many natural waters contain yellow substances in solution

to which the name Glebstoff is applied. These substances absorb energy particularly in the ultraviolet but also in parts of the visible spectrum.

The energy necessary for photosynthesis and accumulated as chemical energy in the organic matter, is derived from light. It is generally accepted that chlorophyll *a* is the photosynthetically active pigment, but with the rapid attenuation of light in the ocean especially of the longer wavelengths the role of the accessory pigments in trapping other wavelengths becomes increasingly important. The minimal and simplest functional composite structure, comprised of two photosystems and their light-harvesting components, has been termed a photosynthetic unit (PSU) (Prézelin, 1981). The light-harvesting components (phycobiliproteins) of cyanobacteria are packed in special aggregated structures, termed phycobilisomes which are themselves aligned in rows along the thylakoids (Gantt, 1975; Cohen-Bazire and Bryant, 1982). The phycobiliproteins function as antennae for light-energy trapping by chlorophyll in the spectral region 500-650nm where light absorption by chlorophyll is poorest and they are organised such that the energy from the trapped photon is transmitted stepwise from phycoerythrin (if present) via phycocyanin and allophycocyanin to the reaction centre of photosystem II (Gantt, 1975; Alberte et al., 1984). Greater than 95% of the light energy absorbed by phycobilisomes is initially transferred to photosystem II (Gantt, 1981; Bryant, 1986).

Among the different *Synechococcus* species there are clonal variaties in the composition and organisation of the photosynthetic apparatus. Various spectral types of phycoerythrin have been found among 40 *Synechococcus* isolates in the Woods Hole culture collection;

pigment types are characterised by the ratio of phycourobilin to phycoerythrobilin chromophores. Phycoerythrins with absorption maxima at approximately 490 and 550nm can be distinguished; these maxima indicate the presence of phycourobilin and phycoerythrobilin prosthetic groups in the protein (Ong et al., 1984). The presence of phycoerythrobilin-like chromophores (A_{max} 558nm) appears to be diagnostic of this marine cyanobacterial group (Alberte et al., 1984); however some *Synechococcus* strains contain phycoerythrin with both phycoerythrobilin and phycourobilin chromophores. The phycourobilin chromophore has an absorption maxima at 492nm and its presence enhances light harvesting ability at blue wavelengths of natural light which reach the bottom of the photic zone (Wood, 1985). Several marine *Synechococcus* strains have been shown to have high phycourobilin:phycoerythrobilin ratios (Alberte et al., 1984). Ong et al., (1984) reported that phycoerythrin purified from a laboratory culture of *Synechococcus* WH8103 had a higher phycourobilin content than any other component of the phycoerythrin. Phycoerythrin-rich clones grown at low irradiances showed higher photosynthetic performance, larger photosynthetic unit sizes, reaction centre I to II ratios near unity and steeper initial slopes of photosynthesis than non phycoerythrin containing clones (Alberte et al., 1984), thereby providing a light-harvesting advantage at low irradiances.

An immunofluorescence assay with antisera directed against *Synechococcus* WH7803 showed that not all clones react with the anti-7803 serum and that different serogroups (i.e. clusters of strains labelled by one antiserum) could be recognised (Campbell and Carpenter, 1987). A negative reaction was observed when ten strains with high phycourobilin:phycoerythrobilin ratios were tested for

cross reactions with anti-7803 serum (Campbell and Iturriaga, 1988). In natural assemblages from the Sargasso Sea the same anti-serum failed to label most cells throughout the photic zone, suggesting that strains of high phycourobilin:phycoerythrobilin ratio were dominant (Campbell and Iturriaga, 1988).

The water-soluble phycobiliprotein pigment phycoerythrin is confined to three planktonic groups: cryptophytes, cyanobacteria and the few planktonic rhodophytes. The pigments degrade almost immediately upon the death of the cell and are not found associated with detrital material (Stewart and Farmer, 1984) and are therefore ideal markers of the abundance and distribution of particular groups of phototrophs within the plankton. Sensitive fluorometric methods have been developed for the quantitation of phycoerythrin (Moreth and Yentsch, 1970). Glover et al., (1985a) found these methods unsuitable for marine *Synechococcus* species, and phycobilin mixtures (Stewart and Farmer, 1984). Wood et al., 1985 have also developed a system using a combination of scanning spectroscopy, epifluorescence microscopy and flow cytometry, for the differentiation and measurement of the major phycobilins present in each clone. Flow cytometry has been used to study the distributions and optical properties of *Synechococcus* cells in the Atlantic ocean (Olson et al., 1988). These authors found that virtually all *Synechococcus* cells in the open ocean contain phycourobilin and most have very high relative phycourobilin contents. The distinct A_{545} peak-height values in the *in vivo* absorption spectra proved most useful to trace the amount of red-pigmented picocyanobacteria rapidly (Zavenboom, 1986).

Green wavelengths (500 to 575nm) are absorbed maximally by phycoerythrin and not effectively by phycocyanin and allophycocyanin.

Ong and Glazer (1987) reported the first phycoerythrobilin-containing phycocyanin of cyanobacterial origin, with absorption maxima at 533 and 554nm and a subsidiary maximum at 615nm. This phycocyanin with a high content of green-absorbing bilin was still capable of efficient energy transfer and represents a striking example of adaptation (Ong and Glazer, 1987).

Zeaxanthin has been reported as the main carotenoid in marine *Synechococcus* species (50 to 80% of the total carotenoid) (Guillard et al., 1985; Stockner and Antia, 1986). Kana et al., (1988) showed that *Synechococcus* WH7803 responded to changes in irradiance by altering their concentration of β-carotene but not zeaxanthin, and concentrations of zeaxanthin have been correlated with the occurrence of *Synechococcus* (Gieskes and Kraay, 1983). As no other phytoplankton contain more than traces of zeaxanthin (Guillard et al., 1985) it has been suggested that zeaxanthin may be useful as a characteristic cyanobacterial marker of picoplankton in the marine environment (Guillard et al., 1985; Kana et al., 1988). The constant cellular zeaxanthin concentration reported by Kana et al., (1988) is consistent with zeaxanthin being located primarily in the cytoplasmic or outer membrane, as has been shown for other cyanobacteria (Omata and Murata, 1983). This implies that zeaxanthin is not involved in the photosynthetic process but may serve as protection against photo-oxidation (Paerl, 1984), thereby increasing the photosynthetic performance of cyanobacterial assemblages at or near the water surface.

Cyanobacteria are the only group of micro-organisms with clearly established macromolecular reserves of nitrogen: the phycobiliproteins and cyanophycin. Cyanophycin was shown to be

absent in several strains of *Synechococcus* including the marine *Synechococcus* WH7803 (Newman et al., 1987).

Kursar et al., (1981) reported that only 8% of the phycoerythrin from *Synechococcus* WH7803 sedimented with the phycobilisome fraction in sucrose gradients. In addition a high phycoerythrin fluorescence was observed (Kursar et al., 1981; Barlow and Alberte, 1985; Wyman et al., 1985) suggesting that a free pool of phycoerythrin existed *in vivo*, that was not part of the photosynthetic system. Phycoerythrin may serve two functionally distinct roles : as a collector of quanta for photosynthesis and as a nitrogen reserve (Wyman et al., 1985). Natural assemblages of picoplankton studied by Lewis et al., (1985) showed action spectra for photosynthesis which gave no indication of participation of either phycocyanin or phycoerythrin in photosynthetically effective light absorption, hence the phycoerythrin was behaving in the natural environment as it did in laboratory culture (Fogg, 1986).

1.5 Contribution of picoplankton to total phytoplankton production.

The light saturation curve plays a central role in studies of the productivity of phytoplankton (Parsons et al., 1984; Falkowski, 1980; Harris, 1980; Prézelin, 1981): it relates photosynthetic productivity to the irradiance. Several formulations (Platt and Jassby, 1976) have been produced to define the photosynthesis-irradiance curve, partly because no single mathematical formulation can precisely define such a complex series of events in one equation. Figure 1.4 shows a diagrammatic photosynthesis-irradiance relationship. Productivity is usually normalised to chlorophyll

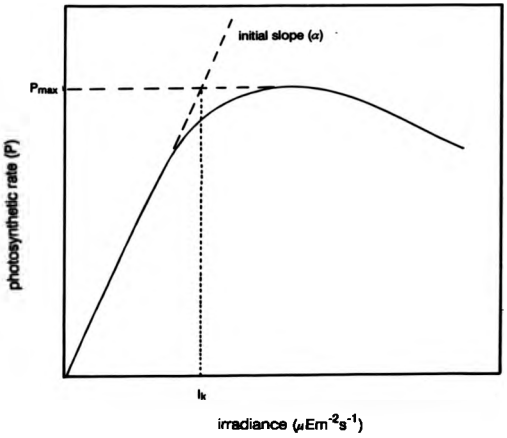

Fig. 1.4. General representation of a photosynthesis-irradiance curve (P-I).

content; parameters thus normalised are given the superscript B, and at the optimal illumination the maximum production P^B_m is expressed in mgC (mg chl a)$^{-1}h^{-1}$, termed the assimilation number. In the absence of limiting factors, P^B_m reflects the output of the dark reactions of photosynthesis (Platt et al., 1980). Photosynthesis is proportional to irradiance up to a certain point where photosynthesis becomes light saturated. The initial slope α^B in [mgC (mg chl a)$^{-1}$ h^{-1}][μEm^{-2}s^{-1}]$^{-1}$ is a function of the light reaction: the rate at which light is absorbed and the quantum efficiency with which absorbed light is converted to photosynthate. Other factors effect α^B through their effects on chlorophyll a and concentrations of accessory pigments (Perry et al., 1981).

Values of α^B and P^B_m have been found to be inversely related to cell size (Taguchi, 1976) but the size-dependency of photosynthetic parameters is modified by environmental effects (Malone and Neale, 1981). P^B_m has been correlated with temperature (Platt and Jassby, 1976; Malone, 1977) and also varies with nutrient concentration (Curl and Small, 1965; Malone, 1971; Takahashi et al., 1973; Glover, 1980), light history (Beardall and Morris, 1976), the time of day (Harding et al., 1981a & b) and the species composition (Dunstan, 1973). It is also believed to be influenced by the structural changes associated with light reactions (Harris, 1978). Separation into size classes enables the effect of environmental factors to be investigated whilst size effects are reflected in how the photosynthetic parameters vary relative to one another (Malone and Neale, 1981).

The rate of carbon assimilation in most phytoplankton species tends to remain constant with considerable further increase in illumination, but at much higher irradiances photosynthetic activity

begins to decrease. Photosynthetic-irradiance curves also take into account any photoinhibition effect at high irradiance, with the parameter β.

Talling (1957) proposed I_k which is the irradiance at the intersection of an extension from the initial slope and P_m^B, and represents the irradiance at which saturation of photosynthesis first occurs. I_k is frequently used to describe the physiological adjustments of algal assemblages to changing environmental conditions (Beardall and Morris, 1976) and in particular for distinguishing between the sun and shade adapted phytoplankton (Yentsch and Lee, 1966). Environmental factors influencing I_k include temperature, species composition and previous light history (Côté and Platt, 1983). Prézelin and Matlick (1980) found in culture populations of a dinoflagellate, that changes in I_k closely paralleled the changes in the growth potential of the organism.

The photosynthesis-irradiance curve is an indication of environmental effects on photosynthesis and can be used to explain certain properties of algal species or natural assemblages of phytoplankton.

It has become apparent that photosynthetic picoplankton play a major role in oceanic primary production particularly in the oligotrophic oceanic regions. Gieskes et al., (1979) were one of the first to report significant carbon fixation by cells passing through a 1.0 μm pore-size filter. They found 20-30% of ^{14}C and 43-53% of chlorophyll *a* passed through a 1.0 μm filter. Takahashi and Bienfang (1983) found that phytoplankton <3μm in waters off Hawaii accounted for 75 to 80% of the total biomass on a chlorophyll basis and 77 to 82% of the photosynthetic activity. In addition a high specific

growth rate was estimated. Li et al., (1983) also found a high proportion of the biomass in the tropical ocean in the <1μm fraction (picoplankton) and a high percentage of the primary productivity was attributed to this fraction. They showed that 20% of the ^{14}C fixed in surface waters and as much as 75% of the ^{14}C fixed in the bottom region of the euphotic zone was due to small coccoid cyanobacteria. Values in the literature vary considerably however, in general picoplankton accounts for 20 to 30% of the summer production in temperate regions, >50% in the tropics and subtropics and 10 to 25% in the Artic summer (Table 1.2). Much of the variation seen in Table 1.2 is caused by differences in methodology, size fractions or values being quoted for individual depths rather than primary production for integrated values (Waterbury et al., 1986).

In general there is a correlation with temperature such that at high temperature there is high productivity, however other factors such as nutrient concentrations and the influence of season need to be taken into consideration. In the tropics water temperature does not vary a great deal, however production does and this is associated with variations in the physical system, associated with trade winds, current speeds etc.

The relative success of *Synechococcus* in nutrient-poor, low light environments may well be related to high affinities for nutrients including light these being well established characteristics of fresh-water cyanobacteria (Zevenboom, 1986). Low nutrient concentrations are by definition characteristic of oligotrophic waters where picoplankton appear to be abundant. Joint and Pomroy (1983) observed picoplankton production to be highest in Spring, immediately after the spring diatom bloom and again in August

Table 1.2. Estimates of primary production in the picoplankton fraction from different geographical locations.

location	% primary production	size–fraction	reference
Santa Barbara Channel	75	<5µm	Putt & Prezelin (1985)
Hawaiian embayments	34 – 63	<3µm	Bienfang et al., (1984)
Sargasso Sea	51 – 85	<3µm	Glover et al., (1985b)
Subtropical Hawaiian waters	82 – 88	<3µm	Takahashi & Bienfang (1983)
Coral Sea	43 – 63	<2µm	Furnas & Mitchell (1988)
North Pacific Ocean	65	<1.5µm	Iturriaga & Mitchell (1986)
Sargasso Sea	60 – 95	<1.5µm	Iturriaga & Marra (1988)
North Atlantic Ocean	12 – 30	<1µm	Douglas (1984)
Northwest Atlantic Ocean			
Neritic front	6	<1µm	Glover et al., (1986a)
Warm core eddy	25	<1µm	Glover et al., (1986a)
Wilkinson's basin	46	<1µm	Glover et al., (1986a)
Celtic Sea	20 – 30	<1µm	Joint & Pomroy (1986) (summer)
Celtic Sea	13	<1µm	Joint et al., (1986) (Winter)
Tropical Pacific Ocean	20 – 80	<1µm	Li et al., (1983)
Mozambico Channel (Madagasacar)	21 – 82	<1µm	Magazzu & Hull (1985)
Strait of Messina (Mediterannean)	24 – 43	<1µm	Maguzzu et al., (1987)
Tropical North Atlantic Ocean	60	<1µm	Platt et al., (1983)
Lago di Faro (saline lagoon)	10	<1µm	Platt et al., (1983)
Canadian Arctic	10 – 25	<1µm	Smith et al., (1985)
Southern Ocean	0 – 32	<1µm	Weber & El-Sayed (1987)
Sargasso Sea			
White light	57 – 61	*	Prezelin et al., (1989)
Blue–green light	73 – 84	*	Prezelin et al., (1989)
Sargasso Sea (North)	18 – 20	<1µm	Waterbury et al., (1986)
Sargasso Sea (North)	10 – 25	<1µm	Waterbury et al., (1986)
South of Cape Cod	5 – 15	<1µm	Waterbury et al., (1986)

* total of Synechococcus species

when nutrient levels were again low. Bienfang et al., (1984) found that picoplankton isolated from island embayments demonstrated a capacity for high growth rate, however the relative contribution of this fraction to the total biomass was lower in these nutrient rich waters than from oligotrophic waters surrounding the islands. Low ambient nutrient conditions are exploited most efficiently by small cells which have high surface area:volume ratios and negligible sinking rates, whereas relatively high nutrient concentrations create an environment more favourable to larger phytoplankton. Magazzú and Hull (1985) found that the contribution of surface picoplankton to total production increased from the coast towards offshore and that primary production in the picoplankton fraction accounted for between 21 and 82% (average 54%) in the North-Western coast of Madagascar.

Different water masses also affect the contribution of picoplankton to total ^{14}C fixation. At a neritic front primary production was only 6% of the total; at a warm-core eddy 25% and at Wilkinson's Basin 46% (Glover et al., 1986a). In general *Synechococcus* species make a greater contribution to primary production in water masses where >20μm phytoplankton do not thrive (i.e. oligotrophic waters).

Although the contribution of picoplankton to total primary production is significant throughout the euphotic zone, it generally increases with decreasing light intensity (Li et al., 1983; Platt et al., 1983; Glover et al., 1985b) so that at the 1% light level values of 50 to 60% may be achieved. Glover (1985) suggested that the relative contribution of picoplankton to water column production would be greatest around sunrise and sunset, with increasing depth in the euphotic zone and when daily irradiance was low. For all size

fractions sampled near the bottom of the euphotic zone the highest photosynthetic capacity was observed at dawn (Vézelin et al., 1986). Putt and Prézelin (1985) reported that cyanobacteria exhibited diurnal patterns of photosynthesis i.e., maximal rates of light-saturated photosynthesis occurred at mid-day. The composition of picoplankton varied with depth and below the surface mixed layer larger cyanobacteria became more dominant and the eukaryote component became more important (Glover et al., 1985b). As noted previously eukaryotes in the picoplankton size-fraction are more often found at the 0.5% light depth where they have been found to photosynthesize even more efficiently than the cyanobacteria (Murphy and Haugen, 1985).

Although in the majority of *Synechococcus* species cells were observed in the 0.6 to 1.0μm fraction an increasing proportion of the total *Synechococcus* cells were found in the 1 to 5μm fraction as nitrate concentrations increased near the base of the thermocline. In deeper water an increase in the primary production could be due to an influx in nutrients (Glover et al., 1985b). From immunofluorescence analyses (Glover et al., 1986a) determined that the increasing proportion of larger *Synechococcus* species cells at depth was not the result of a change in strain composition and may therefore be associated with increasing cell volume due to the enhanced nutrient supply. Glover et al., (1988a) reported that high surface productivity in stratified Sargasso Sea water was supported by nanomolar concentrations in nitrate concentrations supporting the concept of a nitrate-dependent transient bloom.

Low surface contribution may be caused by photoinhibition or photorespiration. Two laboratory strains of *Synechococcus* have

demonstrated a potential for photorespiration (Morris and Glover, 1981; Glover and Morris, 1981). Photorespiration is defined as the light-dependant oxygen-sensitive evolution of carbon dioxide from photosynthetic organisms. Natural assemblages of *Synechococcus* species therefore may well exhibit photorespiration at high irradiances and high oxygen concentrations. The presence of polyhedral bodies in electron micrographs of *Synechococcus* have been observed (Johnson and Sieburth, 1979, 1982; Joint and Pipe, 1984; Takahashi and Hori, 1984) and have been assumed to be carboxysomes. A suggested role for carboxysomes is to protect the carboxylase function of ribulose-1,5-bisphosphate carboxylase from competitive inhibition by oxygen (Codd and Marsden, 1984., cited in Joint, 1986). Low numbers of carboxysomes have been found in exponentially growing cultures of *Synechococcus* strains, whereas in natural assemblages significant numbers have been observed. The importance of photorespiration in natural assemblages has still to be determined, however the available data implies that it may not be as significant as laboratory cultures have suggested.

Photoinhibition has been observed frequently in laboratory cultures (Barlow and Alberte, 1985) and natural assemblages (Platt et al., 1983; Li et al., 1983; Glover et al., 1985a). Differences in the degree of photoinhibition at high irradiance between strains WH7803 and WH8081 were observed (Barlow and Alberte, 1985); with WH7803 being markedly more inhibited. Kana and Glibert (1987b) however observed no inhibition of *Synechococcus* WH7803 when it was suitably pre-adapted and picoplankton in tropical oceans can apparently adapt to virtually the full range of natural submarine light levels (Furnas and Mitchell, 1988). These workers found photoinhibition of surface-

adapted populations limited to the upper five metres of the water column under midday full-sun conditions. Picoplankton from around 100m depth became photoinhibited at irradiances of between 200 to 400 $\mu Em^{-2}s^{-1}$. Neritic and oceanic assemblages of photoautotrophic picoplankton in temperate and subtropical waters have also been found to photosynthesise rapidly at near-surface irradiance levels (Putt and Prézelin, 1985; Iturriaga and Mitchell, 1986; Glover et al., 1986a; Prézelin et al., 1986; Gieskes and Kraay, 1986). Waterbury et al., (1986) suggested that examples of photoinhibition found in natural assemblages were bought about as a result of sample containment at high irradiances over long periods of time (five to six hours). In natural assemblages this situation is unlikely to occur because of continual mixing of surface layers and therefore the cells are not subjected to high irradiance long enough to induce photoinhibition. The results obtained by Furnas and Mitchell (1988) suggest that in oceanic regions of the Coral Sea, near-surface picoplankton were adapted to utilise the high irradiances present in tropical surface layers with minimal apparent detriment. This infers that the picoplankton remained near the surface long enough to adapt to high irradiance levels (Furnas and Mitchell, 1988).

The assimilation number P_m^B and the initial slope α were found to be higher for picoplankton than larger phytoplankton and the I_k lower (Platt et al., 1983; Prézelin et al., 1986; Magazzù et al., 1987), and this is consistent with picoplankton being shade-adapted.

Diel variability in photosynthetic parameters in coastal phytoplankton has been observed (Côte and Platt, 1983). Cyanobacteria and larger nanoplankton from various water masses exhibited similar diurnal patterns of photosynthesis (Putt and Prézelin, 1985; Prézelin

et al., 1986) with P^B_m occurring at mid-day. A given growth rate is attained as a result of how well matched an organism's metabolic and structural configurations are to the prevailing environmental conditions. Bienfang et al., (1984) reported rapid growth rates (μ) of picoplankton from subtropical embayments, ranging from 0.97 to 3.62 day^{-1}, and Bienfang and Takahashi (1983) 1.3 to 2.5 day^{-1}. Growth rate was measured by isolating the <3μm fraction and monitoring the rate of change of chlorophyll biomass in the absence of grazers. These rates may be higher than other calculated values as nutrient concentrations were non-limiting. Kuosa (1988) reported a growth rate of 1.09 day^{-1} for picoplankton isolated from the Baltic Sea and this compares well with values reported previously (Campbell and Carpenter, 1986, 0.42 - 0.86 day^{-1}; Iturriaga and Mitchell, 1986, 1.6 day^{-1}; Waterbury et al., 1986; Joint and Pomroy, 1986, 2.8 day^{-1}; Prezelin et al., 1987a, 0.83 day^{-1}; Weisse, 1988, 1.2 day^{-1}; Carpenter and Campbell, 1988, 1.56 day^{-1}). Glover and Morris (1981) reported a value of 1.5 day^{-1} for a laboratory grown phycoerythrin-containing *Synechococcus* species.

High growth rates in the surface mixed layer and the observation that cells collected from the surface or depth grew faster when incubated at surface irradiance (Campbell and Carpenter, 1986), are supported by the lack of photoinhibition of photosynthesis in *Synechococcus* assemblages growing in the surface mixed layer (Iturriaga and Mitchell, 1986; Joint and Pomroy, 1986; Prézelin et al., 1986). However Barlow and Alberte (1987) reported inhibition of photosynthesis at high irradiances in laboratory cultures and inhibition was observed in near-surface picoplankton at irradiances >2200$\mu Em^{-2}s^{-1}$ in the Coral Sea (Furnas and Mitchell, 1988).

The average size and fluorescence of cyanobacteria increased with depth (Li and Wood, 1988). *Synechococcus* size distribution shifted towards larger cells, typical of those from oceanic and neritic nitraclines during a bloom in the Sargasso Sea, suggesting that the *Synechococcus* assemblage was nitrogen sufficient (Glover et al., 1988a).

1.6 Grazers of *Synechococcus* species

The relative abundance of *Synechococcus* species and their autotrophic capacity has prompted speculation that single-celled marine cyanobacteria may constitute an important component of the oceanic pelagic food web.

Population abundance is a balance between growth and loss. Raven (1986) predicted that a 0.5μm radius cell would sink only 2.6mm day^{-1} and Takahashi and Bienfang (1983) indicated that there was virtually no loss of picoplankton biomass from the photic zone due to sedimentation. Physiological cell death is generally low in actively growing phytoplankton assemblages (Reynolds, 1984) and therefore grazing is considered the most important single loss factor.

Crustacean zooplankton are usually considered as the primary group of grazing organisms in the sea, but are probably unable to graze particles as small as picoplankton (Conover, 1978). Coccoid cyanobacteria have been reported in both the gut and faecal pellets of copepods (Sieburth, 1979; Silver and Alldredge, 1981; Johnson et al., 1982) but though they were ingested there was no degradation of the cells and they concluded that cyanobacteria did not contribute significantly to the nutrition of the copepod. Undigested faecal pellets from copepods (Johnson et al., 1982) and other organisms

provide another mechanism by which *Synechococcus* cells can be transported to greater depths. Faecal pellets sink rapidly and may be important in the deep sea food chain (Johnson et al., 1982). *Synechococcus* cells can also be transported to greater depth via 'marine snow', the name given to aggregations which form microenvironments rich in organisms and nutrients, with sinking rates of 50 to 100 m day^{-1} (Silver and Alldredge, 1981; Lochte and Turley, 1988).

The present evidence is that the cellular carbon of *Synechococcus* species may enter the marine food web directly and exclusively via the protozooplankton (Sieburth et al., 1978; Glover, 1985), however some loss from the system may be via extracellular excretion of organic materials which enter the food web via bacterial heterotrophic uptake. The bacterial component may play an important role in transferring energy through the marine food web from primary producers to higher trophic levels (Williams, 1981; Azam et al., 1983).

Johnson et al., (1982) demonstrated that phycoerythrin-rich *Synechococcus* species were suitable as food for micrograzers such as the helioflagellate *Actinomonas* species and the ciliate *Uronema* species. Although the small size of picoplankton prohibits their ingestion and efficient utilisation by many forms of herbivores, microflagellate, protozoan and ciliate protozooplankters appear to be the link by which picoplankton carbon is transferred to higher trophic levels (Fenchel, 1982; Sieburth and Davies, 1982; Azam et al., 1983). Sherr et al., (1986) identified aplastidic flagellates and aloricate ciliates less than 20 μm as the dominant consumers of picoplankton and Iturriaga and Mitchell (1986) found that a diverse

assemblage of microheterotrophs grazed 30 to 40% of the standing crop of picoplankton daily. More recently Waisse (1988) identified ciliates, heterotrophic nanoflagellates and rotifers from Lake Constance as major consumers of picoplanktonic cells.

Estimates of the grazing pressure of heterotrophic nanoplankton on *Synechococcus* species have been reported (Landry et al., 1984; Campbell and Carpenter, 1986), but although it is clear that picoplankton are an important food source for pelagic protozoans, their nutritive value is still controversial, (Porter et al., 1985; Sherr et al., 1986).

1.7 Scope of the present study

This study involved an investigation into the carbon metabolism of the marine unicellular cyanobacterium *Synechococcus* WH7803 in laboratory cultures and natural assemblages.

It was proposed to determine the respiratory rates and heterotrophic potential of laboratory cultures and to attempt measurements in natural assemblages. The distribution and abundance of *Synechococcus* species would also be examined and the rate of photosynthesis and other physiological/ecological properties measured in the temperate waters around the British Isles.

An investigation into the influence of light quantity on the pigment composition and photosynthetic parameters in laboratory culture would be carried out to determine a basis for comparison with natural assemblages.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

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2.1 Culture strains and origins

The organism on which most of this work was carried out was *Synechococcus* species WH7803 (DC-2); DC-2 refers to an experimental designation which was employed in early work and used in some current papers, a better term is WH7803 which refers to its number in the Woods Hole Culture Collection. The organism was initially isolated by Dr J.B. Waterbury of the Woods Hole Oceanographic Institute, Massachusetts, USA.

The work carried out at the University of Warwick employed a culture of *Synechococcus* WH7803 obtained via Dr W. Zevenboon, from Woods Hole Oceanographic Institute. The work carried out at Plymouth Marine Laboratory used a culture of *Synechococcus* WH7803 obtained from the Scottish Marine Biological Association culture collection, Oban, Scotland.

Anacystis nidulans (PCC6301), *Anabaena (PCC7120)*, *Agmenellum quadruplicatum (PR6)* and *Aphanocapsa (PCC6714)* were the other cyanobacteria used and were from stocks maintained at Warwick University.

Alteromonas haloplanktis, a marine heterotrophic bacterium, was from the National Collection of Industrial and Marine Bacteria Ltd, Torry Research Station, Aberdeen, Scotland.

2.2 Culture conditions

2.2.1 *Synechococcus* strain WH7803.

Stock cultures were maintained on ASW agarose slopes (section 2.3.5) at 24 ± 2°C in continuous light of approximately 10$\mu Em^{-2}s^{-1}$. 100ml liquid cultures in 250ml Erlenmeyer flasks were also maintained. Experimental cultures were grown under the same conditions except that irradiance was varied as described.

2.2.2 Other cyanobacteria.

These were maintained on BG11 agar slopes at room temperature in continuous light of approximately 10$\mu Em^{-2}s^{-1}$. Experimental cultures were grown as described.

2.2.3 *Alteromonas haloplanktis.*

Alteromonas haloplanktis was grown at 20 ± 2°C in a Gallenkamp Orbital Incubator, shaking at approximately 100rpm.

2.3 Culture media

It has been found in this laboratory that the quality of the purified water is critical for the success of growing oceanic cyanobacteria. Routinely water was deionised and then passed through a four stage purification system (Elga). It is considered that one of the important features of the process is the removal of small organic molecular residues by the carbon filtration process.

2.3.1 Artifical seawater (ASW) (Wyman et al., 1985).

NaCl	- 25.00 g l^{-1}
$MgCl_2.6H_2O$	- 2.00 g l^{-1}
KCl	- 0.50 g l^{-1}
$NaNO_3$	- 0.75 g l^{-1}
$MgSO_4.7H_2O$	- 3.50 g l^{-1}
$CaCl_2.2H_2O$	- 0.50 g l^{-1}
Trizma	- 1.10 g l^{-1}
$K_2HPO_4.3H_2O$	- 0.02 g l^{-1}
Trace elements	- 1.00 ml l^{-1}

Chemicals were dissolved in 1 litre of purified water and the pH adjusted to 8.0 with concentrated hydrochloric acid. This solution was autoclaved and stored at room temperature.

2.3.2 Trace elements (Wyman et al., 1985).

H_3BO_3	- 2.86 g l^{-1}
$MnCl_2.4H_2O$	- 1.81 g l^{-1}
$ZnSO_4.7H_2O$	- 0.222 g l^{-1}
$Na_2MoO_4.2H_2O$	- 0.390 g l^{-1}
$CuSO_4.5H_2O$	- 0.008 g l^{-1}
$Co(NO_3)_2.6H_2O$	- 0.0049 g l^{-1}
Ferric chloride hexahydrate	- 3.0 g l^{-1}
EDTA (disodium magnesium salt)	- 0.5 g l^{-1}

This solution was autoclaved and stored at 4°C.

2.3.3 BG11 medium (Stanier et al., 1971).

$NaNO_3$	= 1.50 g l^{-1}
$K_2HPO_4.3H_2O$	= 0.04 g l^{-1}
$MgSO_4.7H_2O$	= 0.075 g l^{-1}
$CaCl_2.2H_2O$	= 0.036 g l^{-1}
Trace elements	= 1 ml l^{-1}

The pH was adjusted to 8.0, autoclaved and stored at room temperature.

If the cultures were to be gassed with carbon dioxide-enriched air, 1g l^{-1} of filter sterilised $NaHCO_3$ was added to the medium immediately before inoculation.

2.3.4 Marine broth (Board and Lovelock, 1973)

Bactopeptone	= 5.00 g l^{-1}
Ferric phosphate	= 0.10 g l^{-1}
ASW (pH8.0)	= 1.00 litre

The pH was adjusted to 8.0, autoclaved and stored at room temperature.

2.3.5 Solid media

ASW agar (1.5%) plates were prepared to test for microbial contaminants. ASW (×2 concentration) and 3.0% nutrient agar (Difco) were autoclaved separately, mixed, and poured into sterile plates. These were stored at 4°C.

Stocks of other cyanobacteria were maintained on slopes of 1.5% BG11 agar, which was prepared in a similar way.

Stocks of *Synechococcus* WH7803 were maintained on 0.75% ASW agarose. ASW (×2 concentration) and 1.5% agarose were autoclaved separately and mixed together. Slopes were prepared in 'Steriline' plastic tubes and stored at 4°C.

2.3.6 Sterilisation

All sterile media (up to 2 litre volumes) and equipment were sterilised by autoclaving for 15 minutes at 121°C (approximately 15lbs in^{-2}). Large volumes (2 - 20 litres) of media were autoclaved at full pressure for 45 minutes. To prevent precipitation, phosphate and trace metals were autoclaved separately and added asceptically to greater than 2 litres of ASW media.

Filter sterilisation was achieved by passing through sterile 0.22μm pore diameter Millipore filter units.

2.3.7 Chemicals

All the chemicals were of Analar (analytical) grade where possible. The general chemicals used in the media were supplied by BDH, Sigma or Fisons. Vitamins and bovine serum albumin (BSA) were obtained from Sigma, fluoroacetic acid (sodium salt, 98%) from Aldrich Chemical Co Ltd.

Radiochemicals were obtained from Amersham International plc, and scintillants were supplied by LKB (Optimcint Safe and Optisorb) and Dupont (UK) Ltd, NEN Products division (Aquasol).

2.4 Routine growth of organisms

Stock cultures were grown in 100ml ASW medium (section 2.3.1) in 250ml cotton-stoppered conical flasks at a temperature of 24 ± 2°C and with continuous illumination from cool white fluorescent tubes. Neutral density screens were used to ensure that cultures were maintained at a low irradiance of 8 to 10$\mu Em^{-2}s^{-1}$. These stocks were subcultured whilst still in exponential growth (every 14 to 21 days) using a 5ml inoculum to give a concentration of approximately 10^6 cells ml^{-1}.

Light intensity was measured at Warwick University with a Macam photometer/radiometer model 3000 (Macam Photometrics Ltd, Livingston, Scotland) and at Plymouth Marine Laboratory with a LICOR light meter which gave a reading in mV. 1mV was equal to 260.5$\mu Em^{-2}s^{-1}$.

The cultures were checked periodically for contamination by bacteria and fungi, by growth on agar, in test media and by visual inspection.

2.4.1 Tests for contaminants

16ml yeast extract (3%), 100ml glucose (10%) and 384ml ASW were autoclaved separately, mixed and dispensed asceptically in 5ml volumes into sterile plastic bijoux bottles. These were stored at 4°C.

Culture suspension (0.5ml) was inoculated into sterile test media. This was incubated in the dark at 25°C for up to seven days and if the media remained clear by comparison to a control vial, the culture was assumed to be axenic. This procedure was also carried out on solid test media (section 2.3.5). The plates were incubated at

24°C in continuous illumination ($10\mu Em^{-2}s^{-1}$) for up to seven days. The absence of bacterial growth was taken as an indication that the culture was axenic.

2.4.2 Visual inspection

4'6-diamidino-2-phenylindole (DAPI) was used to visualise bacteria in a sample (Porter and Feig, 1980). This stain allows combined detection and counts of bacteria and cyanobacteria; the former fluorescing bright blue and the latter a pale yellow.

A working solution of $5\mu g\ l^{-1}$, prepared in purified water was maintained at 4°C; 0.1ml was added per 1ml of culture to be examined. After addition, the mixture was shaken, left for 5 minutes to obtain even and bright staining, and then filtered and mounted as in section 2.6.

The same fluorescent microscope system as in section 2.6 was used, but with a blue filter block (I_2). (A BP 450-490 exciting filter, RKP 510 beam-splitting mirror and LP 515 suppression filter.)

2.4.3 Measurement of the growth of *Synechococcus* WH7803 at different irradiances

Experiments were initiated by inoculating exponentially growing stock cultures into 100ml fresh ASW media, to a cell density of approximately 10^6 cells ml^{-1}.

A range of irradiances from 10 to $90\mu Em^{-2}s^{-1}$ was achieved by the use of neutral density filters and by positioning flasks at different distances from the fluorescent tubes. The irradiance was

calculated as the irradiance received at the nearest surface of the flask to the light source.

Growth of the culture was monitored by the increase in cell numbers using an epifluorescence microscope (section 2.6). Counts were carried out once a day or more frequently at higher irradiances until the stationary phase of growth was reached.

2.5 Dry weight estimations

Dry weights were determined by filtering 1ml of well mixed culture suspension concentrate, onto glass fibre filters (previously washed in purified water and dried to a constant weight). The cells were washed three times with ASW, purified water or ammonium formate (3%) (Holland and Hannant, 1973; Fisher and Schwarzenbach, 1978). Ammonium formate was the only solution to successfully remove excess sea salts without any apparent bursting of the cells. The filters were prepared in triplicate together with control filters which were washed only. These were dried at 100°C to a constant weight (overnight) and reweighed on a Cahn Electrobalance.

The weight of 1ml of culture was determined by subtraction of the dry weight of the filter and the control filter. The control filter accounts for any insoluble compounds in the culture media. Dilutions of culture suspension with ASW media were prepared and the OD_{750} of each one was recorded. A dilution curve of OD_{750} versus dry weight of (mg ml^{-1}) culture in each dilution was plotted. There was a good linear relationship between OD_{750} and dry weight over a wide range of optical densities.

2.6 Enumeration by epifluorescence microscopy

Phycoerythrin-rich *Synechococcus* species can be identified and counted directly by epifluorescence microscopy due to their phycoerythrin autofluorescence and small size (0.2 - 1.0μm diameter).

Samples were processed as soon as possible after collection, although some were stored for up to six hours at 4°C in the dark. The sample size to be filtered varied in natural populations. Typically 30-50ml was filtered, which was sufficient to give 20-40 cells in the eye-piece graticule. For laboratory cultures 1-10ml of a sample dilution, prepared in autoclaved medium, was filtered to give 20-60 cells in the eye-piece graticule.

To provide a dark background for improved visibility of fluorescing bacteria, polycarbonate Nuclepore filters (0.2μm pore-size, 25mm diameter) were stained in a solution of Irgalan black (2.0 g l^{-1} in 2% acetic acid) for twelve hours or more (Daley and Hobbie, 1975). These were rinsed in distilled water and stored in a solution of 2% glutaraldehyde until use.

Samples were filtered onto stained Nuclepore filters under a low vacuum (100-125mm Hg). The filter was mounted, sample side up on top of a thin film of immersion oil (Nikon) on a microscope slide. When the filter became transparent, an additional drop of immersion oil was placed on the filter, followed by a cover slip. The preparation could be counted immediately or kept at 4°C in the dark for up to twelve hours without apparent loss of autofluorescence.

Phycoerythrin-rich *Synechococcus* fluoresce bright orange in green light. These cyanobacteria were counted using a Leitz Ortholux 2 epifluorescence microscope with 50W HBO light source and equipped with a Ploempak 2.2 fluorescence vertical illuminator, containing a

BP 515-560 exciting filter, RKP 580 beam-splitting mirror and LP 580 suppression filter (filter block N2.1) and an NPL Fluorotar 100/1.32 oil objective.

Cell concentration was calculated as follows:

[mean cell number (field)$^{-1}$/vol of sample filtered] x

[filter area/field area] = cells ml^{-1}

The internal diameter of the filter funnel was 15.00mm

so, area of filter used (πr^2) = 176.7146mm^2

area of graticule (field area) = 0.008464mm^2

(filter area/field area) = 20878.38 (conversion factor)

2.7 Oxygen electrode

The oxygen electrode used was the Hansatech D.W. Clark-type oxygen electrode based on a design by Delieu and Walker (1972) which was developed for the measurement of oxygen evolution and uptake. The main features of this apparatus are shown in figure 2.1. It consisted of an electrode disc, located in the base of a water-jacketed reaction chamber. The disc comprised a platinum cathode embedded in the dome of the disc and a silver anode in the wall of the disc. The anode was immersed in and linked to the cathode by a saturated solution of potassium chloride over the surface of the electrodes. A cigarette paper "spacer" was placed beneath an oxygen permeable PTFE membrane, to provide a uniform layer of electrolyte between anode and cathode and this was secured by a rubber O-ring placed over the dome.

The contents of the reaction chamber were stirred by a magnetic follower, "flea", which spun directly above the membrane-covered

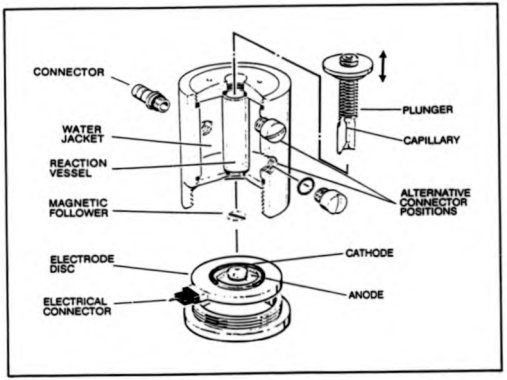


Fig. 2.1. Features of the Hansatech D.W. Oxygen Electrode Unit (from Hansatech brochure).

platinum electrode, preventing local oxygen depletion. A plunger removed trapped air and allowed adjustment of the volume of the reaction chamber between 0.5 and 2.0ml. Samples were removed and substrates added by microsyringe through a central hole in the plunger, without disturbing the recorded trace.

2.7.1 Calibration

The reaction chamber was filled with 2.0ml aerated purified water, and left for about 30 minutes until the recorded trace stabilised. This was established as an electrically convenient "air-line" by closing the chamber with the plunger. To remove all traces of oxygen from the sample and establish an oxygen minimum, a few crystals of sodium dithionate were added. This consumes oxygen according to the equation, $Na_2S_2O_4+H_2O+O_2 = NaHSO_4+NaHSO_3$. The output dropped rapidly and gradually reached a stable minimum value, "N_2-line".

As dithionate can damage or poison the membrane it was removed as soon as possible. The electrode was rinsed five times with purified water to remove the dithionate solution and then replaced by 2ml aerated purified water at 25°C. The electrical output rose to the "air-line". The difference between the "air-line" and "N_2-line represented the oxygen concentration. The oxygen content of air saturated water at 25°C is 0.253μmole ml^{-1} (8.11ppm), (Deliau and Walker 1972); each division of the chart represented,

$(2 \times 0.253)/$("air-line"-"N_2-line" divisions)

$= nmols\ O_2\ (division)^{-1}(2ml\ culture)^{-1}$.

2.7.2 Measurement of respiration rate

The oxygen electrode was calibrated with 2ml aerated purified water. The reaction chamber was maintained at 25°C by the water jacket at Warwick university and at Plymouth Marine Laboratory the whole unit was maintained in a constant temperature laboratory. Cells were centrifuged and resuspended in fresh ASW media to give an optical density at 750nm of between 0.1 and 0.2. These were kept under the original growth conditions.

2ml of culture suspension was placed in the electrode chamber. The vessel was sealed with the plunger and the whole unit darkened. To ensure similarity of stirring all experiments were mixed at maximum rate. A trace was recorded and the oxygen uptake was calculated as follows.

[nmols O_2 division^{-1} 2ml^{-1}] × [(divisions up)/(cm across)] ×

trace speed × sensitivity = oxygen uptake (nmols O_2 h^{-1} 2ml^{-1})

where trace speed was in units of cm h^{-1} and sensitivity refers to the expansion of the trace scale.

The respiratory rate in terms of Qo_2 [μl O_2 h^{-1}(mg dry weight)$^{-1}$] was also calculated,

1 nmol of gas occupies 22.414 nl,

[nmols O_2 h^{-1} (2ml culture)$^{-1}$][2 × dry weight (mg ml)$^{-1}$]$^{-1}$ × 22.414

= μl O_2 h^{-1} (mg dry weight)$^{-1}$.

2.7.3 Assessment of the effect of different concentrations of substrates and inhibitors.

The oxygen electrode was calibrated with 2ml aerated purified water at 25°C. The cells were prepared as in section 2.7.2 and

maintained at the original growth conditions. An oxygen uptake rate was measured with 2ml of suspension. Substrate was pipetted directly into the culture suspension and the new oxygen uptake rate recorded. To obtain increasing concentrations of added substrate, aliquots were added sequentially and the sum of all previous additions plus the present one was equal to the new concentration. 1 to 20mM substrate was added.

The same procedure was used for assessing the effect of different concentrations of the inhibitors, arsenite, fluoracetate and malonate (all sodium salts) on *Synechococcus* WH7803.

At Plymouth Marine Laboratory a slightly different procedure was used. A rate for 2ml of suspension was obtained, then 20μl of one of the substrates was added via the central plunger (final concentration of 10mM) and a trace recorded. A further 80μl was added (final concentration of 50mM) and another trace recorded. This was repeated twice for each substrate and finally the electrode was recalibrated to ensure no "drift".

2.7.4 Assessment of the effect of 10mM substrate or inhibitor over several hours.

Cells were prepared as in section 2.7.2 and the suspension was divided into aliquots. Substrate was added to one flask to give a final concentration of 10mM, whilst the other was kept as a control culture without any additions. The two flasks were darkened by covering them in foil and maintained at their original growth temperature (25 ± 2°C).

Samples were removed at recorded time intervals (0 to 8h)

without allowing light to enter either the incubation or the oxygen electrode and an oxygen uptake rate was recorded. Between samples the electrode was rinsed thoroughly with five changes of purified water.

This procedure was also used to assess the effect of 10mM inhibitor. The cultures were maintained at 25 ± 2°C and at a low irradiance ($8\mu Em^{-2}s^{-1}$) during incubations with inhibitors.

2.7.5 Determination of the effect of inhibitors on a natural assemblage

10L of seawater from 30 metres depth were filtered onto a 1.0μm Nuclepore filter. This was then filtered onto a 0.6μm Nuclepore filter and this filter resuspended in 650ml deionised water. In this way species larger than 1.0μm were removed and the sample was concentrated × 15. NUNC bottles (60ml) were filled with the concentrate and three sets of incubations were prepared.

a) 100 μCi ^{14}C.

b) approx 1.5mM sodium malonate + 100 μCi ^{14}C.

c) approx 1mM fluoracetate + 100 μCi ^{14}C.

The bottles were covered in foil and kept at 10°C. 10ml samples were removed at timed intervals and filtered through a 0.2μm Nuclepore filters. The filters were placed in vials in a dessicator until counted.

2.8 Control of turbidostat cultures

Turbidostat systems were set up (Munson, 1970; Loogman et al., 1980) in which a constant cell density was maintained by dilution

with a continuous supply of fresh media. The cell density was kept low (optical density at 750_{nm} <0.200) in order to maintain an even light distribution in the culture vessel.

Photocells were attached to the outside of a transparent water bath (Figure 2.2). The sample photocell measured light transmitted through the culture from the growth lights, whilst the reference photocell measured the growth irradiance directly. When the difference between the voltages generated by the light reaching both the photocells fell to a pre-selected value, a peristaltic pump was operated and fresh medium transferred from a reservoir to the growth vessel. Constant volume was achieved by means of an overflow system. The speed of the pump was adjusted, until there was no change in cell density at a steady dilution rate.

From chemostat theory when there is no change in cell density, $\mu = D$, where μ is the specific growth rate of the culture and D is the dilution rate and a steady state exists. The dilution rate D, was calculated from $D = F\ V^{-1}$, where F was the flow rate of fresh medium and V was the volume of culture in the vessel.

Illumination was provided by a bank of warm white fluorescent tubes and adjusted to give different growth irradiances by the addition of netting which acted as a neutral density filter. An accurate description of the growth irradiance was found difficult to measure with the light meter due to factors such as light-scattering and selective absorption. The different specific growth rates which were a result of the different growth irradiances were used as a scale by which the changes in the measured parameters could be related. Temperature was kept constant at 25°C by circulating water from a Grant circulator and cooler. A high constant rate of stirring

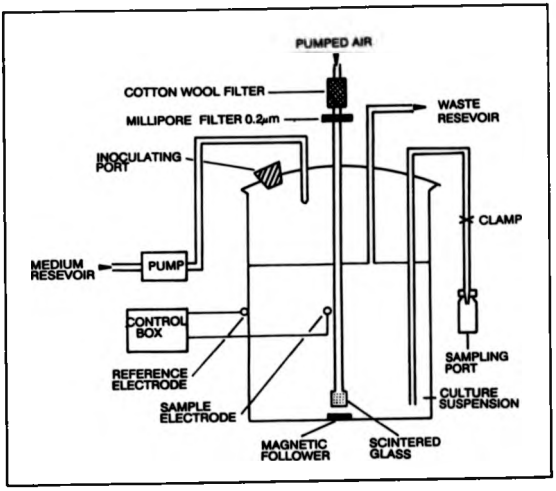


Fig. 2.2. Diagrammatic representation of a turbidostat vessel.

was maintained by a teflon-coated magnetic stirrer. Air from a small air pump was fed into the culture via a 0.2μm sterile Millipore filter and a sterile cotton wool air filter. Both the stirring and aeration helped to prevent wall growth.

Experiments were started by inoculating exponentially growing stock cultures (maintained at $10\mu Em^{-2}s^{-1}$) into a sterile culture vessel to a cell density of approximately 10^6 cells ml^{-1}. Cell biomass was determined by the measurement of optical density (OD) at a wavelength of 750_{nm} in a LKB spectrophotometer. The culture was grown without dilution to an optical density of approximately 0.200. The flow rate of added media was adjusted manually until a constant biomass was achieved with a continuous flow rate. Steady states at each specific growth rate were maintained for at least two generation times before harvesting the culture to measure respiratory and photosynthetic rates, pigment concentrations, nucleic acid and protein concentrations.

2.8.1 Measurement of the rate of photosynthesis

The rate of respiration and photosynthesis were determined at 25°C in a Clark-type oxygen electrode (section 2.7).

The electrode was calibrated with 2ml of purified water. Cells were concentrated and resuspended in fresh ASW media to give a optical density of approximately 0.8, and 2ml were placed in the electrode chamber.

Respiration was determined for a period of 5-10 minutes with the electrode unit completely darkened. Oxygen evolution (photosynthesis) was determined after removing the foil used to

darken the electrode, in irradiances between 4 and 1050$\mu m^{-2}s^{-1}$, provided by a slide projector and neutral density filters. Irradiances were measured by a Macam photometer with the probe positioned at a point equivalent to the distance measured as the centre of the electrode.

Photosynthesis was calculated as the sum of oxygen evolution in the light, plus oxygen consumption in the dark.

2.8.2 Chlorophyll *a* estimation

25ml of cells were centrifuged (6000rpm for 15 minutes). The supernatant was discarded and the pellet resuspended by vortexing with 5ml methanol (90%). The suspension was left at room temperature in the dark for 1 hour to allow elution of the pigment, and then centrifuged to remove the cell debris. The absorbance of the extract was read at 665nm, measured with a path length of 1cm. As chlorophyll is less stable in methanol than in acetone the readings were taken as near to the hour as possible. The equation of Talling and Driver (1963) was used to calculate the concentration of the chlorophyll *a*.

chlorophyll *a* ($\mu g\ ml^{-1}$) = $13.9 \times OD_{665} \times v/l$,

where v was the volume of resuspension (5ml) and l was the original volume (25ml).

2.8.3 Phycoerythrin estimation

25ml of cells were centrifuged at 6000rpm for 15 minutes. The cell pellet was resuspended in 10ml of 50mM potassium phosphate buffer (pH 8.0) and the suspension was poured into glass homogeniser

tubes containing glass beads 0.10 to 0.11mm diameter. 10 x 45 second bursts with a 30 second rest period between each burst in a CO_2 cooled Braun MSK cell homogeniser, were required to break 100% of the cells. This was confirmed microscopically.

To obtain a cell free aqueous extract, the material was centrifuged in an ultracentrifuge at 40000g for 15 minutes. The absorbance of the extract was read at 548nm, measured in a path length of 1cm. An extinction coefficient of 105 was used (Alberte et al., 1984) to calculate the concentration of phycoerythrin ($\mu g\ l^{-1}$).

$OD_{548} \times 105 \times v/l$,

where v was the volume in which the cells were extracted (10ml) and l was the original volume (25ml).

2.8.4 RNA estimation

RNA was estimated by a modification (Taggart, 1967) of the Orcinol reaction of Schneider (1957).

The cell pellet from a 100ml sample was resuspended in 10ml of absolute ethanol at 0-3°C. The suspension was transferred to a glass universal bottle and centrifuged at 1800rpm for 15 minutes. The pellet was washed once more in absolute ethanol at 0-3°C and resuspended in 5ml NaCl (0.2M) and 0.2ml perchloric acid (PCA, 60% v:v). The sample was incubated at 70°C for 80 minutes; the caps of the universal being placed on loosely to reduce evaporation. After cooling, the cell debris was removed by centrifugation at 0°C and 1800 rpm for 15 minutes. The top 75% of the supernatant was removed and either stored at -15°C, or assayed immediately by the Orcinol method.

Standard solutions of D-ribose (0-25$\mu g\ ml^{-1}$) were prepared in a perchloric acid/sodium chloride solution (4ml 60% PCA:96ml 0.2M NaCl).

1ml of each standard and sample solution was added to 0.1ml ethanolic orcinol (6% w:v) and 1ml ferric chloride (0.05% w:v) in concentrated hydrochloric acid. Both solutions were made up fresh. The mixture was heated at 100°C for 30 minutes in covered tubes. Once these had cooled, 1ml ethanol (20% v:v in distilled water) was added to stabilise the yellow/green colour. The absorbance of each tube was read at 650nm in an LKB spectrophotometer.

The quantity ($\mu g\ ml^{-1}$) of D-ribose present in each sample was read from the standard curve, and the amount of RNA estimated as follows:

If we assume 1μg ribose = 4.3μg RNA, (Mann and Carr, 1974)

then $x\ \mu g$ D-ribose $\times\ 4.3 \times {}^{v}/_{V} = \mu g\ ml^{-1}$ RNA,

where v was the volume of resuspension (5ml), V was the volume of culture (100ml) and x was the value read from the standard curve given the OD_{650} of the sample.

2.8.5 DNA estimation

DNA was estimated by a procedure based on the modified diphenylamine reaction of Abraham et al., 1972.

The pellet obtained from a 100ml sample was resuspended in 5ml perchloric acid (0.25M) in a glass universal and left for 15 minutes on ice (0-3°C). The suspension was centrifuged at 1800rpm for 15 minutes and washed again in 5ml 0.25M perchloric acid. After the second wash and subsequent centrifugation, the remaining pellet was

resuspended in 5ml 0.5M perchloric acid. The universals were covered and incubated at 70°C for 80 minutes. After cooling, the suspension was centrifuged as before. The top 75% of the supernatant was carefully removed and either stored at -20°C until analysis at a later date or assayed immediately, using the diphenylamine reaction described below.

Diphenylamine (1.5g) was dissolved in 100ml glacial acetic acid. 1.5ml concentrated sulphuric acid (H_2SO_4) was added followed by 1ml of acetaldehyde (8mg ml^{-1}).

Standard solutions of deoxyribose (0-10μg ml^{-1}) were prepared by dissolving in 0.5M perchloric acid. To 1ml of each standard and sample, 2ml of freshly prepared diphenylamine reagent was added and the tubes inverted to mix. The tubes were left overnight (16-20 h) at room temperature in the dark, to allow a blue colour to develop.

The colour of the samples was read at 650nm in an LKB spectrophotometer. A standard curve of deoxyribose (0-10μg ml^{-1} in 0.5M perchloric acid) was produced for each set of determinations. The quantity (μg ml^{-1}) of deoxyribose present in each sample was read from the standard curve and the amount of DNA present in the sample was estimated as follows,

If we assume 1μg deoxyribose = 5.6μg DNA, (Mann and Carr, 1974)

then x (μg deoxyribose) $\times$ 5.6 $\times$ v/V = μg ml^{-1} DNA.

where v was the volume of resuspension (5ml), V was the volume of culture (100ml) and x was the value read from the standard curve given the OD_{650} of the sample.

2.8.6 Protein estimation

A "Biorad" microassay was used (commercial assay).

To prepare a standard curve, several dilutions of bovine serum albumin (protein standard) containing 1 to 25$\mu g\ ml^{-1}$ were made up. 25ml samples were prepared and homogenised as in the phycoerythrin procedure. The homogenised suspension was used for protein estimations.

0.8ml of the standards and appropriately diluted samples were pipetted into clean dry test-tubes. 0.8ml of sample buffer (ASW media) was placed in the "blank" test-tube. 0.2ml of the Dye Reagent Concentrate was added, and the samples mixed by gentle inversion of the tube. After a time period of from 5 minutes to one hour the absorbance at 595nm was read versus the reagent blank.

OD_{595} was plotted against the concentration of the standards to produce a standard curve. The protein concentration of the diluted sample was read from this.

The concentration of protein in the original sample was calculated as follows,

x ($\mu g\ ml^{-1}$) protein x dilution x 1/0.8 x 10/25,

where x ($\mu g\ ml^{-1}$) protein was the reading form the standard curve.

2.9 Sampling at sea.

All samples for photosynthesis-irradiance curves and carbon incorporation experiments were collected from the surface mixed layer at 10 metres using NIO water bottles. These bottles which were fitted with a closing device, were lowered open to the desired depth and

then closed automatically. Closure was activated by a drop-weight, "messenger" which slid down the supporting wire. Other discrete samples for vertical profiles of cyanobacteria and chlorophyll *a* were obtained from various depths, using a conductivity, temperature, depth (CTD) rosette, and surface samples were collected whilst underway from a subsurface pump at a depth of @ 1.5 metres.

2.9.1 Size-fractionation

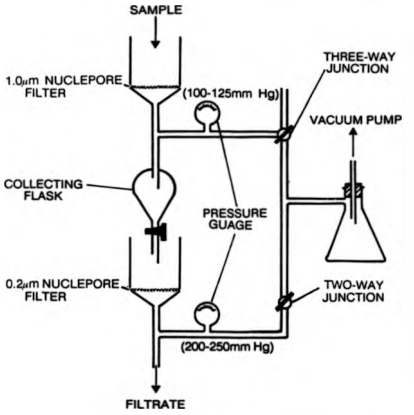
Natural assemblages were separated into two size-fractions, >1.0μm phytoplankton and 1.0-0.2μm phytoplankton (picoplankton) using a filter tower built of Sartorius glassware (Figure 2.3). Nuclepore filters (47mm diameter), of pore size 1.0μm (top) and 0.2μm (bottom) were used. A vacuum from a vacuum pump of 100 - 125mm Hg was applied to the upper filter and the filtrate was collected in a pear-shaped flask. The pressure was released and a valve opened to allow the filtrate to drain onto the 0.2μm filter. A vacuum of 200 - 250 mm Hg was applied to the bottom filter. A gentle vacuum was used in both filtration steps to avoid disruption of the cells. The filters were removed and treated as described for each procedure.

2.9.2 Chlorophyll *a* measurement (natural populations)

100ml samples were collected and filtered in duplicate within six hours of sampling using the size-fractionation apparatus (section 2.9.1). These were processed immediately or placed in separate labelled plastic bags and kept frozen until return to the laboratory.

The filters were extracted in glass vials with 10ml 90% acetone

(a)



(b)

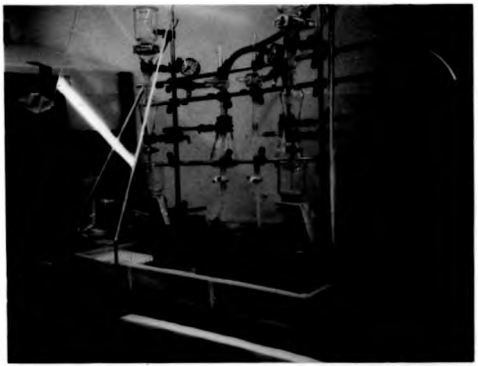


Fig. 2.3. Size-fractionation apparatus used in the separation of natural assemblages. (a) diagrammatic representation, and (b) photographic record.

at 4°C and incubated overnight in the dark. Fluorescence of the extracts was measured in a Turner fluorometer. The measurement before adding acid was recorded (R_b); 2 drops of 6N hydrochloric acid (HCL) were added, the vial inverted to mix the contents and the new reading was recorded (R_a).

The concentration of chlorophyll *a* was calculated according to Strickland and Parsons (1968).

mg chl *a* m^{-3} ($\mu g\ l^{-1}$) = $58.280(R_b - R_a) \times$ dilution factor

where R_b = before acid

R_a = after acid

100ml samples were filtered and 10ml 90% acetone was used for each extraction, therefore the dilution factor was 10/100 = 0.1.

$[58.280(R_b - R_a)] \times (10/100)$

2.9.3 Carbon-14 assimilation.

Time course incubations were carried out in 60ml tissue-culture bottles (NUNC), which were incubated at constant light intensity (white light fluorescent tube) and cooled by circulating water at ambient temperature. Bottles were filled with water from a discrete sample, inoculated with 10μCi/bottle $Na^{14}HCO_3$ (Amersham International) and placed in an incubator for between zero and forty-eight hours. At the end of each incubation period, four bottles were size-fractionated (section 2.9.1). Two pairs of filters were placed in scintillation vials, 3ml cold methanol/acetic acid (95:5, v:v) was added and the vials were stored at -20°C until further analysis (cellular fractionation, section 2.9.4) in the laboratory. The other two pairs were transferred to scintillation vials and dried in a

desiccator with active silica gel. Total ^{14}C incorporation was calculated using Optiscint Safe (LKB) as a scintillant.

2.9.4 Cellular fractionation

The distribution of ^{14}C in low molecular weight metabolites, lipids, polysaccharides and proteins was determined using the extraction procedure of Li et al., (1980). A schematic representation of this procedure is shown in Figure 2.4.

Chloroform (1.5ml) was added to the filter in the methanol/acetic acid mixture (section 2.9.3). The suspension was vortexed for 1 minute, kept at 4°C for 10 minutes, and then filtered through a Whatman GF/F filter (25mm diameter) under a vacuum of <100mm Hg. The filter was washed with a further 1.5ml chloroform and 1.5ml distilled water was added to the combined filtrate. This was subsequently vortexed for 1 minute before being centrifuged at 2600 rpm for 10 minutes. 1ml of the bottom chloroform layer, which contained the lipid fraction, was placed in a scintillation vial, dried, and 10ml scintillation fluid added (Aquasol, New England Nuclear (NEN)). Scintillant was also added to 1ml of the upper methanol/ water layer, which contained the low molecular weight metabolites. The residue on the filter was incubated at 95°C for 30 minutes in 4ml 5% trichloroacetic acid (TCA), filtered through a second GF/F filter (25mm diameter), and washed with a further 4ml 5% trichloroacetic acid. The combined filtrates were vortexed and 2ml aliquots were dried in scintillation vials. The dried extracts were resuspended in 1ml distilled water, and Aquasol scintillant added. This fraction was referred to as the polysaccharide fraction but also

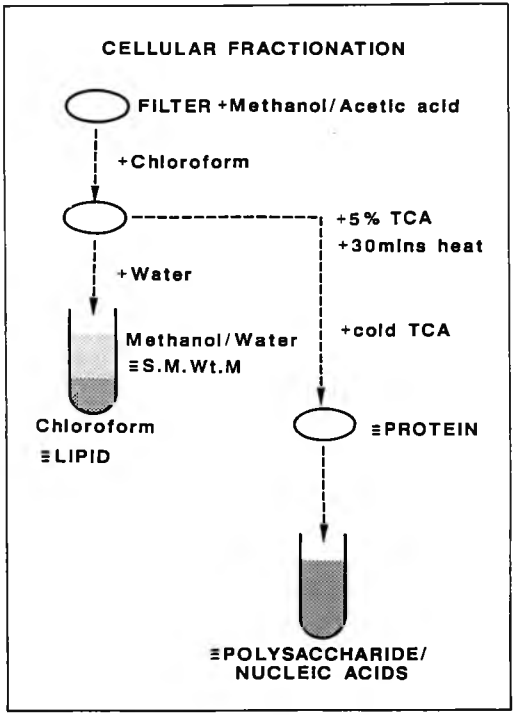


Fig. 2.4. Schematic representation of the extraction procedure according to Li et al., (1980).

contained nucleic acids. Scintillant was added to the remaining filter, which contained the protein fraction.

2.9.5 Measurement of primary production

Primary production was measured by the Steemann-Nielsen ^{14}C method. Photosynthesis-irradiance (P-I) experiments were carried out in 60ml NUNC tissue culture bottles. These were filled to the brim with the water sample within 30 minutes of sampling. (Air bubbles in the bottles can cause inconsistencies in the light measurements). 100μl of 100μCi ml^{-1} $Na^{14}CO_3$ (Amersham International plc), was added to each bottle (ie: 10μCi bottle^{-1}) and incubated for between four and six hours in a light gradient, cooled with circulating water at the ambient temperature from which the samples were taken. All samples on any particular day were inoculated with ^{14}C from the same stock ampoule.

The light source was a projector (tungsten lamp) and since this was run for some considerable period of time, cooling fans were necessary. A mirror, placed at an angle, was in the light path to deflect the light into the incubator. The light gradient was formed by the bottles and their contents, which were stacked directly on top of one another in racks of 20 bottles. To obtain low light intensities an opal plastic filter was fitted to the bottom of one of the two racks, thus reducing the intensity of the light reaching the bottles. In this way a range of light intensities from approximately 5 to 1500 $\mu Em^{-2}s^{-1}$ was obtained, with some overlap in the middle sections. The light intensity reaching each bottle was measured for every experiment with a LICOR quantum sensor.

At the end of the experiment the samples were size-fractioned

(section 2.9.1) through two different pore size Nuclepore filters. The filters were removed and transferred to scintillation vials which were placed in a dessicator with active silica gel. These filters were left to dry overnight and either counted on board, or kept desiccated until returned to the laboratory. 5mls of a toluene based scintillation cocktail (Optiscint Safe, LKB) was added to the vials, which were counted in a (Packard TRI-CARB 4430) liquid scintillation counter.

Distintegrations per minute (dpm) were automatically produced from the pre-determined quench curve stored on a computer disc.

2.9.6 Determination of radioactivity and quench curves for each of the scintillation cocktails.

Carbon-14 decays by the emission of beta particles, and this was measured in a scintillation counter. At Plymouth Marine Laboratory and whilst at sea a Packard TRI-CARB 4430 liquid scintillation system was used. Several different commercially available scintillants were used and for each a quench curve was prepared. The scintillants (as shown in the text), were Optiscint Safe (LKB), Optisorb-2 (LKB) and Aquasol (NEN).

Filters were left in vials in a desiccator until ready to count. If they were stored with scintillant the counting efficiency was found to deteriorate rapidly.

Quench curves were determined for the Packard TRI-CARB system using the external standard technique for determining the counting efficiency. The Packard Tri-CARB system has fully automated external standard facilities and determines the counting efficiency

automatically.

A set of four vials were prepared containing one of the standards, the volume of liquid and/or filter used in the experiment and the scintillation cocktail. Aliquots of chloroform (a quenching reagent), were added so that a series of forty quenched standards were produced and the efficiency determined. An efficiency calibration curve (efficiency versus SIE) was constructed automatically and stored on disc.

The Spectral Index of External Standard (SIE), is a number obtained from the spectralyser that is calculated from the spectral distribution of the external standard.

2.9.7 Determination of the activity of carbon-14 added

The activity of the ^{14}C from each ampoule of $NaH^{14}CO_3$ used in an experiment was measured daily.

Optisorb (scintillant) was dispensed (10ml) into glass scintillation vials before the cruise and stored away from the light. 100µl of the $NaH^{14}CO_3$ used was pipetted into a 10ml volumetric flask directly after inoculation of the bottles and the volume made up to 10ml with Optisorb. This was mixed well and 100µl of the dilution put into one of the vials containing pre-dispensed Optisorb. Five vials for each dilution were prepared and counted immediately.

When Optisorb was unavailable 10ml of Optiscint Safe containing 100µl phenylethanolamine was used. The phenylethanolamine absorbs $^{14}CO_2$.

2.9.8 Calculation of primary production

Production: mgC m^{-3} h^{-1}

= (dpm counted x 25200 x 1.05)(dpm added x time)$^{-1}$

where the concentration of bicarbonate = 25.2 mgC l^{-1}

= 25200 mgC m^{-3}

This assumes that the bicarbonate content of seawater is 2.1 mmol l^{-1}. The factor 1.05 is put in to allow for the fact that the carbon-14 isotope behaves rather differently from the carbon-12 isotope found in nature (Strickland and Parsons, 1968).

Specific production: mgC (mg chl a)$^{-1}$ h^{-1}

CHAPTER THREE

THE EFFECT OF GROWTH IRRADIANCE ON GROWTH, RESPIRATION AND THE MACROMOLECULAR COMPOSITION OF *SYNECHOCOCCUS* WH7803

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3.1 RESULTS

3.1.1 The different growth rates of *Synechococcus* WH7803

The growth rate of *Synechococcus* WH7803 reaches a maximum at relatively low irradiances (Morris and Glover, 1981). However recent field observations (Joint and Pomroy, 1986; Waterbury et al., 1986; Glover et al., 1988b) and laboratory investigations (Kana and Glibert, 1987a) suggest that *Synechococcus* WH7803 is not confined to dimly illuminated areas of the ocean. The effect of growth irradiance on cultures of *Synechococcus* WH7803 was examined over a range of irradiances from $7\mu Em^{-2}s^{-1}$ to $90\mu Em^{-2}s^{-1}$. No growth occurred at higher irradiances possibly as a result of the sudden change from low light at which the stock culture was maintained ($10\mu Em^{-2}s^{-1}$) to irradiances greater than $90\mu Em^{-2}s^{-1}$. Difficulty in maintaining the temperature at 25 ± 2°C was also found at higher irradiances. Cells were counted at recorded times after inoculation and growth curves were drawn from the data points using a logarithmic scale. A selection of the growth curves obtained and the line of best-fit calculated by linear regression through each set of data points, are plotted in Figure 3.1; Tables 3.1 to 3.5 show the cell count at each time interval for the plotted lines.

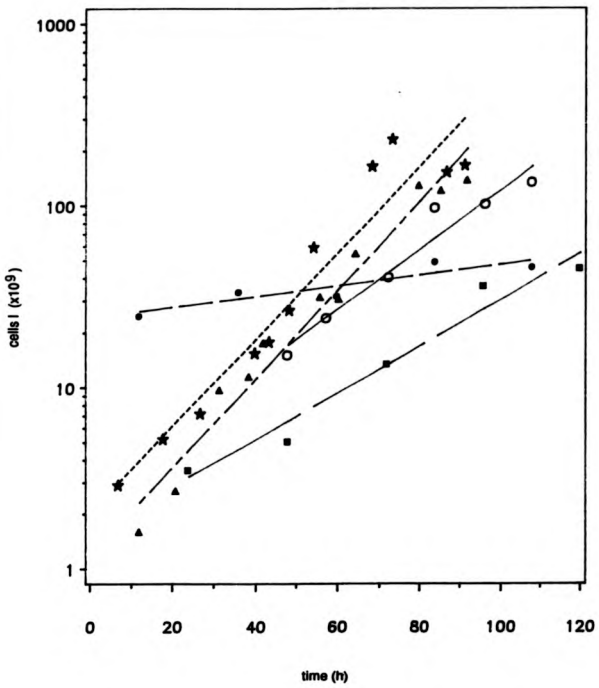


Fig. 3.1. Growth curves of *Synechococcus* WH7803 at various irradiances :
7μEm⁻²s⁻¹ (—●— —●—), 31μEm⁻²s⁻¹ (—■———■—),
50μEm⁻²s⁻¹ (—○———○—), 70μEm⁻²s⁻¹ (--★----★---),
90μEm⁻²s⁻¹ (—▲– –▲—).

Table 3.1. to 3.5. Variations in *Synechococcus* cell density at timed intervals after inoculation, when grown at different irradiances ($7\mu Em^{-2}s^{-1}$ to $90\mu Em^{-2}s^{-1}$)

Table 3.1. Growth irradiance, $7\mu Em^{-2}s^{-1}$.

time (h)	cells l^{-1}
12	$2.50x10^{10}$
36	$3.39x10^{10}$
60	$3.22x10^{10}$
84	$4.96x10^{10}$
108	$6.64x10^{10}$
132	$7.49x10^{10}$
156	$9.92x10^{10}$
180	$1.30x10^{11}$
204	$1.58x10^{11}$
228	$1.39x10^{11}$
252	$2.08x10^{11}$
276	$2.34x10^{11}$
300	$2.36x10^{11}$
324	$1.84x10^{11}$

growth irradiance = $7\mu Em^{-2}s^{-1}$
generation time = 69.5h
specific growth rate = $0.010h^{-1}$

Table 3.2. Growth irradiance, $31\mu Em^{-2}s^{-1}$.

time (h)	cells l^{-1}
24	$3.55x10^{9}$
48	$5.12x10^{9}$
72	$1.36x10^{10}$
96	$3.65x10^{10}$
120	$4.57x10^{10}$
144	$5.07x10^{10}$
168	$4.86x10^{10}$
192	$4.30x10^{10}$

growth irradiance = $31\mu Em^{-2}s^{-1}$
generation time = 16.9h
specific growth rate = $0.041h^{-1}$

Table 3.3. Growth irradiance, $50\mu Em^{-2}s^{-1}$.

time (h)	cells l^{-1}
48.0	$1.53x10^{10}$
57.5	$2.44x10^{10}$
72.5	$4.08x10^{10}$
84.0	$9.83x10^{10}$
96.5	$1.03x10^{11}$
108.0	$1.36x10^{11}$

growth irradiance = $50\mu Em^{-2}s^{-1}$
generation time = 13.6h
specific growth rate = $0.051h^{-1}$

Table 3.4. Growth irradiance, 70$\mu Em^{-2}s^{-1}$.

time (h)	cells l^{-1}
7.0	$2.93x10^{9}$
18.0	$5.26x10^{9}$
27.0	$7.27x10^{9}$
40.0	$1.56x10^{10}$
43.5	$1.80x10^{10}$
48.5	$2.69x10^{10}$
54.5	$5.95x10^{10}$
68.5	$1.66x10^{11}$
73.5	$2.33x10^{11}$
87.0	$1.54x10^{11}$
91.5	$1.68x10^{11}$

growth irradiance = 70$\mu Em^{-2}s^{-1}$
generation time = 11.8h
specific growth rate = 0.059h^{-1}

Table 3.5. Growth irradiance, 90$\mu Em^{-2}s^{-1}$.

time (h)	cells l^{-1}
12.0	$1.60x10^{9}$
21.0	$2.70x10^{9}$
31.5	$9.69x10^{9}$
38.5	$1.15x10^{10}$
42.0	$1.76x10^{10}$
56.0	$3.15x10^{10}$
60.5	$3.17x10^{10}$
64.5	$5.45x10^{10}$
80.0	$1.29x10^{11}$
92.0	$1.38x10^{11}$

growth irradiance = 90$\mu Em^{-2}s^{-1}$
generation time = 12.8h
specific growth rate = 0.054h^{-1}

The doubling time for each growth curve was determined from the regression lines of the points corresponding to the exponential phase of growth and the specific growth rate (μ) was calculated using the equation,

$$\mu = \ln(N_1/N_0)/(t_1/t_0)$$

where N_1 and N_0 were the cell concentrations at times t_1 and t_0. The generation time (g) was determined from $g = \ln 2/\mu$.

Growth rates and generation times of *Synechococcus* WH7803 are shown in Table 3.6 and a plot of these data is shown in Figure 3.2. Initially growth rate (μ) increased linearly with growth irradiance. Above $50\mu Em^{-2}s^{-1}$ only small increases in specific growth rate or a decrease in specific growth rate with increasing irradiance took place, implying that saturation of growth had occurred. There were insufficient data points to determine the exact growth irradiance at which saturation first occurred. However an approximation was determined by calculating the intersection of the linear regression line of all the data points up to and including those at $50\mu Em^{-2}s^{-1}$, with the line produced at μ_{max}: $0.059h^{-1}$ or $0.056h^{-1}$. The highest growth rate obtained was $0.059h^{-1}$ at $70\mu Em^{-2}s^{-1}$. The following two data points at growth irradiances 78 and 90 $\mu Em^{-2}s^{-1}$ had lower specific growth rates, therefore μ_{max} was also calculated as the mean of the last three data points, and a second value for μ_{max}, of $0.056h^{-1}$ was obtained. This gave the growth at which saturation first occurred as between 55.0 and $59.0\mu Em^{-2}s^{-1}$.

Table 3.6. Generation times and specific growth rates (divisions per hour and per day) obtained at different growth irradiances.

growth irradiance ($\mu Em^{-2}s^{-1}$)	generation time (h)	specific growth rate	
		divisions h^{-1}	divisions day^{-1}
6.5	47.3	0.015	0.35
7.0	69.8	0.010	0.24
7.0	37.5	0.019	0.44
7.5	66.7	0.010	0.25
7.5	53.2	0.013	0.31
7.5	41.6	0.016	0.40
31.0	18.1	0.038	0.92
31.0	16.9	0.041	0.98
50.0	14.3	0.048	1.16
50.0	13.6	0.051	1.22
50.0	12.3	0.056	1.35
70.0	11.8	0.059	1.41
78.0	12.6	0.055	1.32
90.0	12.7	0.054	1.31

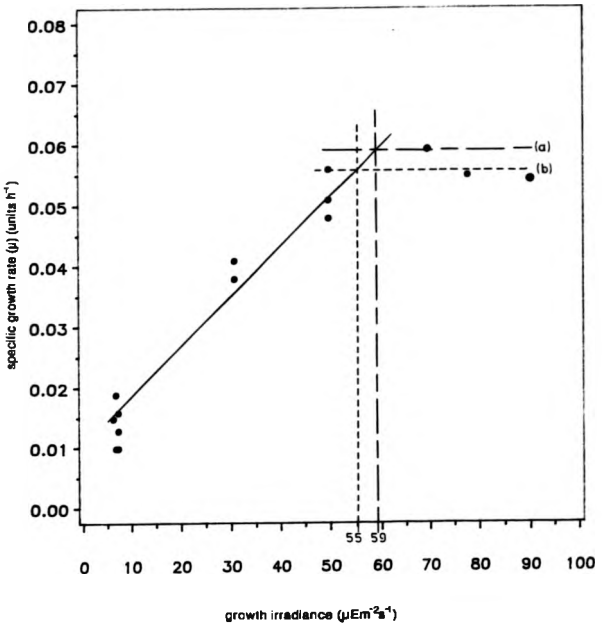


Fig.3.2. Effect of irradiance on specific growth rate (μ), and estimates of the irradiance at which growth became saturated. Two irradiances were calculated, (a) using the maximum specific growth rate obtained (— —), and (b) using the average of the three highest growth irradiances (- - - - -).

3.1.2 Respiratory rates of *Synechococcus*.

The rate of respiration was measured by following the uptake of oxygen per unit time using a Clark-type oxygen electrode. This rate is usually expressed as $\mu l\ O_2\ h^{-1}(mg\ dry\ weight)^{-1}$ and is known as the QO_2 value. Oxygen uptake rates obtained from *Synechococcus* WH7803 cultures grown at Plymouth Marine Laboratory were used for estimating the respiratory rates. To express the oxygen uptake rates in terms of $\mu l\ O_2\ h^{-1}(mg\ dry\ weight)^{-1}$, an estimate of the dry weight of the culture was required.

Many dry weight measurements were carried out, however the results were generally not reproducible. Initially this was because of the difficulty in finding a solution suitable for washing the cells to remove salts in the media. Distilled water produced an osmotic shock on the cells, causing some to burst, easily seen by the pink colouration of the filtrate from the released phycoerythrin. Making an allowance for the salt in the media by filtering an equal volume (1ml) onto a separate filter and subtracting this weight from the unwashed culture filter also yielded inconsistent results. Washing the cells with 3% ammonium formate which elutes the salts (Holland and Hannant, 1973), was found to be the best method and gave consistent results. This method was only tested a few times, and it was decided to use dry weight values, calculated from the carbon content of the cells to express uptake rates in terms of $\mu l\ O_2\ h^{-1}(mg\ dry\ weight)^{-1}$. The carbon content of *Synechococcus* cells, determined using a Carbon Hydrogen Nitrogen (CHN) analyser, ranged from 150 to 290 $fgC\ cell^{-1}$ (Waterbury et al., 1986). It was assumed that the carbon content per cell was 46% of the cell dry weight (Wyman,

personal communication), therefore dry weight was equal to 326 - 630 fg $cell^{-1}$.

In most experiments the absorbance at 750nm and a cell count were carried out. A standard curve of cell number l^{-1} versus absorbance at 750nm (Figure 3.3) was prepared and a regression line plotted through the data points. The data points for this curve were the summation of three sets of data collected under identical conditions. The reproducibility of this data was good and compared well with the results of Cockcroft (1988). In both studies 1.0 OD_{750} unit gave approximately 10^{12} cells l^{-1}. This standard curve provided a means of calculating the number of cells l^{-1} of a culture when direct cell counts were not carried out.

Laboratory cultures have a greater cell diameter (Iturriaga and Mitchell, 1986) and tend to have a higher cell carbon content than natural assemblages of *Synechococcus* species at similar growth rates (Glover et al., 1988a). Therefore in calculating QO_2 values the highest estimate of dry weight ml^{-1} was used; i.e. a dry weight of 630fg $cell^{-1}$. Table 3.7 shows the observed cell counts and the calculated cell counts for several oxygen uptake determinations. Also included in this table are the estimated dry weights, using both of the cell number determinations and the highest dry weight estimation of *Synechococcus* cells suggested by Waterbury et al., (1986). The estimated cell number was always higher than the observed cell number. The dry weights obtained using the ammonium formate procedure were generally within the range of these estimated values.

Table 3.8 shows the mean values of oxygen uptake [(nmols O_2 h^{-1} (2ml culture)$^{-1}$], for several *Synechococcus* WH7803 cultures after growth at different irradiances.

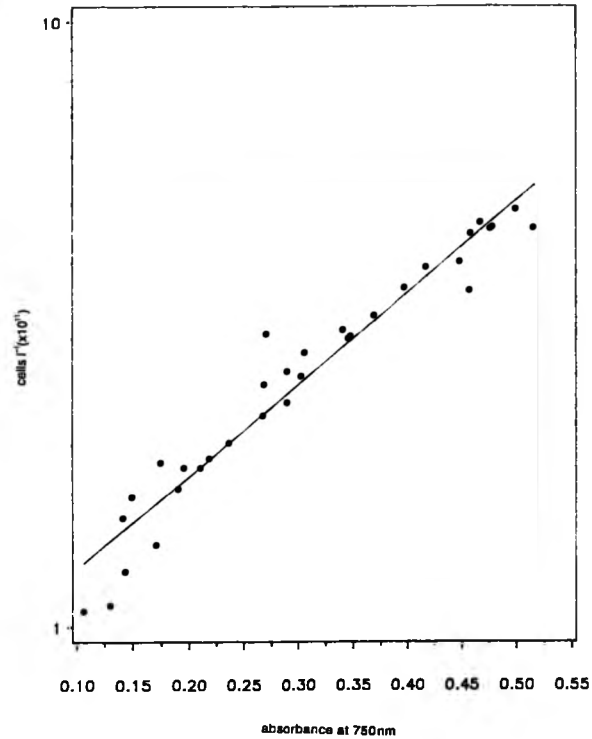


Fig. 3.3. Linear relationship between the number of *Synechococcus* WH7803 cells l^{-1} and the absorbance at 750 nm

Table 3.7. Dry weight determinations calculated from (a) the observed cell counts and (b) from optical density measurements.

(a)		(b)		
observed cell number (cells l^{-1})	dry weight (mg ml^{-1})	OD_{750}	estimated cell number (cells l^{-1})	dry weight (mg ml^{-1})
1.08×10^{10}	0.007	0.025	1.17×10^{10}	0.007
5.95×10^{10}	0.037	–	–	–
1.18×10^{10}	0.007	0.050	3.56×10^{10}	0.022
5.09×10^{10}	0.032	0.080	6.44×10^{10}	0.041
4.30×10^{10}	0.027	0.070	5.48×10^{10}	0.035
3.33×10^{10}	0.021	0.090	7.39×10^{10}	0.047
8.56×10^{10}	0.054	0.120	1.03×10^{11}	0.065
7.70×10^{10}	0.049	0.133	1.51×10^{11}	0.073
3.31×10^{10}	0.021	0.065	4.99×10^{10}	0.031
3.17×10^{10}	0.020	–	–	–
2.44×10^{10}	0.015	0.115	9.79×10^{10}	0.062
1.46×10^{10}	0.092	0.200	1.79×10^{11}	0.113
7.46×10^{10}	0.047	0.100	8.35×10^{10}	0.053
1.84×10^{11}	0.116	0.230	2.08×10^{11}	0.131

A dry weight of 630×10^{-15} g cell (Waterbury et al., 1986) was used to calculate dry weight ml^{-1}.

Table 3.8. Oxygen uptake rates and respiration rates (QO_2 values) for *Synechococcus* WH7803 maintained at different growth irradiances

growth irradiance ($\mu Em^{-2}s^{-1}$)	OD_{750}	oxygen uptake (mean)	number of determinations (n)	respiration rates (mean)	
7	0.025	15	23	24	24
7	0.075	26	13	–	
31	0.050	33	19	17	13
31	0.080	28	20	8	
31	0.070	57	21	18	
31	0.090	39	18	9	
50	0.115	92	14	17	17
54	0.120	77	22	7	17
54	0.133	37	18	6	
54	0.065	103	21	37	
54	0.046	29	21	–	
60	0.230	122	12	10	10
70	0.200	96	13	10	10
90	0.100	69	12	15	15

oxygen uptake – nmols $O_2 h^{-1}$(2ml culture)$^{-1}$
QO_2 – $\mu l\ O_2 h^{-1}$(mg dry weight)$^{-1}$; calculated using dry weights from the estimated cell counts

Mean QO_2 value over the whole range of irradiances tested = 15 $\mu l\ O_2 h^{-1}$(mg dry weight)$^{-1}$

The QO_2 value was calculated for the mean value, using the high dry weight estimation calculated from the OD_{750} measurement. The mean of the QO_2 values at each growth irradiance was also calculated. The mean QO_2 value was 15.0 μl O_2 h^{-1}(mg dry weight)$^{-1}$. The growth irradiance did not appear to effect QO_2 values.

Aphanocapsa 6714, *Anacystis nidulans* (PCC6301), *Agmenellum quadruplicatum (PR6)* and *Anabaena 7120* were grown and oxygen uptake rates [nmols O_2 h^{-1}(2ml culture)$^{-1}$] were determined. The results are shown in Table 3.9. The QO_2 values calculated for *Anacystis nidulans* and *Anabaena 7120* were higher than reported previously in the literature. This might be due to erroneous dry weight measurements, however the values compared well with those obtained for *Synechococcus* WH7803.

3.1.3 Effect of substrates on *Synechococcus* WH7803

All cyanobacteria are photoautotrophs, but some have a limited ability to utilise organic substrates as a source of carbon and energy in the dark. *Synechococcus* WH7803 was examined for heterotrophic activity using a Clark-type oxygen electrode to measure changes in the oxygen uptake rate with the addition of various substrates.

At Warwick University D-glucose, sucrose and sodium acetate (Na-acetate) were added in increasing concentrations (mM) to a 2ml sample of a growing *Synechococcus* WH7803 culture in an oxygen electrode. Table 3.10 shows the oxygen uptake rates obtained at each substrate concentration (1 to 20mM) and each rate as a percentage of the rate with no added substrate.

Table 3.9. Oxygen uptake rates and respiration rates (QO_2 values) for some other cyanobacteria.

cyanobacterium	OD_{750}	dry weight (mg ml^{-1})	O_2 uptake	QO_2
Aphanocapsa 6714	0.929	0.50	196.26	4.4
Anacystis nidulans	1.222	0.47	520.68	12.4
Agmenellum quadruplicatum (PR6)	1.325	0.38	140.18	4.1
Anabaena 7120	0.503	0.45	486.58	14.6

O_2 uptake – nmols O_2 h^{-1}(2ml culture)$^{-1}$
QO_2 – µl O_2 h^{-1}(mg dry weight)$^{-1}$

Table 3.10. The effect of different concentrations of D–glucose, sucrose and sodium acetate on the oxygen uptake rate of *Synechococcus* WH7803.

substrate	concentration (mM)	oxygen uptake rate (a)	%	oxygen uptake rate (b)	%
D–glucose	0	123	–	113	–
	1	127	103%	126	112%
	5	131	107%	139	124%
	10	117	95%	147	130%
	15	115	93%	130	115%
	20	104	85%	126	112%
sucrose	0	174	–	102	–
	1	161	93%	110	108%
	5	130	75%	105	103%
	10	123	71%	104	102%
	15	111	64%	116	114%
	20	126	72%	107	105%
sodium acetate	0	113	–	102	–
	1	123	109%	125	123%
	5	158	140%	120	118%
	10	125	111%	105	103%
	15	125	111%	97	95%
	20	119	105%	102	100%

Oxygen uptake rates (a) and (b) have units of nmols O_2 $ml^{-1}h^{-1}$

$$\% = \frac{\text{oxygen uptake rate with substrate added}}{\text{oxygen uptake rate with no added substrate}} \times 100$$

Although the oxygen uptake rates and hence the percentage values were variable between different experiments, within individual runs the values generally did not vary greatly. Typically a decrease was observed between the addition of 1mM substrate to 20mM. This may be because of the length of time the culture remained in the electrode chamber (not more than 1 hour). It was concluded that D-glucose, sucrose and Na-acetate, up to 20mM concentration had no effect on the oxygen uptake rate of *Synechococcus* WH7803 over a short time period of approximately 1 hour.

During the course of these investigations it became apparent that certain cultures were not axenic. The bacterial biomass was estimated as less than 1% of the total biomass and was not thought to effect the results, particularly as a slight decrease was observed rather than any stimulation in the respiration rate.

Oxygen uptake rates of cultures incubated with 10mM substrate were measured over several hours. The rates obtained are shown in Table 3.11 together with the percentage of a control value.

For D-glucose over seven hours, uptake rates varied from 82% to 127% of the control value; for sucrose 82% to 147%; for Na-acetate 64% to 208%. High percentage values were always followed by a lower percentage at the next sampling time and were not thought to be significant in terms of indicating heterotrophic activity: rather these high values were seen as evidence of the variability that could be found between readings using the oxygen electrode.

At Plymouth Marine Laboratory the substrates D-glucose, Na-acetate, Na-pyruvate, yeast extract and peptone were examined as potential substrates for *Synechococcus* WH7803. Both 10mM and 50mM concentrations of the substrates were used. Cultures were grown under

Table 3.11. The effect of 10mM D-glucose, sucrose and sodium acetate on the oxygen uptake rate of *Synechococcus* WH7803, after timed intervals up to a maximum of eight hours. Half of the original culture was used as a control, with no added substrate.

substrate	time (h)	oxygen uptake rate		
		control	10mM substrate	%
D-glucose (10mM)	0	215	176	82%
	1	184	192	104%
	2	125	143	114%
	3	122	155	127%
	4	160	170	106%
	5	129	160	124%
	6	185	185	100%
	7	198	235	119%
sucrose (10mM)	1	204	163	82%
	2	91	116	97%
	3	35	114	89%
	5	74	111	93%
	6	139	156	147%
	7	185	164	83%
sodium acetate (10mM)	0	106	74	70%
	2	120	121	100%
	3	74	47	64%
	4	88	67	76%
	5	72	81	113%
	6	66	88	133%
	7	80	167	208%
	8	152	194	128%

Control and substrate oxygen uptake rates are in units of nmols $O_2 ml^{-1} h^{-1}$

$$\% = \frac{\text{oxygen uptake rate with added substrate}}{\text{oxygen uptake rate of control}} \times 100$$

several different irradiances at 25°C and substrates were added to 2ml samples of these cultures in the oxygen electrode. There was no significant increase in the oxygen uptake rate under any of the conditions. Yeast extract, (0.05% w/v) was found to stimulate the oxygen uptake on two occasions, however it was thought that in these cases the yeast extract had become contaminated with bacteria.

In similar experiments using the marine heterotroph, *Alteromonas haloplanktis*, oxygen uptake increased by up to four times when nutrient broth (0.05% w/v) was added and doubled when 10mM Na-acetate was added (Table 3.12).

3.1.4 Effect of inhibitors

Oxygen uptake rates and hence respiratory rates of *Synechococcus* WH7803 in laboratory cultures were found to be low in comparison to reported values for heterotrophic bacteria: *Azotobacter* species 2020μl O_2 h^{-1}(mg dry weight)$^{-1}$, *Acetobacter* species 1815μl O_2 h^{-1}(mg dry weight)$^{-1}$, *Pseudomonas* species 1210μl O_2 h^{-1}(mg dry weight)$^{-1}$. (Schlegel, 1981 in Peschek, 1987).

The respiratory rates of natural assemblages, and specific species within that environment have rarely been attempted because of methodological problems. To measure the respiratory rate of natural assemblages of *Synechococcus* species the respiratory activity due to any other species must be removed. The exclusion of heterotrophic activity is commonly attempted by the use of antibiotics, many of whose actions depend on the prokaryotic nature of bacterial metabolism (Li and Dickie, 1985). In view of the fact that it was known that other laboratories were investigating the effect of this

Table 3.12. The effect of various substrates on the oxygen uptake rate of *Alteromonas haloplanktis*.

control rate	substrate	substrate rate	%
1680	nutrient	6250	372%
2060	broth	7500	364%
1562	(0.05%)	6505	416%
1753		7607	434%
1548		5491	355%
1012		4047	400%
767	D-glucose	959	125%
639	(10mM)	1018	159%
931		971	104%
773		798	103%
839	sodium	1801	215%
934	acetate	2210	237%
963	(10mM)	2131	221%
971		2651	273%
1327	succinate	1646	124%
1225	(10mM)	1082	88%

all oxygen uptake rates are in units of nmols $O_2 h^{-1}$ (2ml culture)$^{-1}$

$$\% = \frac{\text{oxygen uptake rate with substrate}}{\text{oxygen uptake rate of control}} \times 100$$

type of inhibitor on natural assemblages, the tricarboxylic acid cycle (TCA) inhibitors arsenite, malonate and fluoroacetate were looked at as a means of removing heterotrophic activity in a sample.

Cyanobacteria do not possess a complete tricarboxylic acid cycle (Pearce and Carr, 1967). Some of the essential tricarboxylic acid cycle enzymes such as α-ketoglutarate dehydrogenase, succinyl CoA synthetase and succinate dehydrogenase are present in extremely low concentrations or are entirely absent in cyanobacteria. This deficiency prevents the cyclic flow of tricarboxylic acid cycle intermediates and the tricarboxylic acid cycle is therefore not important for energy production and growth in the dark.

In natural assemblages the use of tricarboxylic acid cycle inhibitors should reduce the amount of heterotrophic respiratory activity with respect to cyanobacterial respiratory activity.

To test this theory the inhibitors arsenite, malonate and fluoroacetate (sodium salts) were added to cultures of *Synechococcus* WH7803 and a marine heterotroph *Alteromonas haloplanktis*, and the alteration in the oxygen uptake rate assessed.

Table 3.13 shows the effect of different concentrations of arsenite, malonate and fluoroacetate on *Synechococcus* WH7803. Slightly reduced or increased rates may have occurred because different tangents were drawn to similar traces with too steep or too shallow a slope. Also a small reduction in respiratory rate over the experimental time period probably took place. With the addition of up to 10mM arsenite there was no overall reduction in oxygen uptake rate. However at 20mM and 50mM concentrations the rate was approximately 70% of the oxygen uptake rate without added inhibitor. A similar decline in the oxygen uptake rate was observed with the

Table 3.13. The effect of different concentrations (mM) of sodium arsenite, sodium malonate and fluoroacetate on the oxygen uptake rate of *Synechococcus* WH7803.

inhibitor	concentration (mM)	oxygen uptake rate	%
sodium arsenite	0	289	–
	1	236	82%
	5	281	97%
	10	280	97%
	20	200	69%
	50	200	69%
sodium malonate	0	202	–
	1	186	92%
	5	190	94%
	10	174	86%
	20	186	92%
	50	123	61%
fluoro-acetate	0	158	–
	1	165	104%
	5	162	103%
	10	161	102%
	20	160	101%
	50	127	80%

oxygen uptake rate, nmols $O_2 h^{-1}$ (2ml culture)$^{-1}$

$$\% = \frac{\text{oxygen uptake with added inhibitor}}{\text{oxygen uptake without added inhibitor}} \times 100$$

highest concentration (50mM) of malonate and fluoroacetate. The rates were approximately 60% and 80% of the original value respectively.

The effect of 10mM arsenite, malonate and fluoroacetate on a growing culture of *Synechococcus* over a period of seven or eight hours is shown in Table 3.14. Considerable variation between the oxygen uptake rates in the two flasks, of the same culture was observed. Both showed a decline over the time period and on the whole this decrease in rate was fairly constant, however after eight hours the culture containing arsenite showed a marked decrease (50%) relative to the control at that time. This implies that malonate and fluoroacetate did not inhibit *Synechococcus* WH7803, but that 10mM arsenite after eight hours caused some inhibition.

Alteromonas haloplanktis is a marine heterotroph and was grown to carry out control tests to observe the effect of the inhibitors. Only sodium malonate and fluoroacetate were tested as arsenite had shown some definite inhibition of *Synechococcus* WH7803 cultures, and was thought to be of little use in further experiments.

Table 3.15 shows the effect of the two inhibitors on the oxygen uptake of *Alteromonas haloplanktis* with and without added substrates. Sodium malonate showed no inhibitory effect although a slight decrease of 5% to 10% was apparent with nutrient broth (0.05% w/v). With 10mM glucose added to the culture followed by 50mM malonate an increased uptake was observed. Fluoroacetate showed no inhibition when no substrate had been previously added to the culture. However when nutrient broth was present in the sample the oxygen uptake was depressed by 40% to 50%; and when 10mM succinate was present in the sample, fluoroacetate appeared to inhibit

Table 3.14. The effect of 10mM sodium arsenite, sodium malonate and fluoroacetate on the oxygen uptake rate of *Synechococcus* WH7803 at timed intervals up to a maximum of eight hours. Half of the original culture was used as a control, with no added substrate.

time (h)	oxygen uptake rate		%
	control	inhibitor (10mM)	
0.5	290	164	57
1.0	207	124	60
2.0	253	112	44
6.0	158	79	50
8.0	212	55	26
0	201	149	74
0.5	223	207	92
1.0	223	206	92
2.0	163	202	124
5.0	348	322	93
7.0	194	183	94
0	152	69	45
0.5	180	106	59
1.0	179	110	62
1.5	187	125	67
2.0	290	155	53
4.0	216	124	57
6.0	209	146	70

oxygen uptake rate, nmols $O_2 h^{-1}(2ml\ culture)^{-1}$

$$\% = \frac{\text{oxygen uptake rate with added inhibitor}}{\text{oxygen uptake without added inhibitor}} \times 100$$

Table 3.15. The effect of substrates and the inhibitors sodium malonate and fluoroacetate on the oxygen uptake rate of *Alteromonas haloplanktis*.

substrate	control	inhibitor (50mM)	inhibitor rate	%
none	639	malonate	927	145
	1437		727	51
	1054		1133	107
	1007		1117	111
	1018		1232	121
nutrient broth (0.05%)	6250		5803	93
	7500		6750	90
	6505		6199	95
	7607	fluoro-acetate	3482	46
	5491		3571	65
	4047		2678	66
none	811		865	107
	725		1113	154
D-glucose (10mM)	959		1250	130
	1018	malonate	1667	164
	971		1561	161
	798	fluoro-acetate	971	122
sodium acetate (10mM)	1801		2064	115
	2210		2216	100
	2131	malonate	2622	123
	2651		2712	102
succinate (10mM)	1846	fluoro-acetate	1032	83
	1082		785	73
none	592		781	132
	867	malonate	1023	118

all oxygen uptake rates in units of – nmols O_2 h^{-1}(2ml culture)$^{-1}$

$$\% = \frac{\text{oxygen uptake rate with added inhibitor}}{\text{oxygen uptake rate without added inhibitor}} \times 100$$

Alteromonas haloplanktis by 30% to 40%.

Complete inhibition of oxygen uptake by *Alteromonas haloplanktis* did not occur using these two inhibitors and as total inhibition of marine heterotrophs was required in order to measure the uptake rate of *Synechococcus* species in natural assemblages, the theory was rejected.

One attempt to use these inhibitors on natural assemblages was made whilst aboard RRS Charles Darwin in May/June 1986. Figure 3.4 (a) shows the results of the use of sodium malonate and fluoroacetate on the 1.0μm to 0.6μm size-fraction of a sample taken at 0800 h on 1 June 1986, from 30 metres at station CS2 in the Celtic Sea 50°30';07°00'W (Joint and Pomroy, 1983). ^{14}C uptake in the incubations containing the inhibitors was reduced. 1mM fluoroacetate reduced the ^{14}C uptake by approximately half of the control value throughout the seven hours. The incubations containing malonate were approximately 90% of the control value during the first five hours of incubation, but after seven hours this dropped to approximately 75% of the control. In a repeat of this experiment (Figure 3.4 (b)), on 3 June 1986 at the same station, the inhibitory effect of sodium malonate was found to be approximately 80% of the control measurement throughout the time course, whereas fluoroacetate inhibited the ^{14}C uptake by 40%. As these experiments were carried out on different days, the varying inhibitory effects could be accounted for by different species composition of the two samples.

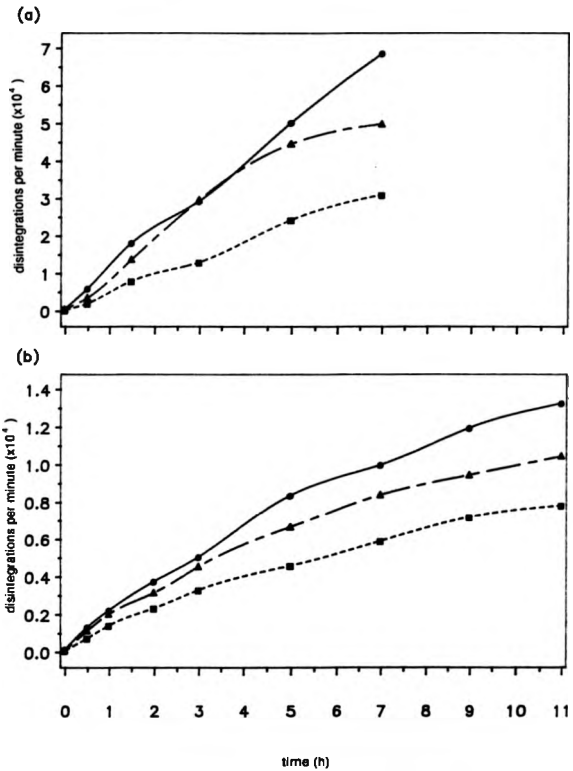


Fig. 3.4. The assimilation of carbon-14 into natural assemblages of phytoplankton <1.0μm isolated from the Celtic Sea and the effect of 1mM sodium malonate (▲ - - ▲), 1.5mM fluoroacetate (■ ····· ■) and a control containing no added inhibitors (● ——— ●). All lines are drawn by eye.

3.1.5 Cellular Fractionation of Laboratory Cultures

The incorporation of ^{14}C label into cellular constituents can be an indicator of physiological activity. Assimilation of label into protein and carbohydrate fractions is dependent upon light and nutrient supply; both result in increased incorporation of ^{14}C label into protein (Morris, 1981; Morris et al., 1974; Morris and Skea, 1978). In exponentially growing laboratory cultures of *Synechococcus* WH7803 nutrients were in excess, therefore the effect of different irradiances (hence growth rate) on the physiological status of the cells could be determined. Cultures of *Synechococcus* WH7803 were grown at three irradiances and labelled with $NaH^{14}CO_3$. Samples were removed after specific time intervals for fractionation into the cellular components: protein, polysaccharide and nucleic acids, lipids and low molecular weight metabolites.

The first set of experiments, shown in Figure 3.5 were not monitored by direct cell counts, consequently it was not known if the cultures were in the exponential or stationary phase of their growth. However by knowing the age of the cultures at the time of starting the experiment and comparing with cultures grown previously under the same conditions, it could be concluded that the cultures grown at irradiances of $12\mu Em^{-2}s^{-1}$ and $60\mu Em^{-2}s^{-1}$ were in stationary phase, whilst the culture grown at $20\mu Em^{-2}s^{-1}$ was probably still in its exponential growth phase.

At $12\mu Em^{-2}s^{-1}$ (Figure 3.5 (a)) the percentage incorporation into polysaccharide and nucleic acids did not decrease or increase significantly and the mean value was 41% over the twenty-four hours. On the other hand it would appear that incorporation into protein did

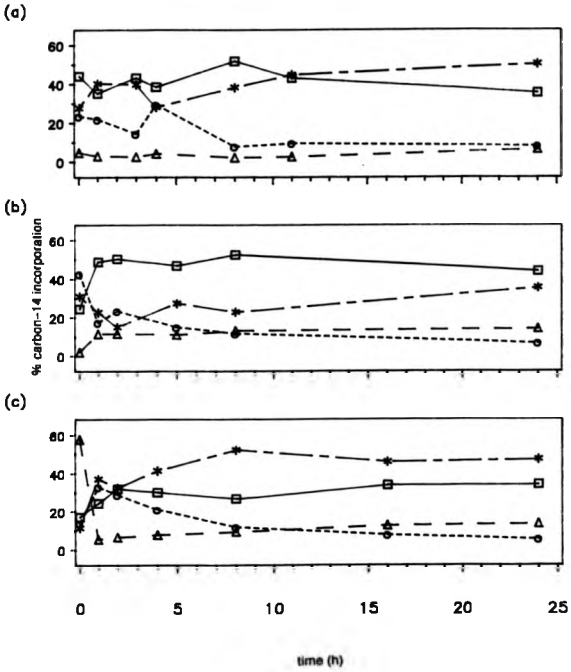


Fig. 3.5. The effect of irradiance on the relative distribution of carbon-14 between low molecular weight metabolites (o - - -o), lipid (* — *), polysaccharide and nucleic acids (□——□) and protein (Δ- - -Δ) in batch cultures of *Synechococcus* WH7803 grown at (a) 12μEm^{-2}s^{-1}, (b) 60μEm^{-2}s^{-1} and (c) 20μEm^{-2}s^{-1}.

increase from approximately 30% to 45%, however it was only after eleven hours that percentage incorporation into protein was higher than that into polysaccharides and nucleic acids. The percentage of label in the low molecular weight metabolites decreased from approximately 30% to 10% after eight hours and thereafter remained constant, whilst the percentage of label in lipids remained constant at about 5% throughout the time course.

After the first hour of incubation at $60\mu Em^{-2}s^{-1}$ (Figure 3.5 (b)), approximately 50% of the ^{14}C label as a percentage of the total label incorporated, was found in the polysaccharides and nucleic acids. Percentage incorporation into protein fluctuated over the time course, however there was an overall increase in incorporation of the label after two hours. Any increase in either of these two components was accompanied by a decrease in low molecular weight metabolites. The fluctuations in the percentage incorporated into protein seemed to affect the low molecular weight metabolites more than the polysaccharide and nucleic acid fraction. Lipid in this incubation was higher than other laboratory incubations, with a mean of 12%.

Figures 3.5 (c) show the results of an incubation at $20\mu Em^{-2}s^{-1}$. Maximum percentage incorporation was into protein with about 50% of the label being incorporated after eight hours. Although incorporation into polysaccharides and nucleic acids increased rapidly over the first two hours, for the rest of the time course this fraction was maintained at around 31%. The extremely high percentage incorporation into lipid (70%), at time zero was assumed to be an artefact bought about by low counts at that time. The other values at time zero were ignored because of the artificially high lipid percentage.

The second set of experiments were sampled during exponential growth of the culture, which was determined by the increase in cell numbers. Figures 3.6 (a), (b) and (c) show the percentage incorporation of ^{14}C into the four fractions.

At the lowest irradiance for growth, $7\mu Em^{-2}s^{-1}$ (corresponding to $1.43x10^{10}$ cells l^{-1}), and after twelve hours incubation time the percentage incorporation into protein was highest at 69% followed by the low molecular weight metabolites at 13.3%, lipid at 10.5% and polysaccharide and nucleic acid at 7.1% (Figure 3.6 (a)). Apart from the initial low value at time zero, incorporation into lipid remained relatively constant at 10%.

At $31\mu Em^{-2}s^{-1}$ (Figure 3.6 (b)) and a cell concentration of $3.44x10^{10}$ cells l^{-1} the highest percentage incorporation was into protein with a maximum of 58%, however a plateau in the percentage incorporation was reached at approximately six hours. Polysaccharides and nucleic acids decreased from 24% after half an hour, to 17% after twelve hours. The percentage label incorporated into low molecular weight metabolites also decreased over the time course from approximately 35% to 17%. Incorporation in lipid remained low at about 8%.

At $50\mu Em^{-2}s^{-1}$ (Figure 3.6 (c)) with $2.82x10^{10}$ cells l^{-1} and after twelve hours, incorporation into protein was highest, at 51.9%, followed by polysaccharides and nucleic acids at 24.1%, low molecular weight metabolites at 14.1% and lipid at 9.9% (Fig.3.6 (c)). The values at six hours for protein and the polysaccharide/nucleic acid fractions suggest a shift to lower irradiances for the period four to six hours. The relative rate of incorporation into protein increased whilst it decreased into the polysaccharide and nucleic acids.

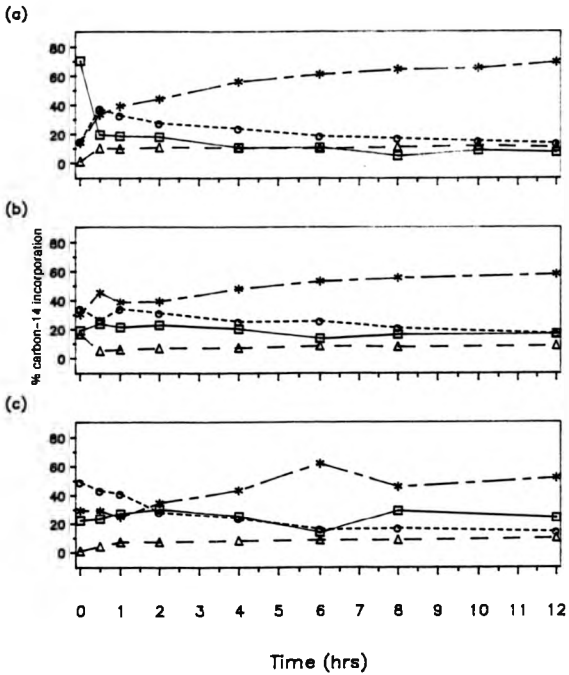


Fig. 3.6. The effect of irradiance on the relative distribution of carbon-14 between low molecular weight metabolites (o---o), lipid (*—*), polysaccharide and nucleic acids (□—□) and protein (Δ- -Δ) in exponentially growing cells of *Synechococcus* WH7803 at (a) 7μEm^{-2}s^{-1}, (b) 31μEm^{-2}s^{-1} and (c) 50μEm^{-2}s^{-1}.

Konopka and Schnur, (1980) reported a similar effect when *Merismopedia tenuissima* (cyanobacterium) was given a shift down in the incubation irradiance. The effect noticed in this incubation may have been bought about by the flask not being returned to its original position in the light field after sampling at four hours.

Finally as a comparison, one sample taken in October 1987 from coastal waters around the Eddystone lighthouse near Plymouth was incubated over a time course at 72$\mu Em^{-2}s^{-1}$ and 10°C (Figure 3.7). This sample contained 1.17×10^4 phycoerythrin-rich *Synechococcus* cells l^{-1}. Percentage incorporation into protein increased over the time course to approximately 40% after sixteen hours. Unlike laboratory cultures of *Synechococcus* species the natural assemblage showed a high percentage incorporation into lipid and this was approximately 25% throughout the time course. The percent incorporation into polysaccharides and nucleic acids also averaged at about 25% although there was a gradual decrease from 36.7% to 23.6% over the forty-eight hours.

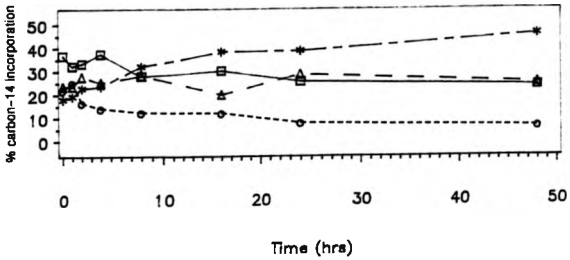


Fig. 3.7. The relative distribution of carbon-14 between low molecular weight metabolites (o---o), lipid (* – *), polysaccharide and nucleic acids (□—□) and protein (▲- - -▲) in the phytoplankton <1.0μm of a coastal sample.

3.2 DISCUSSION

3.2.1 Growth rates of *Synechococcus WH7803*.

The growth curve obtained for *Synechococcus* WH7803 in this study appeared to show a saturation type relationship, similar to that found by Morris and Glover (1981). Growth saturation occurred between 55.0 and 59.0$\mu Em^{-2}s^{-1}$. In other similar studies values of 45$\mu Em^{-2}s^{-1}$ (Morris and Glover, 1981) and 61$\mu Em^{-2}s^{-1}$ (Iriarte, 1986) have been reported.

Growth rates generally showed good agreement with those reported by Morris and Glover (1981), however they obtained a lower maximum specific growth rate (μ_{max}), 0.050h^{-1}. The growth rates reported by Iriarte (1986) were typically slightly lower than found here but the maximum specific growth rate was similar, at 0.058h^{-1}. Iriarte (1986) suggested that the differences in growth rates obtained may have been caused by differences in the calibration of light measuring devices. In this study *Synechococcus* WH7803 was grown at 25 ± 2°C whereas Morris and Glover, (1981) and Iriarte, (1986) grew *Synechococcus* WH7803 at 20 ± 2°C. The difference in growth temperature may explain the higher specific growth rate obtained compared with Iriarte's results.

Unlike these three studies Barlow and Alberte (1985) did not report a growth saturation type relationship. They reported a maximum growth rate of 0.59 divisions day^{-1} at 25$\mu Em^{-2}s^{-1}$ and a decline in growth rate at higher irradiances. The rates that Barlow and Alberte (1985) reported are lower than the corresponding rates found in this and Iriarte's (1986) study at the same growth irradiance. At present,

the reasons for such a difference in the pattern of growth are unclear.

3.2.2 Respiratory rates

Respiration is considered as the complete oxidation of an organic compound (carbohydrate) to carbon dioxide and water with molecular oxygen serving as the ultimate electron acceptor (Gibbs, 1962). Van Liere et al., (1979), reported that the maintenance energy requirements of cyanobacteria are relatively low. Dark endogenous respiration maintains the intracellular ATP pool, and is bought about by oxidative phosphorylation in cyanobacteria (Pelroy and Bassham, 1973). This type of metabolism preferentially consumes intracellular carbohydrate reserves (Matthijs and Lubberding, 1988). Webster and Frenkel (1952), using an *Anabaena* species observed a respiratory rate of 4 to 5μl h^{-1}(mg dry weight)$^{-1}$ at 25°C. Kratz and Myers (1955) reported endogenous respiration values of 8.4μl O_2 h^{-1}(mg dry weight)$^{-1}$ for *Anabaena variabilis* and 1.6μl O_2 h^{-1}(mg dry weight)$^{-1}$ for *Anacystis nidulans* (*Synechococcus* species) using growing cells at 25°C. Both studies showed that there was a reduction in the respiratory rate when the cells were starved for twenty-four hours, although Kratz and Myers (1955) noted that there was only a slight decrease during the first hour of the experiment. Pearce and Carr (1967) observed QO_2 values of 12.3 and 6.8μl O_2 h^{-1}(mg dry weight)$^{-1}$ for *Anabaena variabilis* and *Anacystis nidulans* grown at 34°C.

The results of the investigations on the respiratory rate of *Synechococcus* WH7803 suggest that this cyanobacterium also has a low respiration of approximately 15μl O_2 h^{-1}(mg dry weight)$^{-1}$. This was a

little higher in comparison with other reported values for cyanobacteria.

Aphanocapsa 6714, *Anacystis nidulans (Synechococcus* species), *Agmenellum quadruplicatum (PR6)* and *Anabaena 7120* grown in this study also produced low rates of endogenous respiration. The variability in the results compared with previous data may have been due to erroneous dry weight measurements which as noted previously were not reproducible.

QO_2 values observed in this study were higher than previously reported for *Anabaena 7120* and *Anacystis nidulans*, with values of 12.4 and 14.6μl O_2 h^{-1}(mg dry weight)$^{-1}$ respectively. This suggests that either the dry weights were underestimated or that QO_2 values measured by this method were slightly higher than by manometric procedures.

Paschinger (1977) and Fry et al., (1986) have reported a salt-induced increase in the rate of dark respiration of cyanobacteria. Jeanjean and Joset (1988) found that dark oxygen uptake increased by a factor of 1.5 to 2.0 when salt-tolerant *Synechocystis* species were adapted to higher salt concentrations.

Mackay et al., (1983) proposed that cyanobacteria could be classified as freshwater or marine on the basis of their ability to synthesise and accumulate 2-O-α-D-glucopyranosylglycerol (glucosylglycerol), a major osmoregulatory compound. In their study three *Synechococcus* species were examined and only the marine isolate was found to accumulate glucosylglycerol.

It is suggested that the slightly higher respiratory rate obtained for the marine cyanobacterium *Synechococcus* WH7803 compared with freshwater species may have been due to a requirement for

increased levels of ATP for the extrusion of Na^+ from the cells and/or a requirement for energy to synthesise glucosylglycerol.

3.2.3 Heterotrophic growth in *Synechococcus WH7803*.

All cyanobacteria so far examined use light as an energy source and carbon dioxide as a source of carbon for the synthesis of cell material. The major nutritional mode of cyanobacteria is photoautotrophy (Stanier, 1973), however a few cyanobacterial species are capable of dark aerobic growth on organic substrates: heterotrophic growth (Rippka et al., 1979). *Agmenellum quadruplicatum* PR6 grew heterotrophically in the dark with glucose as a source of carbon and energy (Van Baalen et al., 1971), but only in low irradiances at which photoautrophy did not occur. To grow with organic compounds in the dark a photosynthetic organism must be able to use them as sources of ATP and reducing power, generated through respiration (Stanier et al., 1971). Khoja and Whitton (1971) reported that the majority of strains of filamentous cyanobacteria examined were capable of growth in the dark, and Rippka et al., (1979) found that although many cyanobacterial strains grew with an exogenous carbon source this ability was rarely found among *Synechococcus* or *Synechocystis* isolates.

If substrates are assimilated and are able to be used for growth it might be expected that the respiration rate would increase. Stimulation of respiration alone does not indicate that a compound will support growth but it does indicate that a substrate was assimilated (Danforth, 1962). Utilisation of various substrates by *Synechococcus* WH7803 in the dark was determined by a comparison of

oxygen uptake rates (respiration) with those of control cultures from which the substrate was omitted.

Pelroy et al.,(1972) reported that most of the glucose assimilated by heterotrophic cyanobacteria was stored as glycogen and then metabolised by the pentose phosphate pathway. Other authors have observed that the substrates which support dark growth of cyanobacteria are invariably carbohydrates (Kiyohara et al., 1960; Fay, 1965), or compounds readily convertible to intermediate of the oxidative pentose phosphate pathway (Peschek, 1987). Light-dependent assimilation of organic substrates such as acetate and amino acids is probably carried out by all cyanobacteria (Stanier, 1973). This has been shown in unicellular (Hoare and Moore, 1965) and filamentous strains (Carr and Pearce, 1966).

D-glucose, sucrose, Na-acetate, Na-pyruvate, succinate, yeast extract (mixture of amino acids) and bacteriological peptone were examined as potential substrates of *Synechococcus* WH7803. This selection of carbohydrates, organic acids and amino acids was tested at fairly high concentrations compared with those found in the natural environment. The concentration of dissolved organic carbon in surface water is about 300μM and decreases with depth (Sugimura and Suzuki, 1988). This was determined chemically using a high-temperature catalytic oxidation method followed by analysis by an infra-red (IR) gas analyser.

The oxygen uptake rate did not increase with the addition of any of these substrates up to a concentration of 50mM or at 10mM concentration for seven hours. Yeast extract and peptone were added as potential substrates as these contain a mixture of amino acids and other compounds and it was thought of as a convenient method to test

for several compounds at one time. No stimulation of respiration of *Synechococcus* WH7803 was observed, therefore *Synechococcus* WH7803 was considered to be an obligate phototroph.

Synechococcus WH7803 does not assimilate glucose or thymidine, and while acetate and adenine are assimilated they do not make a significant contribution to the cellular carbon budget (Cuhel and Waterbury, 1984). Typically growth and respiratory rates do not increase when organic substrates are supplied to obligate phototrophs (Leach and Carr, 1970) and this is probably due to problems with assimilation into the cell, or to a lack of enzyme synthesis necessary for substrate utilisation. Kratz and Myers (1955) postulated that a permeability barrier limited the assimilation of readily available exogenous substrates to the organisms examined. Dark growth on exogenous substrates requires enzyme systems for uptake for activation (introduction into intracellular metabolic pathways), for intermediary metabolism and for generation of energy by oxidative phosphorylation.

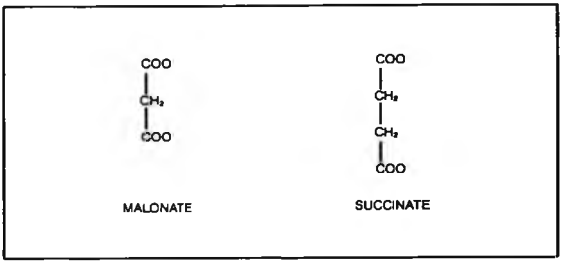
3.2.4 Effect of inhibitors on *Synechococcus WH7803*

To measure the respiratory rate of natural assemblages of *Synechococcus* species, the respiratory rate due to any other species must be eliminated. As cyanobacteria do not possess a complete tricarboxylic acid cycle (Pearce and Carr, 1967) it was expected that tricarboxylic acid cycle inhibitors would have little or no effect on cyanobacteria, but would severely inhibit marine heterotrophs. The effects of three tricarboxylic acid cycle inhibitors, arsenite, malonate and fluoroacetate were investigated

for the cyanobacterium *Synechococcus* WH7803 and the marine heterotroph *Alteromonas haloplanktis*.

Arsenite is known to be an inhibitor of oxidative decarboxylation (Gibbs, 1962). Two oxidative decarboxylation reactions occur in the tricarboxylic acid cycle, one catalysed by the pyruvate dehydrogenase complex and the other by the α-ketoglutarate dehydrogenase complex. Both contain a lipoic acid cofactor associated with one of the enzymes of the complex (Zubay, 1984). Arsenite is known to combine with lipoic acid, thereby preventing its oxidation and reduction and blocking the oxidative decarboxylation of pyruvate or α-ketoglutarate dehydrogenase.

Malonate closely resembles succinate and therefore acts as a



competitive inhibitor of succinate dehydrogenase.

Fluoroacetate and oxaloacetate form fluorocitrate from a condensation reaction catalysed by the TCA cycle enzyme, citrate synthase. Fluorocitrate is a potent inhibitor of aconitase, which catalyses the conversion of cis-aconitate to isocitrate. Fluorocitrate binds to and blocks the active site of aconitase and thereby poisons aerobic metabolism (Zubay, 1984).

Arsenite at 50mM concentrations and after eight hours at 10mM concentration, showed some inhibition of *Synechococcus* WH7803. Low levels of the enzyme complex α-ketoglutarate dehydrogenase may have been present which could explain the reduction in oxygen uptake rate. As inhibition occurred, investigations with this inhibitor were not extended to *Alteromonas haloplanktis*.

Both malonate and fluoroacetate apparently had a small inhibitory effect on oxygen uptake at 50mM, however over seven hours cultures containing 10mM of these inhibitors showed no inhibition.

All the oxygen uptake rates were subject to variation, either because of the insensitivity of the electrode - although this method was initially chosen for the sensitivity of the electrode and its low detection limit; or because of inconsistancies when drawing a tangent to the trace. As these were drawn by eye, an improvement to the system would involve a system whereby this stage was standardised. Investigations with *Alteromonas haloplanktis* showed that no inhibition occurred when the inhibitor was the sole addition. When some subtrates were added previous to the addition of fluoroacetate, inhibitions of up to 50% of the oxygen uptake rate with substrate did occur. This suggests that an uptake mechanism was required in order for the inhibitor to enter the cell. Where substrate appeared to be actively taken up by the cell and stimulated respiration, inhibition also occurred. An explanation for this might be that the inhibitor poisoned the substrate uptake system, however there is no direct evidence for this.

As complete inhibition of the marine heterotroph that was investigated did not occur it could not be assumed that all or any other heterotrophs in a natural assemblages would be inhibited.

3.2.5 Macromolecular composition of *Synechococcus* WH7803.

The exponentially growing cultures of *Synechococcus* WH7803 (Figure 3.6) showed that more carbon was assimilated into proteins than into the other components when the cells were growing under optimum conditions. It would have been good to have carried out this experiment with the same cultures at irradiances higher than the optimal irradiance for growth (55.0 to 59.0 $\mu Em^{-2}s^{-1}$). These results were in agreement with the observation that the high incorporation of ^{14}C label found in the protein fraction of *Synechococcus* assemblages did not alter significantly in response to varying irradiances (Glover et al., 1985). Incorporation of label into the protein fraction was highest at all the tested irradiances; however as the irradiance was increased an increasing proportion of the label was incorporated into polysaccharide. Previous work by Cuhel and Waterbury (1984) with an oceanic phycoerythrin-rich clone of *Synechococcus* showed that assimilated carbon was incorporated primarily into protein and low molecular weight metabolites.

Cuhel and Waterbury, (1984) reported that there was little evidence for polymeric carbohydrate in the hot acid-soluble fraction (polysaccharides and nucleic acids) of rapidly growing cultures of *Synechococcus* spp. It was assumed that the cultures shown in Figure 3.5 were not growing exponentially: hence although alkaline hydrolysis was not carried out and therefore the proportion of RNA in this fraction was not known, it is suggested that the high incorporation of ^{14}C label in the polysaccharide and nucleic acid fraction was due to carbohydrate storage products.

Incorporation into lipid was relatively constant at all the irradiances tested and most probably represents the essential lipid required for structural components of the cell rather than storage products. The carbon allocation required for the essential or structural forms of lipid, such as membrane phospholipid would be expected to display relatively conservative allocation patterns (McConville et al., 1985).

By comparison to the laboratory cultures the natural assemblage showed a different pattern of labelling. Incorporation into protein was highest as before, however unlike the laboratory cultures of *Synechococcus* spp. the natural assemblages showed a high percentage incorporation into lipid; this was approximately 25% throughout the time course. Allocation into low molecular weight metabolites was lower than in the cultures.

Apart from the high values of percentage incorporation into lipid the fractionation carried out on the coastal sample was most like the laboratory culture grown at 50$\mu Em^{-2}s^{-1}$ in saturating nutrient conditions and sampled in exponential growth. This implies that the natural assemblage had a high growth rate and therefore the natural environment that it had been sampled from (surface at 10°C), was promoting a high growth rate.

CHAPTER FOUR

MACROMOLECULAR COMPOSITION AND PHOTOSYNTHETIC PARAMETERS OF LABORATORY CULTURES OF *SYNECHOCOCCUS* WH7803

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MACROMOLECULAR COMPOSITION AND PHOTOSYNTHETIC PARAMETERS OF LABORATORY CULTURES OF *SYNECHOCOCCUS* WH7803.

4.1 RESULTS

4.1.1 Turbidostat cultures of *Synechococcus* WH7803

In order to interpret the data collected from the turbidostat cultures it was necessary to know the light distribution in the culture vessel or a parameter that changed in direct proportion to the growth irradiance. Factors such as light-scattering and selective absorption complicate the measurement of the mean irradiance, so that in dense cultures differences in the irradiance at the surface, and at the centre of the vessel are observed. The cell concentration of the culture, pigment content of the cells, shape of the vessel and the nature of the light source all affect the distribution of light in a vessel.

The irradiance measurements were found to vary in different positions in the culture vessel and the degree of self-shading occurring made the effective irradiance difficult to measure. The turbidostat cultures were maintained at an absorbance at 750nm of approximately 0.2 and this corresponds to between 1×10^{11} and 2×10^{11} cells l^{-1} (Table 4.1). At this concentration the cultures appeared pink to orange depending on the incident irradiance.

The only variable in the growth conditions during these experiments was the incident irradiance. Provided that other factors

Table 4.1. Density of *Synechococcus* WH7803 and cell numbers in turbidostat cultures maintained at different specific growth rates.

specific growth rate	OD_{750}	cells l^{-1} ($x10^{11}$)
0.038	0.235	1.439
0.132	0.223	2.040
0.018	-	1.125
0.024	0.271	2.000
0.025	0.198	1.969
0.033	0.196	2.100
0.033	0.175	2.890
0.033	0.153	1.560
0.035	0.218	0.864
0.0517	0.165	0.830
0.0522	0.163	0.930

are not limiting, the specific growth rate will depend upon the availability of light as well as efficiency of light capture (Wyman and Fay, 1986a). Therefore in this study the change in pigment concentrations, nucleic acid concentrations and photosynthetic characteristics were related to the change in specific growth rate brought about by a change in irradiance.

From chemostat theory (chapter 2) D=F/V, where (D) is the dilution rate, (F) the rate at which fresh medium is added to the culture and (V) is the volume of culture in the vessel. When the culture is in steady state the specific growth rate (μ) equals (D).

The measurement of incident light measured at the surface of the vessel nearest the light source was used merely as a guideline in setting up the turbidostat apparatus. Incident irradiance measured in this way ranged from 20 to 150$\mu Em^{-2}s^{-1}$.

Marine *Synechococcus* have been observed in seawater collected from the euphotic zone in numbers ranging from a few cells l^{-1} to nearly 10^9 cells l^{-1} (Waterbury et al., 1986). The highest value that was obtained from the North Sea in July 1987 (chapter 5) was 1.7×10^8 cells l^{-1}. These laboratory turbidostat cultures were therefore maintained at higher concentrations of cells than occur in natural assemblages; but the higher concentration was required for measuring the various parameters by the methods chosen.

An attempt was made to relate the measured parameters to cell numbers but this was not totally successful. No cell counts were carried out in the earliest turbidostat experiments and cell counts made on the later runs were found to be very variable (Table 4.1). Cell clumps were common despite disruption of the sample by forcing it through a needle and syringe three times, and these clumps

contributed to the variability of the cell count. It was not possible to express measurements on a per cell basis using these values because of the variability and incompleteness of the data, therefore all parameters were normalised to culture optical density, OD_{750}.

Table 4.2 shows the specific growth rates (h^{-1}) obtained, the OD_{750} at which these cultures were maintained and the concentrations of chlorophyll *a*, phycoerythrin (PE), protein, DNA and RNA extracted from them ($\mu g\ l^{-1}$ normalised to unit biomass). The concentrations of the pigments, nucleic acids and protein were normalised to unit biomass (1.0 OD_{unit} at 750nm). Also included in Table 4.2 are the chlorophyll *a*:phycoerythrin (chl *a*:PE) ratio and the DNA:RNA ratio.

The optical density of the growing culture and the sample used in the oxygen electrode for measuring the rates of photosynthesis were not the same. The growing cultures were maintained at an absorbance at 750nm of 0.2 (Table 4.2), whereas the samples used in the oxygen electrode were concentrations of the growing cultures giving an absorbance at 750nm of approximately 0.8. The concentration of culture for experiments using the oxygen electrode was necessary to obtain gradients of sufficient slope from which to calculate the rates of photosynthesis. All the photosynthetic parameters were normalised to 1.0 OD_{unit} at 750nm using the OD_{750} of the concentrate.

The specific growth rates ranged from 0.004 h^{-1} to 0.052 h^{-1}, and these correspond to 0.091 and 1.25 divisions day^{-1}, similar to growth rates of natural assemblages. Carpenter and Campbell (1988) measured maximal growth rates of *Synechococcus* species in natural assemblages of 1.56 divisions day^{-1}; this was a single growth rate found in early summer, however during the summer months the growth rate was more usually about 0.6 divisions day^{-1}. Although a wide

Table 4.2. Concentration of chlorophyll a (chl), phycoerythrin (PE), protein, DNA and RNA ($\mu g\ l^{-1}$) and the calculated chl:PE and DNA:RNA ratios for *Synechococcus* WH7803 maintained at different specific growth rates.

specific growth rate	culture OD_{750}	chl a ($\mu g\ l^{-1}$)	PE ($\mu g\ l^{-1}$)	chla:PE	protein ($\mu g\ l^{-1}$)	DNA ($\mu g\ l^{-1}$)	RNA ($\mu g\ l^{-1}$)	DNA:RNA
0.004	0.235	3.11	86.26	0.036	228.38	3.83	8.68	0.44
0.013	0.223	3.77	79.06	0.048	207.40	3.72	10.36	0.36
0.014	0.230	3.79	75.65	0.050	-	2.43	8.18	0.30
0.021	0.170	3.32	71.00	0.147	159.59	-	-	-
0.025	0.195	3.85	91.08	0.042	225.23	3.64	11.59	0.31
0.033	0.196	3.45	63.01	0.055	133.93	3.64	18.67	0.19
0.033	0.175	3.31	41.71	0.079	177.60	-	-	-
0.035	0.218	1.97	16.38	0.120	150.22	-	-	-
0.041	0.200	2.32	44.05	0.053	176.65	3.70	13.55	0.27
0.045	0.228	3.90	56.05	0.070	70.18	3.68	14.61	0.25
0.048	0.169	3.95	46.86	0.084	53.85	5.33	14.08	0.38
0.052	0.165	1.92	23.27	0.083	101.27	-	-	-
0.052	0.163	2.21	22.58	0.098	110.74	-	-	-
0.061	-	2.47	21.04	0.118	-	-	-	-

specific growth rate (h^{-1})
PE – phycoerythrin
chl a, PE, protein, DNA and RNA concentrations ($\mu g\ l^{-1}$) were all normalised to unit biomass (1.0 OD unit at 750nm).

range of specific growth rates from the turbidostats were examined, studying higher specific growth rates might have helped to provide an explanation for the different vertical distributions found in natural assemblages. However it was difficult to maintain the highest specific growth rates; this may have been because the cells were grown under constant illumination and some physiological consequence of the diel cycle may have been disturbed.

Although some growth rates were replicated in different experiments, the measured parameters did not always give good agreement (Tables 4.2 and 4.3). It is possible that there was incomplete extraction of cellular components or that the methods chosen were suboptimal for *Synechococcus* species. The variability in the data is shown clearly in the plots of the data.

4.1.2 Pigment content of *Synechococcus* WH7803

Optimisation of photosynthesis under the prevailing conditions is bought about by maximising light energy capture and transfer (Perry et al., 1981). The altering content of the light harvesting pigments in the photosynthetic apparatus maximise photosynthesis, hence changes are adaptive.

The physiological alterations to a change in the ambient irradiance were first investigated by assessing the changes in pigment concentration in *Synechococcus* WH7803. Figures 4.1 (a) and (b) show the concentration of the pigments, chlorophyll *a* (chl *a*) and phycoerythrin (PE) ($\mu g\ l^{-1}$ normalised to unit biomass) versus the steady state specific growth rate (h^{-1}).

Table 4.3. Photosynthetic parameters of Synechococcus WH7803 normalised to unit biomass, chlorophyll a and protein for the turbidostat cultures at each specific growth rate.

	unit biomass		chlorophyll a		protein		
specific growth rate	P_{max}	alpha	P_{max}	alpha	P_{max}	alpha	I_k
0.004	779.8	16.25	250.7	5.23	3.4	0.07	47.99
0.013	1025.3	18.99	272.8	5.04	4.9	0.09	53.99
0.018	1025.2	19.61	–	–	–	–	52.28
0.021	1080.7	21.67	325.5	6.53	6.8	0.14	49.87
0.025	1247.2	21.46	323.9	5.57	5.5	0.10	58.12
0.033	1821.6	15.51	528.0	4.46	10.3	0.09	117.97
0.033	1500.9	11.16	456.2	3.39	11.2	0.08	134.49
0.052	1840.2	10.34	958.4	5.39	18.2	0.10	177.97
0.052	2134.4	8.95	965.8	4.05	19.7	0.08	238.48
0.061	2181.6	14.96	883.2	6.06	–	–	145.83

specific growth rate (h^{-1})
P_{max} – nmols $O_2 h^{-1}$ ml^{-1}
alpha – [nmols O_2 h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$
I_k – $\mu Em^{-2}s^{-1}$

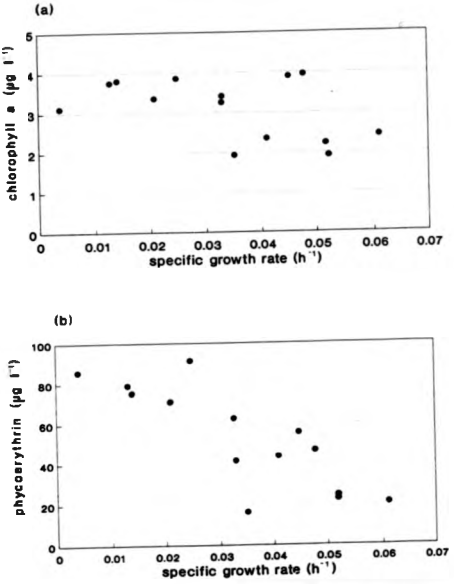

Fig. 4.1. The effect of irradiance on photosynthetic pigment concentrations of *Synechococcus* WH7803. (a) chlorophyll *a* ($\mu g\ l^{-1}$) and (b) phycoerythrin ($\mu g\ l^{-1}$).

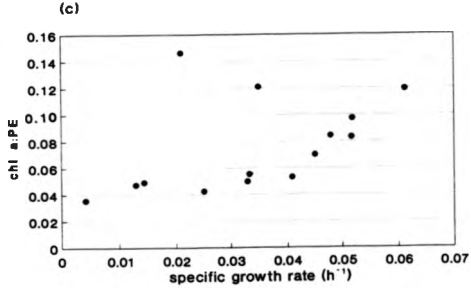

Fig. 4.1 (continued). chlorophyll *a*: phycoerythrin ratio (chl *a*:PE).

Perry et al., (1981) reported that under low irradiances the amount of light-harvesting pigments increased in proportion to reaction centre chlorophyll *a*, hence an increase in photosynthetic unit size. As the low specific growth rates were a consequence of low irradiances, one might expect a higher concentration of chlorophyll *a* at the lower growth rates. Due to the scattered nature of the data points no significant relationship could be found, however a general decline in chlorophyll *a* concentration was observed (Fig. 4.1 (a)). It is not known if this was a true reflection of what was occurring at each specific growth rate or variation caused by incomplete chlorophyll *a* extraction on some occasions.

Mean cell phycoerythrin concentration was greatest at low specific growth rate. There was an almost four-fold decrease in concentration between the highest and lowest specific growth rate, 86.26 to 22.58 $\mu g\ l^{-1}$ phycoerythrin (Fig. 4.1 (b)). Concentrations of phycoerythrin were 8 to 28 times greater than chlorophyll *a* concentrations (Table 4.2), demonstrating that phycoerythrin is the major light-harvesting pigment in *Synechococcus* WH7803. A similar conclusion was drawn by Barlow and Alberte, (1985) for *Synechococcus* WH7803 and another oceanic isolate, *Synechococcus* WH8018.

A plot of the data points of the chl *a*:PE ratio versus specific growth rate (Fig. 4.1 (c)) suggested a possible non-linear relationship. The concentrations of both pigments apparently declined, but the individual pigment concentrations declined at different rates. The pigment ratio was highest at high specific growth rate which implied that there was a decrease in chlorophyll *a* concentration with the decline in phycoerythrin concentration; however the rate of decrease in phycoerythrin concentration was

greater than that of the chlorophyll *a* concentration, hence the observed increase in the size of the light-harvesting component of the cell with decreasing growth irradiance. These observations suggest an increase in the size of the light-harvesting component of the cell with decreasing growth irradiance. At still lower growth irradiances the chl *a*:PE ratio flattens out, implying either constant pigment concentrations or pigment concentrations changing at the same rate. It was noted that the cultures grown at high irradiances appeared bleached and were an orange colour, whereas the cultures grown at low irradiances were pink. This most probably reflects the decrease in the pigments at high irradiance.

4.1.3 Nucleic acids and protein content in *Synechococcus* WH7803

The main interest in nucleic acids and protein in ecological studies is as an index of the physiological status of the cells (Thoresen et al., 1983) and in the possible use of their concentrations in estimating biomass. Figure 4.2 shows plots of the data points of (a) DNA concentration ($\mu g\ l^{-1}$), (b) RNA concentration ($\mu g\ l^{-1}$), (c) the DNA:RNA ratio and (d) protein concentration ($\mu g\ l^{-1}$) versus specific growth rate. No significant relationship was found between the data points of any of the measured parameters although general trends could be established, as indicated.

DNA concentration (Figure 4.2 (a)) remained at approximately 3.7 $\mu g\ l^{-1}$ at most irradiances. There were variations in this value, however there were sufficient points to suggest that over the range of growth irradiances tested no change in DNA concentration occurred. RNA concentration (Figure 4.2 (b)) showed a slight increase with

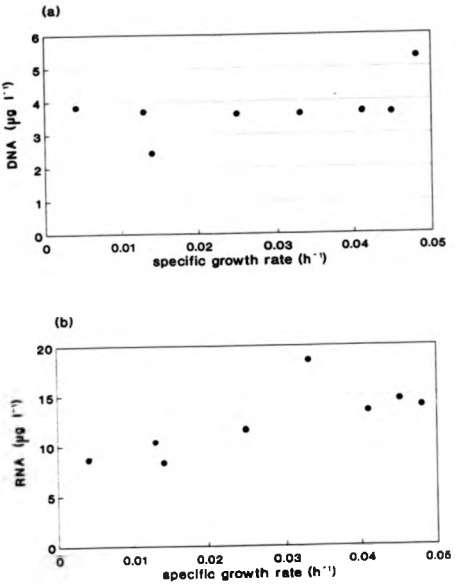

Fig. 4.2. The effect of irradiance on the nucleic acid and protein concentrations of *Synechococcus* WH7803. (a) DNA ($\mu g\ l^{-1}$) (b) RNA ($\mu g\ l^{-1}$).

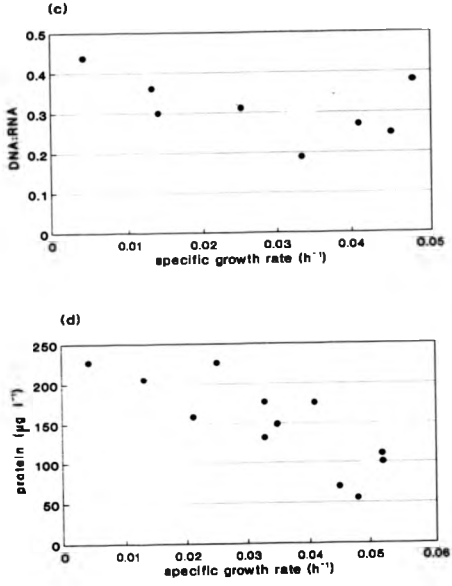

Fig. 4.2 (continued). (c) DNA:RNA and (d) protein ($\mu g\ l^{-1}$).

increasing growth irradiance with values of 8.68 $\mu g\ l^{-1}$ at the lowest specific growth rate and 14.61 $\mu g\ l^{-1}$ at the highest specific growth rate (Table 4.2). RNA concentration was approximately 2.5 times higher than DNA concentration.

These suggested patterns of nucleic acid concentration in *Synechococcus* WH7803 appeared to be confirmed by the trend in the DNA:RNA ratio (Figure 4.2 (c)), which showed a gradual decline with increasing specific growth rate. There was also an apparent decline in protein concentration as specific growth rate increased from approximately 200 $\mu g\ l^{-1}$ to 100 $\mu g\ l^{-1}$. Under optimal conditions, phycobiliproteins may account for up to 50 percent of the total protein in the cyanobacterial cell (Cohen-Bazire and Bryant, 1982). Therefore the reduction in protein may reflect the reduction in pigment which is associated with protein in the phycobilisomes.

4.1.4 Determination of photosynthetic parameters at different specific growth rates.

It has been suggested that characteristic photosynthetic curves are produced by different responses of the photosynthetic unit to changing irradiances (Prezelin and Sweeney, 1979; Falkowski and Owens, 1980; Parry et al., 1981; Ley and Mauzerall, 1982). The relationship of photosynthesis to irradiance was examined for each *Synechococcus* culture to see how the observed pigment changes affected the photosynthetic characteristics. Table 4.3 shows the values of the photosynthetic parameters obtained.

Figure 4.3 (a) and (b) show the photosynthetic-irradiance curves of cultures grown at high (0.052 h^{-1}) and low (0.013 h^{-1}) specific growth rates. In Fig. 4.3 (a) the photosynthetic rates are expressed per unit biomass (1.0 OD_{unit} at 750nm) and in Fig. 4.3 (b) these data have been normalised to the chlorophyll *a* concentration. The photosynthetic capacity normalised to unit biomass was greater in cells grown at high growth rates than at low growth rates. In this example the lower photosynthetic capacity (P_{max}) was approximately half the higher P_{max}. Also the initial slope (α) of cells grown at low specific growth rate (Fig. 4.3 (a)) was steeper than that for the high growth rate cells. On a chlorophyll basis the initial slopes (α^B) were similar, with values of 4.05 and 5.04 [nmols O_2 h^{-1} (μg chl *a*)$^{-1}$][μEm^{-2}s^{-1}]$^{-1}$ for the higher and lower specific growth rate respectively (Table 4.3). The maximum photosynthetic rate normalised to chlorophyll *a* concentration (known as the assimilation number, P^B_m) was again lower in cells with a low specific growth rate. The maximum rate of photosynthesis was higher in high specific growth rate cells when normalised to unit biomass and chlorophyll *a* concentration, whereas the alpha values differed depending on which parameter they were normalised.

The initial slope (α) of the photosynthesis-irradiance curve gives an indication of the efficiency of light absorption and the quantum efficiency with which absorbed light is converted to photosynthate.

Figure 4.4 (a), (b) and (c) summarise the relationship between alpha and specific growth rate normalised to (a) unit biomass (1.0 OD_{unit} at 750nm), (b) chlorophyll *a* concentration (μg l^{-1}) and (c) protein concentration (μg l^{-1}).

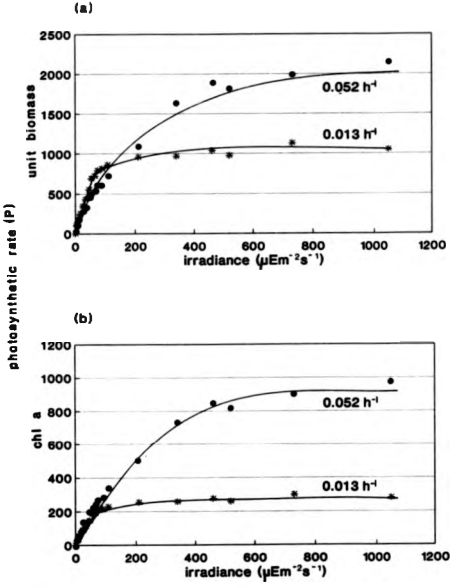

Fig. 4.3 Photosynthesis-irradiance curves of *Synechococcus* WH7803 at specific growth rates of 0.052 h^{-1} and 0.013 h^{-1}, (a) per unit biomass and (b) per chlorophyll a.

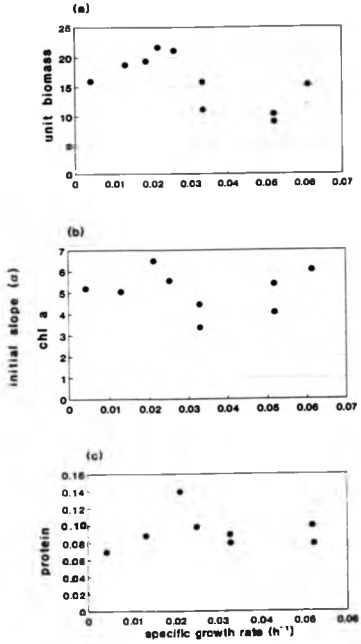

Fig. 4.4. The effect of growth irradiance on the initial slope (α) of the photosynthesis-irradiance curve for *Synechococcus* WH7803, normalized to:
(a) unit biomass, [nmols O_2 (unit biomass)$^{-1}$ h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$,
(b) chlorophyll *a*, [nmols O_2 (μg chl *a*)$^{-1}$ h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$,
and (c) protein, [nmols O_2 (μg protein)$^{-1}$ h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$.

Protein concentration was used for normalisation, as it has been suggested that protein concentration is a good estimate of cell number (Foy and Gibson, 1982b).

No significant correlation was observed between the data points in any of the graphs. In general when normalised to unit biomass, α declined with increasing specific growth rate. When normalised to chlorophyll *a* concentration or protein concentration, alpha remained at the same value at all specific growth rates examined; at approximately 5 [nmols O_2 (μg chl *a*)$^{-1}h^{-1}$][$\mu Em^{-2}s^{-1}$]$^{-1}$ or 0.09 [nmols O_2 (μg protein)$^{-1}h^{-1}$][μEm^{-2} s^{-1}]$^{-1}$.

The dark reactions of photosynthesis described by the maximum rate of photosynthesis (P_{max}), complete carbon dioxide assimilation by utilising the compounds synthesised in the light reactions. Figure 4.5 (a), (b) and (c) summarise the relationship between P_{max} and specific growth rate normalised to (a) unit biomass (1.0 OD_{unit} at 750nm), (b) chlorophyll *a* concentration (μg l^{-1}) (c) protein concentration (μg l^{-1}). All three plots show increases in P_{max} with increasing specific growth rate, but no significant relationship was found between the points. When normalised to unit biomass the increase appeared to be linear, but on a chlorophyll *a* or protein basis, a curved relationship appeared to fit the data points best. Little change in photosynthetic capacity was observed at low specific growth rates up to approximately 0.025 h^{-1}, but above this growth rate and up to the maximum specific growth rate examined the P_{max} values gradually increased. I_k ($\mu Em^{-2}s^{-1}$) which is the parameter derived from the ratio of P_{max}/α, (Talling, 1957) indicates the irradiance at which photosynthesis first became saturated.

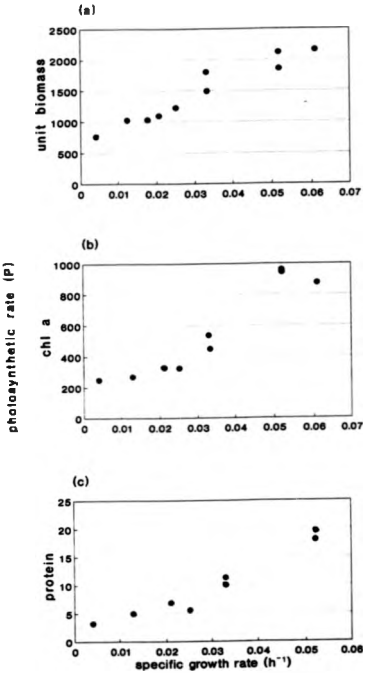

Fig. 4.5. The effect of growth irradiance on the maximum photosynthetic capacity of *Synechococcus* WH7803, normalized to (a) unit biomass, [nmole O_2 (unit biomass)$^{-1}$h^{-1}], (b) chlorophyll *a* concentration, [nmole O_2 (μg chl *a*)$^{-1}$h^{-1}], and (c) protein concentration, [nmole O_2 (μg protein)$^{-1}$h^{-1}].

I_k shown plotted versus specific growth rate (Figure 4.6) was greatest at the highest specific growth rates.

The plotted values show a similar but more pronounced pattern to the P_{max} values when normalised to chlorophyll *a* and protein concentration. There was little variance in the I_k values up to approximately 0.025 h^{-1} ($28 \mu Em^{-2}s^{-1}$), however above this specific growth rate the I_k value increased rapidly. The specific growth rate of 0.025 h^{-1} is the nearest value which can be given using this data set and is not an accurate value for the growth rate at which the increase in I_k first occurs.

4.1.5 Determination of photosynthetic parameters for cells illuminated with blue light.

Growth rate in chromatic light is a general function of the light absorption capacity of the cell (Wyman and Fay, 1986b). Blue and green wavelengths of light penetrate to the deepest part of the euphotic zone and since *Synechococcus* WH7803 contains phycoerythrin which absorbs specifically in the blue and green regions of the spectrum (Wood, 1985) these organisms may have different characteristics when grown at, or illuminated with different qualities of light. The photosynthetic characteristics of *Synechococcus* cultures illuminated in blue light, but grown in white light were investigated. This corresponds most closely to a movement of cells from near surface waters to deep waters within the euphotic zone, however the corresponding reduction in quantity of light which would occur, is not considered. Samples taken from the turbidostat cultures grown under conditions of continuous white light were used

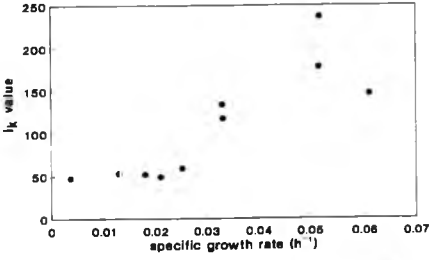

Fig. 4.6. Changes in the light saturation characteristic I_k of *Synechococcus* WH7803 with growth irradiance.

in the oxygen electrode. A blue light gradient was created by taping a blue filter to the front of the projector and then using the neutral density filters to reduce the quantity of light; the number of irradiances used was less than for white light. Table 4.4 shows the values of the photosynthetic parameters obtained when samples were illuminated with blue light.

The photosynthetic parameters obtained from the photosynthesis-irradiance curves generated using incident blue light are shown in Figures 4.7 (alpha values) and 4.8 (P_{max}). Both figures show the two parameters normalised to (a) unit biomass (1.0 OD_{unit} at 750nm) and (b) chlorophyll *a* concentration ($\mu g\ l^{-1}$). Values of α per unit biomass appeared to decline with increasing irradiance and were slightly higher than the corresponding culture illuminated in white light, particularly at low irradiances. On a chlorophyll *a* basis no alteration in α value of approximately 6.0 [nmol O_2 (μg chl *a*)$^{-1}h^{-1}$][$\mu Em^{-2}s^{-1}$]$^{-1}$ was observed.

The overall patterns of P_{max} versus specific growth rate were similar to those obtained for white light; showing an unchanging value up to a specific growth rate of 0.025 h^{-1} followed by a rapid increase with increasing specific growth rate. Values were higher at low specific growth rate in blue light than in white light.

I_k values being the derivative of P_{max}/alpha were slightly lower from blue-light illuminated cells above specific growth rates of 0.025 h^{-1} (Fig. 4.9), and the same as in white light with a value of approximately 50$\mu Em^{-2}s^{-1}$ below 0.025 h^{-1}. The increase in values, with increasing specific growth rate followed the same shape curve.

Table 4.4. Photosynthetic parameters of *Synechococcus* WH7803 for turbidostat cultures illuminated in blue light but grown in white light: normalised to unit biomass and chlorophyll a.

	unit biomass		chlorophyll a		
specific growth rate	P_{max}	alpha	P_{max}	alpha	I_k
0.004	1041.61	33.80	334.92	10.87	30.82
0.013	1393.26	27.66	369.56	7.34	50.37
0.018	1262.11	24.88	–	–	50.73
0.025	1216.90	25.90	316.08	6.73	46.98
0.033	2043.74	22.76	592.39	6.60	89.90
0.033	1389.39	12.17	422.31	3.70	114.17
0.052	1830.66	11.33	953.47	5.90	161.58
0.052	1981.69	10.35	896.69	4.68	191.47

specific growth rate (h^{-1})
P_{max} – nmols O_2 h^{-1} ml^{-1}
alpha – [nmols O_2 h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$
I_k – $\mu Em^{-2}s^{-1}$

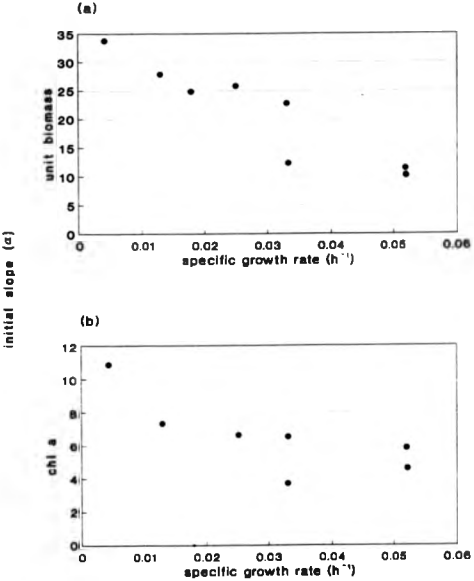

Fig. 4.7. Values of the initial slope (α) determined from blue light P-I curves of *Synechococcus* WH7803, (a) per unit biomass, [nmols O_2 (unit biomass)$^{-1}$ h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$, and (b) per chlorophyll *a* concentration, [nmols O_2 (μg chl *a*)$^{-1}$ h^{-1}][$\mu Em^{-2}s^{-1}$]$^{-1}$.

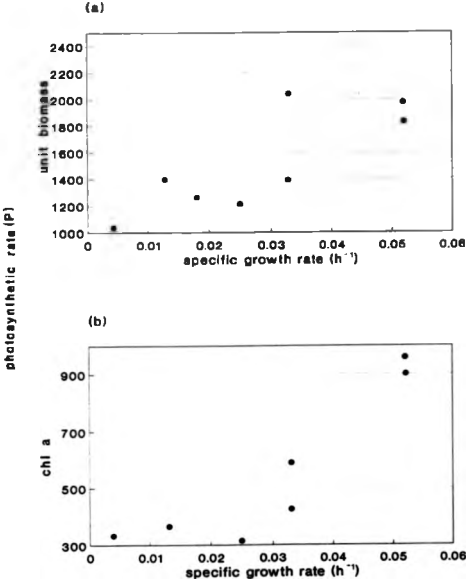

Fig. 4.8. Values of P_{max} determined from blue light P-I curves of *Synechococcus* WH7803, (a) per unit biomass, [nmols O_2 (unit biomass)$^{-1}h^{-1}$] and (b) per chlorophyll a concentration [nmols O_2 (μg chl a)$^{-1}h^{-1}$].

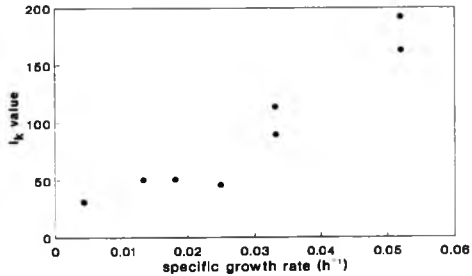

Fig. 4.9. Changes in the light saturation characteristic I_k ($\mu Em^{-2}s^{-1}$) from blue light P-I curves of *Synechococcus* WH7803.

4.1.6 Ratio of respiration to photosynthesis at each specific growth rate.

Cyanobacteria have often been shown to have relatively low respiration rates, and these have been estimated as being approximately 10% of the light saturated photosynthetic rate (McAllister et al., 1964; Humphrey, 1975). Higher ratios of respiration to P_{max} have been found in other phytoplankton (Parsons, et al., 1984) hence the ratio is thought to be a species dependant characteristic. It was difficult to find any pattern with increasing specific growth rate (Table 4.5), however it would appear that the percentage rate is higher than the 10% value predicted, at 16.7% ± 2.8(2SE), supporting the idea that this parameter is a species dependent characteristic.

Table 4.5. Ratios of respiration to P_{max} in the turbidostat cultures.

specific growth rate	respiration:P_{max} %
0.004	27.3
0.013	17.1
0.018	14.3
0.021	12.6
0.025	13.6
0.033	14.9
0.033	18.4
0.033	17.2
0.035	29.2
0.041	14.2
0.045	15.9
0.048	11.1
0.052	15.2
0.052	12.4
mean	16.7

4.2 DISCUSSION

4.2.1 Pigment organisation

Natural phytoplankton assemblages grow in environments where they are subjected to fluctuations in irradiance over a wide range of scales: diel, seasonal, hydrographic and meteorological.

Previous studies have demonstrated that all the major phytoplankton groups alter their pigment complement in response to environmental factors, particularly growth light intensity (Gallagher et al., 1984). Physiological responses of the phytoplankton assemblages to changes in the ambient light maximise the photosynthetic and nutrient uptake rates, and hence growth rates (Perry et al., 1981). Techniques designed to characterise the photosynthetic apparatus can help to explain the mechanisms behind the light induced physiological changes. In this study an attempt was made to relate the changes in pigment concentration of *Synechococcus* WH7803, to the changes in photosynthetic characteristics that were observed in response to changing incident irradiance.

The growth, pigmentation, cell composition and photosynthetic responses of *Synechococcus* WH7803 were investigated by Kana and Glibert (1987a and b) over a range of irradiances from 30 to 1300$\mu Em^{-2}s^{-1}$. They obtained growth rates of 0.018 h^{-1} to 0.077 h^{-1}. A higher irradiance was included but the temperature of the culture rose hence the value was not included. In this study the irradiances tested ranged from approximately 20 to 150$\mu Em^{-2}s^{-1}$ and specific growth rates ranged from 0.004 h^{-1} to 0.052 h^{-1}. As the growth rates

in both studies were similar, comparisons between the two were considered justified.

Siegelman (personal communication in Waterbury et al., 1979) reported that *Synechococcus* WH7803 contained chlorophyll *a* as its major photosynthetic pigment and phycobiliproteins as accessory pigments. A predominance of the phycobiliprotein, phycoerythrin, gave the *Synechococcus* WH7803 cells a red appearance when grown at low irradiances. The cultures became bleached and more orange coloured as irradiance increased, presumably as a result of pigment loss.

In algae the minimal and simplest functional composite structure comprised of two photosystems and their light-harvesting components has been termed a photosynthetic unit or PSU (Prézelin, 1981). Photosystem I is most commonly estimated by P_{700} content, where P_{700} is the reaction centre chlorophyll of photosystem I, and photosystem II is estimated by oxygen flash yields. Changes in the photosynthetic unit size are often expressed as the ratio of light-harvesting pigments to P_{700}. As neither of the aforementioned analytical procedures was available, the concentrations of the pigments, chlorophyll *a* and phycoerythrin were measured. Information could be inferred from changes in the relative proportions of the pigments.

Variation of the cellular concentration and functional organisation of photosynthetic pigments during acclimation to variation in irradiance has been reported for cyanobacteria (Fogg, 1973; Kawamura et al., 1979; Vierling and Alberte, 1980; Wyman and Fay, 1986a) as well as in other phytoplankton groups (Prézelin, 1981; Perry et al., 1981; Foy and Gibson, 1982a & b; Richardson et al., 1983). In general changes in the functional organisation of the

photosynthetic unit include alterations in the photosynthetic unit size, ratio of photosystems I and II reaction centres or numbers of photosynthetic units per cell (Gallagher et al., 1984). Previous studies of cyanobacteria (Kawamura et al., 1979; Raps et al., 1983) have shown that while the number of photosystem I reaction centres per cell decreases with increasing irradiance, there is little change in their overall size. The number of light-harvesting chlorophyll *a* molecules associated with photosystem II is relatively small and appears to be constant (Wang et al., 1977). Although it was not possible to predict the magnitude of the response of *Synechococcus* WH7803, a change in photosynthetic unit size and number was inferred from disproportionate changes in cellular pigment concentrations.

Phycoerythrin concentrations declined four-fold over the specific growth rates 0.004 h^{-1} to 0.052 h^{-1}. A significant relationship was not found for the change in chlorophyll *a* concentration, however an apparent decrease was observed. At most this was only a two-fold difference between the highest and lowest concentrations (Table 4.2). It was assumed that the primary light-harvesting pigment in *Synechococcus* WH7803 was phycoerythrin, as concentrations of phycoerythrin were almost ten-fold higher than chlorophyll *a* concentrations at the highest specific growth rate and over twenty-fold higher at the lowest specific growth rate. These differences in concentrations of the individual pigments were similar to those observed for *Synechococcus* WH7803 by Barlow and Alberte (1985). Changes in photosynthetic unit size were indicated through changes in the amount of phycoerythrin associated with each photosynthetic unit, (ie: changes in the whole cell pigment ratio). Figure 4.1 (c) shows a plot of the chlorophyll *a*:phycoerythrin ratio

against specific growth rate. The line of best-fit through the data points was a curve with the slope increasing towards higher specific growth rates. A straight line through the data points would indicate a uniform decline in the size of the photosynthetic units with increasing specific growth rate. The non-linear relationship suggests that a change in photosynthetic unit size does not entirely explain the observed variation in the pigment concentrations.

If the number of photosynthetic units were to increase, concentrations of both pigments would increase but at the same rate. The pigment ratio, chlorophyll *a*:phycoerythrin would remain unaltered as the composition of the photosynthetic unit remained the same. The lack of change in value of the chlorophyll *a*: phycoerythrin ratio at low irradiances and the concentrations of the pigments which appeared to be increasing, implies that the number of photosynthetic units in *Synechococcus* WH7803 increased at low irradiances.

The data here suggests that the predominant change is in the size of the photosynthetic unit which increases with decreasing incident irradiance. However in conjunction with this size increase, the number of photosynthetic units increase as the specific growth rate declines. This was inferred from the slope of the curve which appeared to flatten out at low specific growth rates.

Cell's in Kana and Glibert's study were harvested between concentrations of 6×10^9 and 15×10^9 cells l^{-1}, whereas in this study they were harvested between 1×10^{11} and 2×10^{11} cells l^{-1}. Because the cultures were maintained and harvested at considerably lower densities in Kana's study a direct comparison of pigment concentration was not possible, however differences between the highest and lowest concentrations were compared.

When the complete range of light intensities was examined phycoerythrin concentrations showed a >20-fold difference and exhibited the greatest plasticity of the pigments measured (Kana and Glibert, 1987a). In this study phycoerythrin was the only pigment concentration measured to show a significant relationship versus specific growth rate. Over the range 0.004 to 0.052 h^{-1} (Table 4.4), a 4-fold decline in phycoerythrin concentration was observed. If comparable growth rates from the study of Kana and Glibert (1987a) were considered (0.018 to 1.058 h^{-1}) a 2.5-fold decline in phycoerythrin concentration was apparent. Most of the decline in phycoerythrin in Kana and Glibert's study was observed at higher growth rates than were studied in this investigation. Above 700$\mu Em^{-2}s^{-1}$ little change in growth rate or pigment concentration occurred. It would have been an advantage to have examined cultures over a wider range of growth rates similar to those obtained by Kana and Glibert (1987a), however even without this additional data the same conclusion concerning the organisation of the pigments in *Synechococcus* WH7803 was inferred. The size and number of photosynthetic units changed in response to changes in growth irradiance. Changes in photosynthetic unit size occurred at irradiances above about 30$\mu Em^{-2}s^{-1}$, whilst changes in numbers occurred at lower irradiances.

The decline in pigments at high irradiance is often interpreted as a mechanism which may reduce the risk of photoinhibition (Harris, 1978). The dramatic photoinhibition of photosynthesis that was observed by Barlow and Alberte (1985) for *Synechococcus* WH7803 was not evident in this study. Some slight inhibition was observed at the highest irradiances used in the light gradient (700 to 1050$\mu Em^{-2}s^{-1}$)

but this was only noticed in cultures grown at the lowest specific growth rates. The inhibition may have been an artefact caused by the difficulty in measuring differences in the recorded traces at the high irradiances. Kana and Glibert (1987a) allowed cultures to acclimatise to higher irradiances using a gradual step-up procedure and did not observe dramatic inhibition at the higher irradiances, however they observed inhibition in low light adapted cells when these were subjected to large and sudden increases in irradiance. The mechanism whereby the concentration of pigments declines at high irradiance (Harris, 1978) is probably functional in *Synechococcus* WH7803. Herbert and Waaland, (1988) reported that sun-adapted algae typically exhibit lower adsorption of light and higher capacity per unit surface area than do shade-adapted algae therefore are less susceptible to photoinhibition.

4.2.2 Response of the photosynthetic parameters to changes in the photosynthetic unit

Increases in the number of light-harvesting pigments per reaction centre enhances the probability of photon capture, however the photosynthetic efficiency per chlorophyll *a* is known to vary within and among species (Perry et al., 1981; Gallagher et al., 1984). The pattern of photosynthetic efficiency per chlorophyll *a* is related to the functional organisation of the photosynthetic unit and it might be expected that an increase in photosynthetic unit size or number would enhance the ability to harvest light energy (ie: alpha, α^B).

It was difficult to determine a clear trend from the plot of α versus specific growth rate. Normalised to unit biomass α appeared to

decline slightly with increasing irradiance (Fig. 4.4(a)), but when normalised to chlorophyll *a* concentration, or protein concentration (Fig. 4.4 (b) and (c)) the data suggested no change or a marginal decrease in alpha value with increasing irradiance.

P_{max} values normalised to unit biomass, chlorophyll *a* and protein concentrations (Fig. 4.5 (a), (b) and (c) respectively) were relatively constant up to a specific growth rate of 0.025 h^{-1}. Above this value P_{max} increased rapidly.

Increases in the concentrations of photosynthetic pigments imply an increase in the light-harvesting potential and hence in photosynthetic efficiency at low irradiance (Wyman and Fay, 1986a). It was inferred from the photosynthetic pigment concentrations obtained during this study that an increase in both photosynthetic unit size and number occurred with decreasing growth irradiance but the initial slope (α) showed essentially no alteration with decreasing irradiance.

Light energy absorbed by phycoerythrin is transferred to reaction centre chlorophyll with up to 90 to 95 percent efficiency in all phycoerythrin containing cyanobacteria so far examined (Gantt, 1981; Cohen-Bazire and Bryant, 1982). However more recently it has been shown that phycoerythrin is not always efficiently coupled to the photosynthetic apparatus in *Synechococcus* WH7803 (Barlow and Alberte,1985; Wyman et al., 1985). A greater proportion of absorbed light energy was lost in the form of phycoerythrin autofluorescence when nutrient-replete cultures of *Synechococcus* WH7803 experienced increasing irradiances, (Wyman et al., 1985). In this study no measurements of phycoerythrin autofluorescence were made, but the unchanging α value may be a result of less autofluorescence than

usual for this species, or an artefact due to the scattered nature of the data.

A decline in α per chlorophyll *a* with increasing irradiance in *Synechococcus* WH7803 has been reported (Barlow and Alberte, 1985: Wyman et al., 1985; Kana and Glibert, 1987b) and this was accompanied by an increase in the assimilation number P_m^B (Wyman et al., 1985; Kana and Glibert, 1987b). Barlow and Alberte (1985) observed an increased P_m^B at low irradiances, which they correlated with an increase in the number of photosynthetic units present per cell and with a reduction in the loss of absorbed light energy as phycoerythrin fluorescence. However unlike other reports for *Synechococcus* WH7803 they also found dramatic photoinhibition of photosynthetic capacity as irradiance increased and these photoinhibited values at high irradiance probably account for the discrepancy in P_m^B values.

Below specific growth rates of 0.025 h^{-1} the efficiency of light energy capture (α) appeared to be sufficient just to maintain a constant P_{max}. At low irradiances enzymatic activity is reduced by comparison with an increased absorption of pigments (Beardall and Morris, 1976), so although no enzyme measurements were made they might be expected to increase in parallel with P_{max}. The maximum photosynthetic capacity (P_{max}) increased rapidly above specific growth rates of 0.025 h^{-1}. The lower limit of P_{max} may therefore be a result of reduced activity of enzymes.

Prezelin (1976) reported similar depressed P_m^B in low light cells in *Glenodinium* species and α values of low light utilisation remaining unaltered. As the low light cells had a greater pigment

content it was inferred that this species was more efficient at low irradiances.

Values of I_k ranged from 48 to 239$\mu Em^{-2}s^{-1}$ but remained at about 50$\mu Em^{-2}s^{-1}$ until a specific growth rate of approximately 0.025 h^{-1} was achieved. The increasing I_k above 0.025 h^{-1} was a result of the rapidly increasing photosynthetic capacity above this value and suggests that although *Synechococcus* WH7803 may be efficient at low irradiances due to its increased pigment content, it is also capable of growth at high irradiances. Beardall and Morris (1976) reported that reduced I_k values often indicates a loss of ability to utilise high irradiance. Therefore the value of approximately 50$\mu Em^{-2}s^{-1}$ below specific growth rates of 0.025 h^{-1}, may imply that *Synechococcus* WH7803 cannot utilise high irradiances efficiently when grown at low irradiances, but as specific growth rate or growth irradiance increases so does the ability to utilise the higher irradiances. Under these circumstances it was inferred that *Synechococcus* WH7803 was not a shade-adapted species.

Prézelin (1981) and Richardson et al., (1983), in their reviews suggested several strategies by which the composition of pigments, activity of enzymes and efficiency of energy transfer might effect the photosynthetic characteristics. *Synechococcus* WH7803 however appeared to conform to more than one of the models, being a combination of increasing size and number of photosynthetic units with increasing irradiance.

The addition of chromophore molecules to the light-harvesting component of a cell of picoplankton size, will always be beneficial in terms of adsorption of additional photons (Raven, 1984), however any additional light-harvesting machinery in a cell of a given volume

and dry weight must displace some other component of the cell. In cyanobacteria the phycobilisomes, the major photosynthetic accessory pigment complexes, comprise 40 to 50 percent of the soluble cell protein under optimal growth conditions (Cohen-Bazire and Bryant, 1982). Therefore reduction in pigments with increasing growth rates amounts to a considerable saving in energy which can be directed towards the synthesis of other cell constituents (Wyman and Fay, 1987).

No allowance was made for a light/dark cycle which would be encountered in a natural environment. Foy and Gibson (1982b) found that the photosynthetic characteristics and biochemical composition of the cells were not the same in cultures of *Oscillatoria redeki* grown either under continuous illumination or a 6:18 light:dark cycle. It was thought that the difficulty in obtaining high growth rates may be attributable to the cultures being grown in continuous light. Under these conditions carbohydrate and not protein is preferentially produced therefore possibly limiting increases in growth rate.

4.2.3 Response of the photosynthetic parameters to illumination in blue light

Recent evidence has shown that spectral quality may be an important factor in determining the relative abundance of different algal classes at different depths in the water column (Wood, 1985: Glover et al., 1986b). Yet the photosynthesis-irradiance response of a given organism should reflect the effective growth irradiance (Kana and Glibert, 1987b). Waterbury (personal communication in Wyman and Fay 1987), reported that none of the *Synechococcus* strains isolated

from open ocean waters has been shown to be capable of chromatic adaptation. In this study α values were slightly higher when the cells were illuminated with blue light compared with white light, whereas P_{max} values were approximately the same except at the lowest specific growth rates where values became slightly higher. Values of I_k were generally slightly lower when illuminated in blue light, however at specific growth rates less than or equal to 0.025 h^{-1}, I_k was maintained at about 50$\mu Em^{-2}s^{-1}$. This was the same value as when illuminated by white light. Although the efficiency of adsorption and transfer of quanta was increased particularly at the low irradiances, only below specific growth rates of 0.025 h^{-1} was there there a corresponding rise in the value of P_{max}. This implies losses by other mechanisms, possibly during the dark reactions of photosynthesis: autofluorescence or enzyme activity.

Yentsch and Lee (1966) suggested that lower I_k values were a response to a physiologically inferior environment. The I_k values obtained in this part of the study suggest that blue light above a specific growth rate of 0.025 h^{-1} corresponding to approximately 28$\mu Em^{-2}s^{-1}$ was an inferior environment for *Synechococcus* WH7803, however below specific growth rates of 0.025 h^{-1} I_k values under both conditions are the same. This may be a coincidance, but may also suggest that irradiances below about 28$\mu Em^{-2}s^{-1}$ provide only enough harvestable energy to maintain the cells. Although these observations may be an artefact introduced by the cells being acclimatised to growth in white light, the evidence in this study suggests that *Synechococcus* WH7803 does not photosynthesise best in blue light except at the lowest specific growth rates.

4.2.4 The respiration:photosynthesis ratio

The respiration:photosynthesis ratios, were measured under optimal conditions. Under suboptimal conditions different values (higher) would be expected, as the P_{max} value declines. The conditions for these cultures were assumed to be optimal in all aspects except irradiance, which was the variable parameter. Irradiance was approaching optimal values for *Synechococcus* WH7803 towards the higher specific growth rates, hence the assumed maximum metabolic rate. Therefore we might anticipate lower respiration:photosynthesis ratios at high specific growth rates. The values ranged from 11.1 to 27.3% with an average of 16.7% ± 2.8(2SE). The ratio is therefore a little higher than the estimated value of 10%.

The ratio of respiration to P_{max} was found to be high in dinoflagellates with values ranging from 35 to 60% (Moshkina, 1961: cited in Parsons et al., 1984). The high respiration rates were attributed to the motility of the flagellates. Waterbury et al.,(1985) reported *Synechococcus* strains capable of swimming motility, however motility in *Synechococcus* WH7803 has not been observed. The slightly higher ratio of respiration to P_{max} is probably insufficient to imply motility of this strain, therefore the high value is attributed to either an increased respiration rate due to sodium ion extrusion from the cells (chapter 3) and/or a species difference.

CHAPTER FIVE

NORTH SEA CRUISE 1987

CHAPTER FIVE

NORTH SEA CRUISE 1987

5.1 Results

5.1.1 Study area and hydrographic conditions.

The data presented here were obtained during a cruise of the RRS "Challenger" in July 1987. The cruise track with stations 1-20 and the position at 1200 (GMT) marked for each day, is shown in Figure 5.1. The object of this cruise was to examine the physiological response of phytoplankton in a temperate shelf sea, the North Sea, and in particular the phytoplankton <1μm fraction.

The North Sea is a particularly good place to study the physiological response of phytoplankton to environmental change since a variety of hydrographic conditions exist. The southern North Sea is tidally well-mixed but in the northern region seasonal stratification occurs, and in the summer the surface waters become nutrient depleted. A frontal boundary between these two regions extends from the German Bight to north of the Humber estuary on the English coast. This is referred to as the Flamborough Front (Pingree et al., 1978) shown in Figure 5.1. Fronts occur where water masses of distinctly different properties meet (Barnes and Hughes, 1988) and in general a discontinuity will exist in the horizontal temperature, salinity and density fields (Fearnhead, 1975).

Information gained from the Conductivity, Temperature and Depth (CTD) analyser and continuous logging systems indicated that a front was present in this area during July 1987.

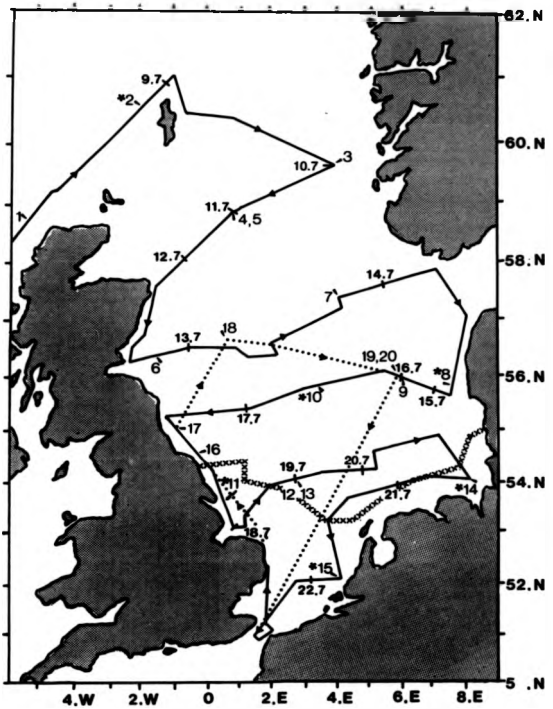

Fig. 5.1. Position at 12.00 GMT and station positions (1-20) in the North Sea during July 1987. Cruise track (————) first leg and (·····)second leg, position of the frontal boundary (xxxxxxxxxx) from Pingree et al., (1978) and the occurrence of photoinhibition (*) are indicated.

In some areas of the southern North Sea the water column appeared weakly stratified, which might well have been due to the relatively calm weather over this period. Pingree et al., (1978), found that chlorophyll *a* concentrations were low in the surface waters of a well-developed thermocline (northern region). The data obtained during the cruise for total chlorophyll *a*, supports this observation as the concentration of chlorophyll *a* in the northern North Sea was $<0.5\mu g\ l^{-1}$ except in coastal regions. South of the Flamborough front chlorophyll *a* concentrations were $1.0\text{-}0.5\mu g\ l^{-1}$ except in coastal regions where concentrations were even higher (Owens, unpublished data) and Table 5.1.

5.1.2 Distribution and vertical profiles of cyanobacteria in the North Sea.

Figure 5.2 shows the numbers and distribution of cyanobacteria found in the surface waters of the North Sea. Numbers ranged from 2.5×10^6 cells l^{-1} to 1.7×10^8 cells l^{-1} and were comparable to cyanobacterial numbers found in oceanic and shelf waters at this water temperature (Joint, 1986). As with other phytoplankton the distribution was patchy, but lowest numbers generally occurred in U.K. coastal waters, and were $<10^6$ cells l^{-1}; elsewhere in the North Sea, the number of cyanobacteria was more usually $>10^7$ cells l^{-1}.

Statistical analysis was carried out on the surface sample data of Table 5.1 to see if the patchy distribution of cell numbers was comparable with any of the other available data. The data included temperature, salinity, total chlorophyll *a*, size fractionated chlorophyll *a* (1.0 - 0.2μm fraction), nitrate and ammonia

Table 5.1. Surface sample data

date	Time (GMT)	temperature (C)	salinity	chl a (total)	chl a (0.2 – 1.0μm)	NO_3 (μgl^{-1})	NH_4 (μgl^{-1})	cells l^{-1}
12/7/87	1430	11.47	35.18	0.87	0.0787	0.027	0.01	$7.2x10^6$
12/7/87	1900	12.35	34.69	0.94	0.0787	0.006	0.01	$3.9x10^6$
12/7/87	2300	11.33	34.53	1.07	0.0729	0.022	0.01	$2.5x10^6$
13/7/87	0300	13.03	34.30	0.87	0.0816	0.014	0.01	$6.2x10^6$
13/7/87	0700	13.27	34.65	0.48	0.0466	0.015	0.01	$5.3x10^6$
13/7/87	1500	14.68	35.10	0.05	0.0175	0.010	0.01	$1.7x10^6$
15/7/87	0300	14.14	32.35	3.30	0.1137	4.270	0.55	$4.1x10^7$
15/7/87	0700	14.14	31.82	4.97	0.4022	5.930	2.16	$1.1x10^8$
15/7/87	1100	13.49	31.98	6.56	0.5187	7.590	2.46	$2.7x10^7$
15/7/87	1500	14.17	33.74	0.69	0.1632	0.023	0.23	$4.3x10^7$
15/7/87	1650	–	–	–	0.1250	–	–	$4.1x10^6$
17/7/87	2124	13.72	34.27	0.21	–	0.032	0.18	$1.3x10^7$
18/7/87	0500	12.10	34.25	3.50	0.1108	0.080	0.24	$5.3x10^6$
18/7/87	0800	11.45	34.27	4.14	0.0816	0.083	0.12	$1.9x10^6$
18/7/87	1100	14.26	34.08	1.05	0.1341	2.170	1.07	$3.7x10^6$
18/7/87	1700	12.33	34.32	1.56	0.1283	0.610	0.48	$4.7x10^6$
18/7/87	2100	12.61	34.79	0.96	0.0671	0.015	0.03	$2.2x10^7$
20/7/87	1253	14.87	34.82	0.35	0.0870	0.040	0.10	$3.6x10^7$
21/7/87	1958	15.56	33.86	1.41	0.1020	0.272	1.63	$4.8x10^7$
22/7/87	0300	14.86	34.60	0.71	0.4313	0.022	0.10	$1.7x10^8$
22/7/87	0728	17.85	27.60	10.31	0.1428	17.30	2.53	$3.9x10^7$
22/7/87	1100	16.31	31.49	6.14	0.4634	0.570	12.84	$4.8x10^7$

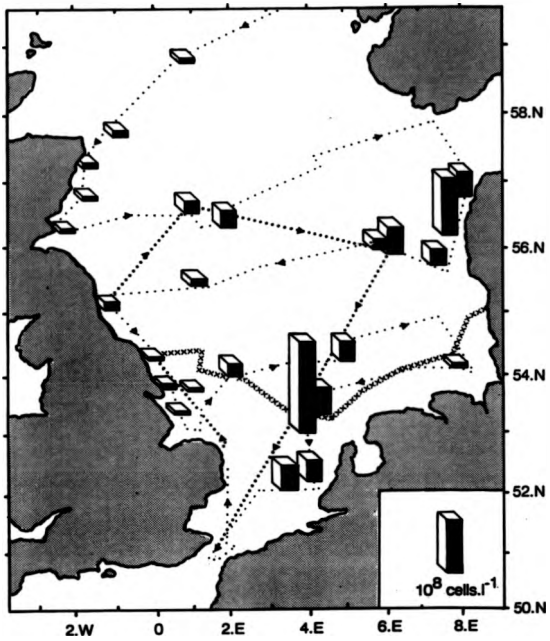

Fig. 5.2. The abundance and distribution of cyanobacteria in the North Sea during July 1987. The cruise track (· · · · ·) and (·····), and position of the frontal boundary (xxxxxxxx) from Pingree et al., (1978) are indicated.

concentrations.

Each set of data was ranked as shown in Table 5.2, with twenty samples in each group. The correlation coefficient for cells l^{-1} with each of the other ranked sets of data was calculated and the significance tested by the student's t test (Table 5.3). Both temperature (°C) and chlorophyll *a* in the <1.0μm phytoplankton fraction gave values of t greater than 1%, and salinity values greater than 2% when ranked against cells l^{-1}; hence it was concluded that the degree of agreement between the three sets of variables was significant. Nitrate and ammonium concentrations showed some correlation with cells l^{-1}, but the degree of agreement was not so good, with values of t greater than 10% and 5% respectively (Table 5.3).

If cyanobacteria were true shade phytoplankton, then they might be expected to be more abundant in the thermocline region of stratified waters where irradiance is low. Vertical profiles of cyanobacterial numbers and chlorophyll *a* concentrations in the phytoplankton <1.0μm and the phytoplankton >1.0μm were obtained. Some of these are shown in Figure 5.3 (a)-(f).

At most stations the highest number of cyanobacteria were found in the surface mixed layer. The vertical profile in Fig 5.3 (a) was sampled off the East coast of Scotland (14.30 GMT 12/7/87) and the maximum number of cells was found at 10 metres with a value of 7.4×10^6 cells l^{-1}. The vertical distribution of the chlorophyll *a* concentration in this size fraction also peaked at 10 metres with a value of 0.1μg l^{-1}. In contrast the maximum concentration of chlorophyll *a* in the >1.0μm fraction was at 20 metres at the thermocline with a value of 1.5μg l^{-1}.

Table 5.2. Ranking of surface sample data

ranked temperature (°C)	ranked salinity	ranked chl a (total)	ranked chl a (0.2 – 1.0µm)	ranked NO_3	ranked NH_4	ranked cells l^{-1}
3	20	6.5	5.5	9	4	10
6	16	8	5.5	1	4	5
1	13	11	4	6.5	4	3
8	11	6.5	7.5	3	4	9
9	15	3	2	5	4	7.5
15	19	1	1	2	4	1
11.5	5	14	12	17	14	15
11.5	3	17	17	18	17	19
10	4	19	20	19	18	12
13	6	4	16	8	11	16
4	9	15	11	11	12	7.5
2	10	16	7.5	12	10	2
14	8	10	14	16	15	4
5	12	13	13	15	13	6
7	17	9	3	4.5	8	11
17	18	2	9	10	9	13
18	7	12	10	13	16	17.5
16	14	5	18	6.5	4	20
20	1	20	15	20	19	14
19	2	18	19	14	20	17.5

The rank correlation coefficient was calculated.
This is defined by

$$R = \frac{1 - 6\Sigma d}{n(n-1)}$$

where Σd is the sum of the squares of the rank differences, and n is the number of samples ranked
The significance of the rank correlation coefficient was tested by the Student's t test

$$\text{Student's } t = R\sqrt{\frac{n-2}{1-R^2}}$$

with n – 2 degrees of freedom

Table 5.3. Summary of rank correlation coefficients and their significance

	R	Student's t test	df	level of significance
temperature	0.598	3.186	18	>1%
salinity	–0.474	–2.284	18	>2%
chl a (total)	0.200	0.866	18	ns
chl a (0.2 – 1.0µm)	0.650	3.627	18	>1%
NO_3	0.380	1.743	18	>10%
NH_4	0.459	2.192	18	>5%

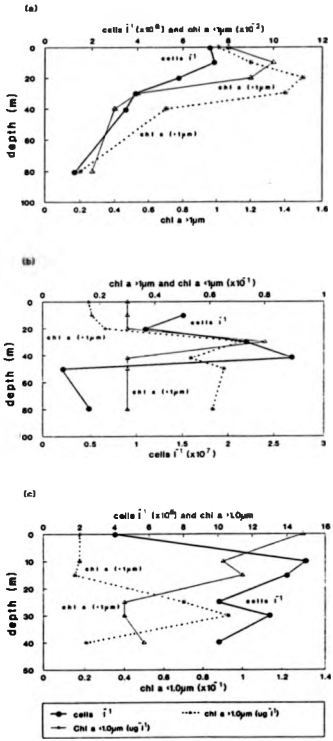

Fig. 5.3. Numbers of cyanobacteria and vertical distribution of chlorophyll *a* in the $>1\mu m$ phytoplankton fraction and $<1\mu m$ phytoplankton fraction at (a) 14.30 GMT on 12th July 1987, (b) 17.55 GMT on 12th July 1987 and (c) 16.50 GMT on 15th July 1987.

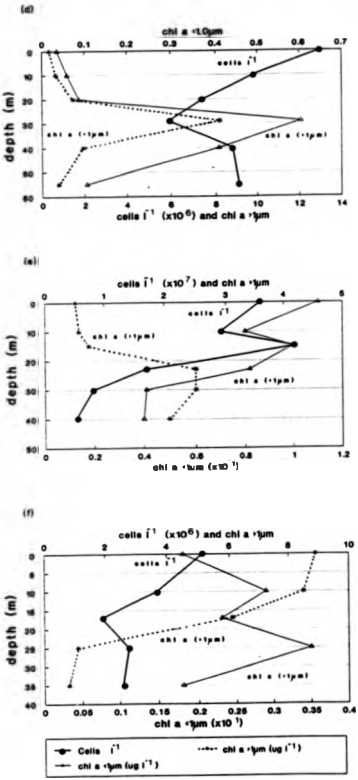

Fig. 5.3 (continued). (d) 21.25 GMT on 17th July 1987, (e) 12.55 GMT on 20th July 1987, and (f) 03.35 GMT on 21st July 1987.

The only station to show a subsurface peak in cyanobacteria number was in the mid northern North Sea (17.56 GMT 12/7/87) (Fig. 5.3 (b)). At this station cyanobacterial numbers were low in the surface mixed layer and the highest numbers were found at 42 metres, 2.7×10^{7} cells l^{-1}, just below the thermocline; chlorophyll *a* concentrations for the phytoplankton <1.0μm and >1.0μm were maximal at 30 metres with values of 0.07μg l^{-1} and 0.7μg l^{-1} respectively.

Figure 5.3 (c) shows the vertical distribution at a station off the Dutch coast (16.50 GMT 15/7/87) where the thermocline was well established. The minimum number of cells was at the surface, 4.1×10^{6} cells l^{-1} where the phytoplankton <1.0μm, chlorophyll maximum was observed. The maximum number of cells was however within the mixed surface layer at 10 metres, with a value of 1.5×10^{7} cells l^{-1}. A secondary peak of 1.3×10^{7} cells l^{-1} was found at a depth of 30 metres at the base of the thermocline where the concentration of chlorophyll in the >1.0μm fraction peaked; at this depth the chlorophyll *a* concentration in the <1.0μm fraction was at a minimum of 0.04μg l^{-1}.

The vertical distribution shown in Fig. 5.3 (d) was sampled at a station on the boundary of the Flamborough front (21.24 GMT 17/7/87) where a thermocline still existed. Maximum numbers of 1.3×10^{7} cells l^{-1} were at the surface, these declined to a minimum of 5.9×10^{6} cells l^{-1} at 30 metres and then increased to 9.1×10^{6} cells l^{-1} at 50 metres. Chlorophyll *a* concentrations in the >1.0μm fraction and <1.0μm fraction peaked at 30 metres at the base of the thermocline with concentrations of 8.2μg l^{-1} and 4.6μg l^{-1} respectively. Although the chlorophyll *a* concentration in the <1.0μm fraction was far higher than that found in any other profile, the

number of cells at that depth did not reflect the large increase in chlorophyll *a* concentration; in fact cell numbers were at a minimum at 30 metres. However it was noted that some detritus and other organisms were present on the prepared slide which could have contributed towards the high chlorophyll *a* concentration. The high concentrations of chlorophyll obtained in both fractions were probably a result of the conditions evolved by the front.

The vertical profile in 5.3 (e) was sampled at a station in the mid southern North Sea (12.53 GMT 20/7/87) where the water column was found to be stratified. Cell numbers peaked in the surface mixed layer at 15 metres with 4.2×10^7 cells l^{-1}, however high numbers were also found at the surface, 3.6×10^7 cells l^{-1}, as was the maximum chlorophyll *a* concentration in the phytoplankton <1.0μm, with a value of 0.1μg l^{-1}. Maximum chlorophyll concentration in the >1.0μm fraction was at the base of the thermocline at approximately 25 metres.

Figure 5.3 (f) which was sampled on the frontal boundary (03.36 GMT 21/7/87), near to the Dutch/German coast has a similar vertical profile to Figure 5.3 (d). Numbers were highest at the surface, 5.1×10^6 cells l^{-1}, and decreased to a minimum of 1.9×10^6 cells l^{-1} at the base of the thermocline. Chlorophyll *a* concentrations in this fraction peaked at 25 metres with a value of 0.04μg l^{-1}, whilst for the >1.0μm fraction the concentration was highest at the surface, 9.00μg l^{-1}.

5.1.3 Cellular fractionation.

The physiological state of the cyanobacterial assemblages was investigated by following the incorporation of ^{14}C into various cellular constituents of two size fractions, the phytoplankton >1.0μm and the phytoplankton <1.0μm (0.2μm - 1.0μm). Incubations were carried out under constant illumination in an attempt to avoid the complication of diel variation in activity.

Figure 5.4 shows typical results obtained for both size fractions. Assimilation of ^{14}C into the various constituents of both the fractions increased approximately linearly, therefore comparison of the pattern of photosynthetic ^{14}C fixation in *Synechococcus* species and the larger phytoplankton was possible. Most radioactivity was found in the protein with little incorporation into low molecular weight metabolites. The specific rate of ^{14}C incorporation by phytoplankton <1.0μm into all the cellular components was considerably greater than into phytoplankton >1.0μm when normalised to chlorophyll *a* content of the different size fractions (Table 5.4).

The difference in uptake rates between the two fractions was high, therefore to compare the labelling patterns in the two assemblages data were expressed as percentage incorporation: [dpm in a fraction / (Σdpm in all fractions)] × 100.

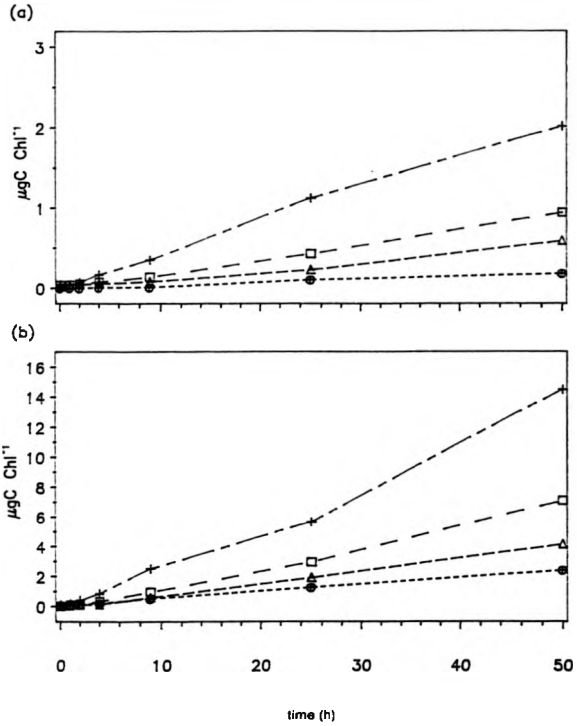

Fig. 5.4. Time course of carbon-14 incorporation into the low molecular weight metabolites (—●—), lipid (— □— —), polysaccharide and nucleic acids (— — —▲— — —) and protein (— — —+— —) by (a) phytoplankton (< 1µm) and (b) phytoplankton (> 1µm) incubated with a constant irradiance of 156µEm^{-2}s^{-1}. Initial chlorophyll a concentrations were (a) 0.02µg l-1 for the picoplankton fraction with 7.4x10^{6} cells l^{-1} and (b)0.28µg l^{-1} chlorophyll a in the > 1µm fraction .

Table 5.4. Time course of carbon-14 incorporation into the low molecular weight metabolites, lipids, polysaccharides and nucleic acids, and proteins, by (a) phytoplankton <1μm and (b) phytoplankton>1μm, at station 5 on 12/07/87.

(a) phytoplankton <1μm

time	LMW		lipids		polysaccharides and nucleic acids		proteins	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
0	0	0	0.035	1.50	0.018	0.78	0.045	1.94
1	0	0	0.037	1.60	0.033	1.42	0.045	1.97
2	0	0	0.052	2.28	0.045	1.94	0.073	3.17
4	0.008	0.36	0.076	3.30	0.054	2.33	0.166	7.20
9	0.012	0.50	0.138	6.00	0.080	3.49	0.350	15.20
25	0.106	4.60	0.428	18.60	0.229	9.95	1.120	48.70
50	0.182	7.90	0.937	40.74	0.587	25.50	2.010	87.40

(b) phytoplankton >1μm

time	LMW		lipids		polysaccharides and nucleic acids		proteins	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
0	0.08	0.28	0	0	0.03	0.10	0.12	0.42
1	0.08	0.28	0.09	0.32	0.05	0.18	0.19	0.67
2	0.15	0.53	0.15	0.50	0.10	0.35	0.40	1.40
4	0.21	0.72	0.32	1.09	0.12	0.40	0.84	2.90
9	0.52	1.80	0.95	3.30	0.58	2.00	2.51	8.70
25	1.27	4.40	2.95	10.20	1.91	6.60	5.66	19.60
50	2.40	8.30	7.08	24.50	4.13	14.30	14.48	50.10

(I) μgC l^{-1} incorporated
(II) mgC (mg chl a)$^{-1}$ incorporated
LMW – low molecular weight metabolites

Table 5.5 summarises the percentage of label incorporated into the different components after twenty-four hours under constant light conditions of 156$\mu Em^{-2}s^{-1}$. Most of the label was incorporated into protein in both size fractions, with a mean of 54.2% (±[2SE] 5.3) for the <1.0μm fraction and 46.2% (±[2SE] 4.1) for >1.0μm phytoplankton, although there were variations in relative proportions in each fraction.

Incorporation into low molecular weight metabolites was low in all cases; with mean percentages of 7.8% (±[2SE] 1.7) for the phytoplankton <1.0μm, and 10.9% (±[2SE] 2.2) for >1.0μm phytoplankton. The percentage label incorporated into the lipid and polysaccharide plus nucleic acid fractions was similar at 21.1% (±[2SE] 3.2) for the lipid and 17.4% (±[2SE] 3.1%) for the polysaccharide plus nucleic acids in the <1.0μm fraction, and 21.9% (±[2SE] 3.1) for the lipid and 21.9% (±[2SE] 4.8) for the polysaccharide plus nucleic acid in the >1.0μm fraction.

When the data for each station and fraction were plotted the graphs looked very similar. So to obtain a general representation of the ^{14}C incorporation into the various cell components the mean percentage values calculated at each incubation time were plotted. Figure 5.5 shows the two graphs obtained by plotting these mean values versus time. In both fractions the percentage incorporation into protein was highest and into low molecular weight metabolites lowest. The mean values of percentage incorporation into protein for the phytoplankton <1.0μm were 49.4% to 55.6% and varied very little throughout the time course. The >1.0μm phytoplankton similarly varied very little (41.0% to 45.9%) over most of the time course, but there was a significant drop from 51.8% to 41.4% over the first hour.

Table 5.5. The percentage distribution of carbon-14 into cellular constituents of phytoplankton <1μm and >1μm in the North Sea after twenty-four hours incubation at a constant irradiance of 156μEm^{-2}s^{-1}.

	phytoplankton <1μm				phytoplankton >1μm			
station	LMW	lipid	polysaccharide	protein	LMW	lipid	polysaccharide	protein
2	14.7	22.3	17.0	46.1	12.8	18.8	30.6	37.8
3	6.7	18.9	11.8	62.6	23.1	6.1	9.9	60.9
4	7.4	21.8	10.2	60.7	8.8	22.1	18.3	50.8
5	5.6	22.8	12.1	59.5	10.8	25.1	16.1	48.0
6	9.3	36.8	23.5	30.4	7.4	23.1	17.0	52.5
7	8.7	15.8	15.3	60.2	9.7	23.6	17.3	49.4
8	7.1	23.4	13.8	55.7	9.1	17.3	33.6	40.0
9	4.7	20.9	23.5	50.9	8.9	27.1	17.6	46.4
10	7.3	17.5	12.3	62.9	10.8	24.4	14.2	50.6
11	10.7	24.2	22.4	42.7	10.0	26.2	17.5	46.3
12	10.0	14.8	21.5	60.3	11.0	20.3	22.0	46.7
13	6.6	14.7	27.3	51.4	7.6	22.4	34.6	35.4
14	2.8	21.0	15.6	60.6	11.8	16.3	36.0	35.9
mean±2SE	7.8±1.7	21.1±3.2	17.4±3.1	54.2±5.3	10.9±2.2	21.0±3.1	21.9±4.8	46.2±4.1

LMW – low molecular weight metabolites

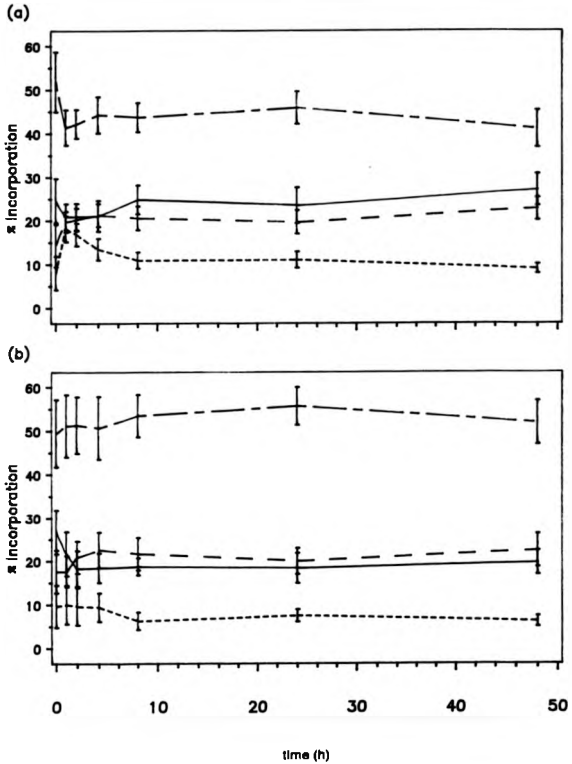

Fig. 5.5. General representation of the relative distribution of C−14 between low molecular weight metabolites ------, lipids ———, polysaccharides and nucleic acids — — and protein – ——— in (a) < 1.0μm phytoplankton, (b) >1.0μm phytoplankton.

This was probably a result of the low level of incorporation of label into all the components at time equal to zero; small variations in radioactive counts can create large differences in the calculations when counts are low. Incorporation into low molecular weight metabolites appeared to change very little, particularly in the <1.0μm fraction, with values ranging from 6.2% to 10.0%. The larger phytoplankton varied from 8.0% to 18.1, and as in the percentage incorporation into protein in the >1.0μm phytoplankton there was a large difference between the values at time zero; 8.0% to 18.1%.

The major difference between ^{14}C incorporation into the components of the two fractions was the percentage incorporation into lipid and polysaccharides plus nucleic acids. The general representation showed that percentage incorporation into lipid in the phytoplankton <1.0μm was higher than that into polysaccharide and nucleic acids, whereas in the other fraction percentage incorporation into polysaccharide was greater than into lipid. The label incorporated into the lipid component ranged from 17.5% to 22.5% in the <1.0μm fraction and 14.5% to 23.0% in >1.0μm fraction; this compares with the label incorporated into polysaccharide and nucleic acids which ranged from 18.3% to 26.7% and 20.9% to 27.0% in the <1.0μm and >1.0μm phytoplankton respectively. There were variations in the relative proportions of these components. Typically there was more percentage incorporation of label into lipid than polysaccharides and nucleic acids at stations 2 to 10, whereas at stations 11 to 19 relatively more label was incorporated into polysaccharides and nucleic acids than lipids.

In the larger phytoplankton an exception to this generalisation was at station 8 where percentage incorporation into polysaccharides

and nucleic acids was always greater than that into lipids. At most stations, (including stations 2 to 10) for the <1.0μm fraction and at time equal to zero the percentage of label incorporated was greatest into polysaccharides and nucleic acids. The pattern of distribution previously noted for the lipids and polysaccharides and nucleic acids in the <1.0μm fraction was attained over the next two hours.

Comparison of the general representations of the results with the graphs generated for the individual stations show no major differences in the incorporation of label into all the four components, except at station 6 (off the East coast of Scotland) in the <1.0μm fraction. The percentage incorporation into the cellular components at this station are shown in Figure 5.6. At this station percentage incorporation into lipid in the phytoplankton <1.0μm was higher than into any other component ranging from 28.0% to 43.2%, with a mean of 37.3%, whereas for protein the range was from 22.7% to 47.3% with a mean of 31.3%. Percent incorporation into the low molecular weight metabolite and polysaccharide plus nucleic acid fractions followed a distribution similar to that of the general representation.

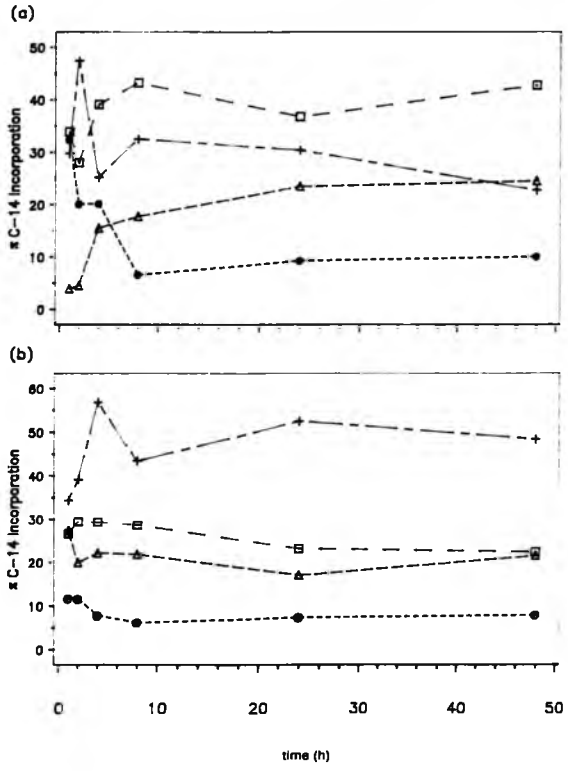

Fig. 5.6. Relative distribution of carbon-14 between four chemical fractions : low molecular weight metabolites (—•---), lipid (—□ —), polysaccharide and nucleic acids (—+—) and protein (—+ —) by (a) phytoplankton < 1.0μm and (b) phytoplankton >1.0μm at Station 6 on 13 July 1987.
The percentage of carbon-14 label found in the lipid fraction of the <1μm phytoplankton was high compared with all other stations.

5.1.4 Photosynthesis-irradiance measurements

The second procedure adopted to investigate the physiology of the picoplankton assemblages was the determination of photosynthetic parameters. All samples were taken shortly after dawn each day to minimise diel variations in photosynthetic characteristics.

A computer program (LSFITS), was prepared by M. Carr (PML) using the equation of Platt et al., (1980) to plot a curve through the data points. Figure 5.7 shows typical examples of the curve fitting to the data for both fractions. The parameters of interest in this figure are the initial slope α^B, the assimilation number P^B_m, and the derivative I_k (P^B_m/α^B), the irradiance at which saturation of photosynthesis first occurs.

Table 5.6 shows the alpha values calculated by LSFITS, and linear regression using data points 2 to 14 at low irradiance. The linear regression was carried out in the expectation of obtaining a better fit to the points and therefore more accurate description of alpha. Due to a lot of scatter of the data points many of the linear regressions, particularly those in the <1.0μm fraction, did not give significant fit to the points. This was evident from the significance level P of the F ratio. Where the F value was equal to or exceeded the tabulated value for $P \geq 0.001$ (and F value of 19.69 with 11 degrees of freedom) it was concluded that the linear component in the relationship was very highly significant. Where $P < 0.050$ the linear regression was not significant (ns).

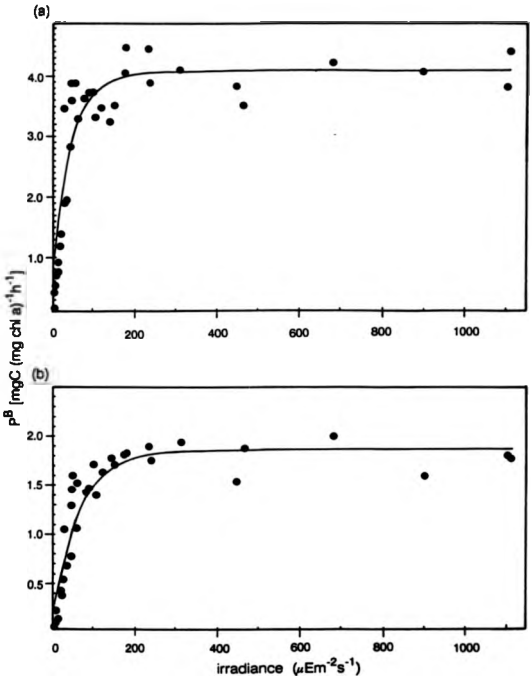

Fig. 5.7. Typical photosynthesis-irradiance curves obtained during the cruise for (a) phytoplankton (< 1μm), and (b) phytoplankton (> 1μm), at station 20 on 30th July 1987.

Table 5.6. Alpha values calculated by a computer model (LSFITS) and by linear regression

		phytoplankton <1µm				phytoplankton >1µm			
station	date	alpha LSFITS	alpha LREG	F ratio	p	alpha LSFITS	alpha LREG	F ratio	p
1	08/07/87	0.630	0.092	2.33	ns	0.033	0.020	9.58	≤0.050
2	09/07/87	0.278	0.012	0.08	ns	0.029	0.028	67.62	≤0.001
3	10/07/87	0.274	0.004	0.04	ns	0.041	0.023	6.36	≤0.050
4	11/07/87	0.458	0.042	1.06	ns	0.089	0.028	16.26	≤0.010
5	12/07/87	0.280	−0.005	0.03	ns	0.061	0.071	34.45	≤0.001
6	13/07/87	0.107	0.115	557.29	≤0.001	0.059	0.066	820.07	≤0.001
7	14/07/87	0.289	0.124	20.41	≤0.001	0.060	0.030	2.08	ns
8	15/07/87	0.045	0.036	195.62	≤0.001	0.051	0.033	377.56	≤0.001
9	16/07/87	0.226	0.149	51.89	≤0.001	0.038	0.026	402.25	≤0.001
10	17/07/87	0.174	0.080	143.14	≤0.001	0.050	0.029	193.02	≤0.001
11	18/07/87	0.121	0.064	199.85	≤0.001	0.053	0.030	337.05	≤0.001
12	19/07/87	0.122	0.059	36.22	≤0.001	0.034	0.024	21.51	≤0.001
13	20/07/87	0.194	0.054	10.85	≤0.001	0.039	0.016	207.38	≤0.001
14	21/07/87	0.094	0.011	1.36	ns	0.028	0.021	172.67	≤0.001
15	22/07/87	0.157	0.031	41.12	≤0.001	0.055	0.026	216.53	≤0.001
16	26/07/87	0.088	0.037	33.10	≤0.001	0.042	0.027	100.96	≤0.001
17	27/07/87	0.067	0.049	11.29	≤0.001	0.046	0.030	256.22	≤0.001
18	28/07/87	0.103	0.014	1.72	ns	0.034	0.014	116.44	≤0.001
19	29/07/87	0.158	0.063	206.24	≤0.001	0.047	0.028	192.75	≤0.001
20	30/07/87	0.092	0.049	68.46	≤0.001	0.029	0.018	192.70	≤0.001

LREG – linear regression
ns – not significant
p <0.001 – significant at the 1% level

The mean α^B for the phytoplankton <1.0μm calculated by the linear regression model was 0.054 and for the phytoplankton >1μm 0.029 [mgC (mg chl a)$^{-1}$h^{-1}] [μEm^{-2} s^{-1}]$^{-1}$.

As almost half of the initial slopes estimated by linear regression in the <1.0μm fraction were considered to be insignificant or poor fits to the data, all the values of α^B and P_m^B in Table 5.7 were calculated according to the LSFITS programme. Table 5.7 summarises the photosynthetic parameters obtained for both fractions in this study. Ninety five percent confidance limits of the data were <10% of the estimated values of α^B and P_m^B.

Parameters of light-dependent photosynthesis tended to be low and less variable in the phytoplankton >1.0μm than the phytoplankton <1.0μm assemblages. Values of α^B were always low for the >1μm size fraction and ranged from 0.028 to 0.089 with a mean of [0.046 mgC (mg chl a)$^{-1}$ h^{-1}][μEm^{-2}s^{-1}]$^{-1}$; this compares with values of α^B for the <1.0μm fraction which varied from 0.045 to 0.630 with a mean of 0.198 [mgC (mg chl a)$^{-1}$h^{-1}][μEm^{-2}s^{-1}]$^{-1}$. Similarly P_m^B values for phytoplankton <1.0μm fraction, with values ranging from 1.32 to 5.83 with a mean of 2.89 mgC (mg chl a)$^{-1}$h^{-1}, compared with 1.91 to 11.00 and a mean of 6.01 mgC (mg chl a)$^{-1}$h^{-1}.

I_k values were usually higher in the phytoplankton >1.0μm and ranged from 45.52 to 84.15 with a mean of 62.24μEm^{-2}s^{-1} compared to a range of 13.71 to 90.91 and a mean of 36.57μEm^{-2}s^{-1} for the phytoplankton <1.0μm.

Table 5.7. Photosynthetic parameters derived from photosynthetic-irradiance curves for phytoplankton <1μm and phytoplankton >1μm: initial slope (α^B) [mgC (mg chl a)$^{-1}$h^{-1}] [μEm^{-2}s^{-1}]$^{-1}$: P_m^B [mgC (mg chl a)$^{-1}$h^{-1}]; I_k [μEm^{-2}s^{-1}].

		phytoplankton <1μm			phytoplankton >1μm		
station	date	α^B	P_m^B	I_k	α^B	P_m^B	I_k
1	08/07/87	0.630	8.64	13.71	0.033	1.68	50.91
2	09/07/87	0.278	* 4.90	17.63	0.029	* 1.32	45.52
3	10/07/87	0.274	9.21	33.61	0.041	3.19	77.80
4	11/07/87	0.458	8.69	18.97	0.089	5.83	65.51
5	12/07/87	0.280	8.87	31.68	0.061	4.08	66.89
6	13/07/87	0.107	6.40	59.81	0.059	3.58	60.68
7	14/07/87	0.289	9.77	33.81	0.060	3.62	60.33
8	15/07/87	0.045	* 2.51	55.78	0.051	2.64	51.76
9	16/07/87	0.226	8.67	38.36	0.038	2.04	53.68
10	17/07/87	0.174	* 5.83	33.51	0.050	2.81	56.20
11	18/07/87	0.121	* 11.00	90.91	0.053	4.46	84.15
12	19/07/87	0.122	5.83	47.79	0.034	2.38	70.00
13	20/07/87	0.194	8.02	41.34	0.039	2.29	58.72
14	21/07/87	0.094	* 2.68	28.51	0.028	1.72	61.43
15	22/07/87	0.157	* 3.06	19.49	0.055	3.71	67.45
16	26/07/87	0.088	3.81	43.30	0.042	3.02	71.90
17	27/07/87	0.067	1.91	28.51	0.046	3.33	72.39
18	28/07/87	0.103	2.97	28.83	0.034	1.77	52.06
19	29/07/87	0.158	3.39	21.46	0.047	2.49	52.98
20	30/07/87	0.092	4.08	44.35	0.029	1.87	64.48
mean		0.200	6.01	36.57	0.046	2.89	62.24

* indicates data which could be fitted to a photoinhibited curve

Figures 5.8 (a) and (b) show α^B and P_m^B values respectively, from Table 5.7 in the form of histograms. P_m^B in the <1.0μm fraction exhibited generally high values up to and including station 13 and then low values for the rest of the study. α^B was also high initially in the <1.0μm fraction, until about station 9 and then decreased to lower values. All stations sampled during the second leg of the cruise had lower than mean P_m^B values and low α^B values. P_m^B and α^B for the >1.0μm phytoplankton were low and showed little deviation from the mean value throughout the study. Although I_k values were generally higher in the >1.0μm phytoplankton, as shown by the higher mean value (Figure 5.9 (a)), I_k in both fractions increased gradually from the beginning of the study, until station 11 and then remained at a generally high value.

Figure 5.9 (b) and (c) show the chlorophyll *a* concentrations for each station. The tendency for higher photosynthetic parameters in the earlier part of the cruise may also be related to reduced chlorophyll *a* concentration at the beginning of the cruise.

Examples of photosynthesis-irradiance curves expressing photoinhibition are shown in Figure 5.10 (a) - (f). This occurred on only seven occasions during the study, even though the maximum irradiance experienced in the incubator was $1000\mu Em^{-2}s^{-1}$. Full sunlight is approximately $1700\mu Em^{-2}s^{-1}$ (Richardson et al., 1983). Stations where photoinhibition occurred are marked (*) in Figure 5.1 and Table 5.7. Station 2 which was positioned West of the Orkney Isles, was the only station to exhibit photoinhibition in both the phytoplankton >1.0μm and <1.0μm fractions, however as shown by Figure 5.10 the slope of the inhibited portion of the curves was always slight.

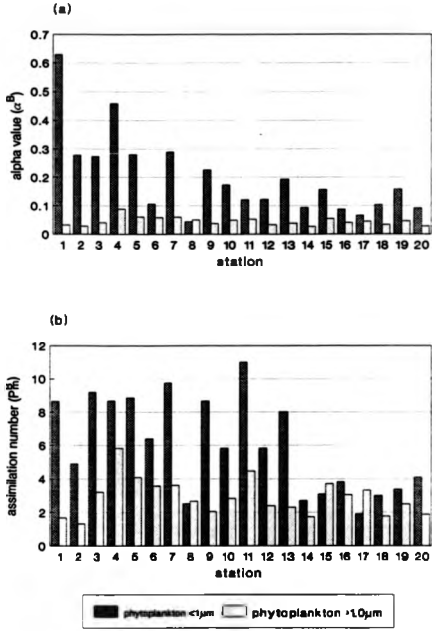

Fig. 5.8. Comparison of (a) alpha values (α^B), [mgC (mg chl a)$^{-1}$h^{-1}][μEm^{-2}s^{-1}]$^{-1}$ and (b) assimilation numbers (P^B_m), [mgC (mg chl a)$^{-1}$h^{-1}], calculated for the phytoplankton< 1μm, and the phytoplankton > 1μm at 20 stations in the North Sea during July 1987.

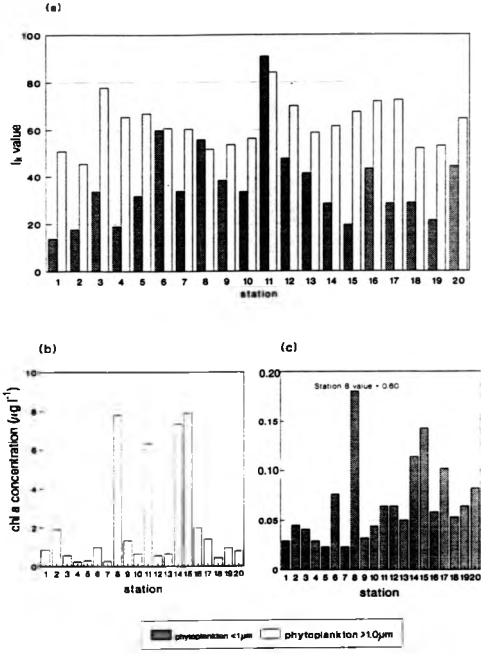

Fig. 5.9. Comparison of (a) I_k values, (b) chlorophyll *a* concentrations in the phytoplankton $> 1\mu m$ and (c) chlorophyll *a* concentrations in the phytoplankton $< 1\mu m$, at 20 stations in the North Sea during July 1987.

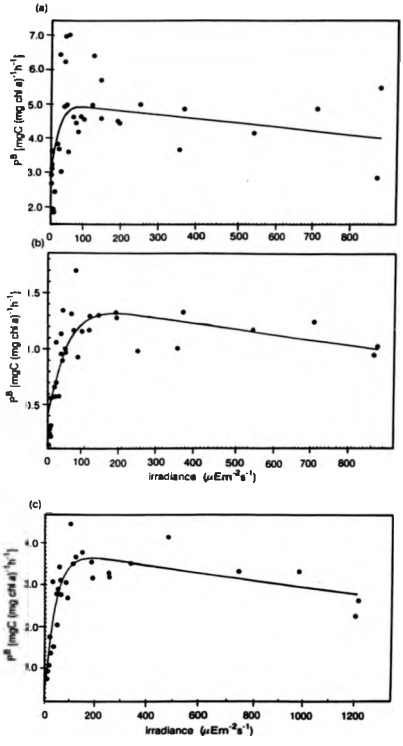

Fig. 5.10. Photosynthesis-irradiance curves obtained during the cruise showing photoinhibition of photosynthesis at high irradiances at (a) station 2 in the phytoplankton < 1μm, (b) station 2 in the phytoplankton > 1μm, and (c) station 15 in the phytoplankton < 1μm.

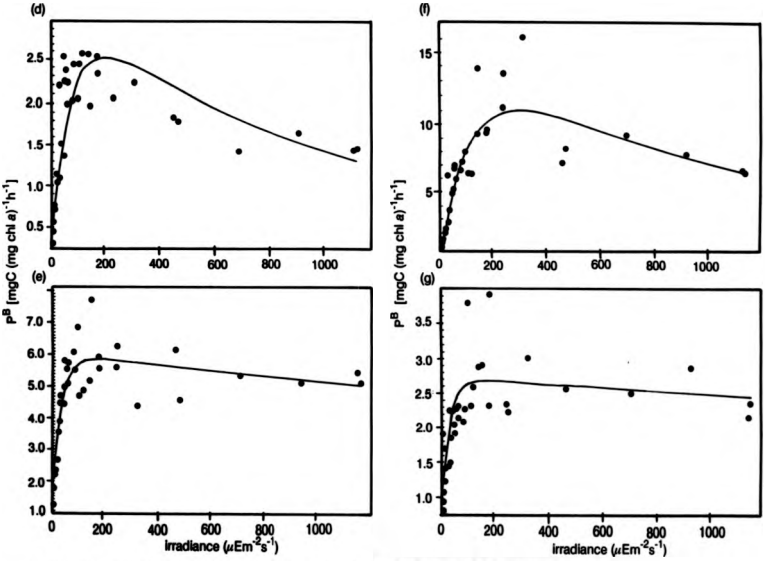

Fig. 5.10. (continued) (d) station 8, (e) station 10, (f) station 11, and (g) station 14 in the phytoplankton < 1μm.

The values of α^B values in the >1.0μm phytoplankton were always lower than in the <1.0μm fraction except at station 8 where values of 0.051 and 0.045 were found respectively. Both α^B and P_m^B were lower than the mean in the smaller size fraction and approaching the mean value for the >1.0μm phytoplankton. The unusually high concentration of chlorophyll *a* (0.60 $\mu g\ l^{-1}$) in the <1.0μm fraction and the relatively high concentrations obtained for the larger phytoplankton (0.78 $\mu g\ l^{-1}$) may explain the low values of the photosynthetic parameters at this station (Figure 5.10).

Stations 4 and 5, 12 and 13, 19 and 20 were sampled on consecutive days. In general the assimilation numbers and I_k values were similar within each pair, but alpha values appeared to be more variable.

5.2 DISCUSSION

5.2.1 Abundance and distribution of cyanobacteria in the North Sea

There have been very few reports on the significance of cyanobacteria to the ecology of the North Sea. Gieskes and Kraay (1983) reported that the pigment pattern of samples from the North Sea suggested the presence of cyanobacteria, but they did not quantify the numbers of cyanobacteria. Schmaljohann (1984) observed *Synechococcus* species up to 2.3×10^7 cells l^{-1} in the surface waters of the Baltic Sea, Kattegat and Skagerrak which flow into the North Sea. He observed the highest abundance of unicellular cyanobacteria during late summer and early autumn, and found only a few cells in winter and spring. Joint and Pomroy (1986) counted 1.5×10^8 cells l^{-1} in surface samples from the Celtic Sea and El Hag and Fogg (1986) reported maxima of 1.5×10^8 cells l^{-1} from inshore waters (Menai Straits) and 8.1×10^7 cell l^{-1} from offshore waters (Irish Sea).

As can be seen from Fig. 5.2, there were areas of the North Sea in July where cyanobacteria were extremely abundant and numbers were as high or higher than previously reported (1.7×10^8 cells l^{-1}). In general values were typical of those observed for the North Atlantic (Murphy and Haugen, 1985) at that time of year. No relationship was apparent between cyanobacterial abundance and hydrographic regime; although the station with the highest numbers of *Synechococcus* was located in the frontal region, there were other areas of the frontal and transitional waters which had very low cell numbers. Similarly, cell densities in tidally well-mixed waters of the southern North Sea

were not significantly different from those in the seasonally stratified northern North Sea. The only consistent feature of cyanobacterial distribution appeared to be that numbers were particularly low along the eastern coast of the U.K.

Glover et al., (1986b) found that cyanobacteria were most abundant at 80 metres just above the chlorophyll maximum and there have been numerous other observations suggesting that cyanobacteria are adapted to photosynthesise at low irradiance. Data from laboratory cultures (Morris and Glover, 1981: this study) has shown that growth of marine *Synechococcus* is saturated at low irradiances and suggests that these cyanobacteria may be most abundant at depths where the irradiance is low, ie: shade adapted.

Vertical profiles showed that at most stations in the North Sea cyanobacteria were most abundant in the near surface water and therefore these profiles do not support the hypothesis that cyanobacteria are shade-adapted. The formation of a thermocline at 5 to 15 metres at most of the stations in the southern North Sea suggests a high degree of physical stability of the water column at this time, therefore the maximum numbers of cyanobacteria might be expected to be found where the conditions were optimum for growth; this appears to be in the surface waters. The only vertical profile to show a subsurface maximum at the base of the thermocline was located in the mid northern North Sea. Similar profiles have been obtained by Waterbury et al., (1986) and they suggested that the population making up the subsurface maximum was benefiting from nutrients supplied from below the mixed layer.

5.2.2 Macromolecular components of natural assemblages

In order to determine whether or not natural picoplankton assemblages are genotypically or phenotypically adapted to growth at low irradiance, two types of measurement which indicate the physiological status of the phytoplankton, photosynthetic parameters and the cellular fractionation of ^{14}C incorporation were measured.

Phytoplankton, like all organisms, must synthesise an array of macromolecules and polymers to grow and reproduce. Variation in the relative quantities of these cellular products may be induced by a number of environmental conditions such as light quality and quantity, temperature, pH and nutrient availability. Labelling patterns interpreted with respect to the immediate cell environment can be used as an indication of the cellular physiological state (Hitchcock, 1983). Factors such as the age of the cells, the stage of the life cycle and the previous culture or cell history also have an important influence on rate of uptake and distribution of assimilated ^{14}C into the products of photosynthesis (Olive and Morrison, 1967).

In an attempt to understand the factors controlling phytoplankton distribution and to find evidence of their physiological state, the allocation of ^{14}C into the macromolecular constituents of phytoplankton >1.0μm and <1.0μm was examined and the results from the two fractions compared.

Increasing degrees of nutrient limitation and low light conditions results in increased incorporation of ^{14}C into protein (Morris, 1981; Morris et al., 1974; Morris and Skea, 1978; Smith and Geider, 1985): however under conditions of extreme nutrient limitation incorporation into protein is prevented by the lack of

available nitrogen and there is increased ^{14}C incorporation into polysaccharides and lipids (Fogg, 1956). Nutrient limitation and high irradiance also result in reduction in the size and number of photosynthetic units (Perry et al., 1981) and hence an increase in the value of the assimilation number P_m^B. Given these responses to light and nutrient stress, these two measures of phytoplankton physiology might have the potential to suggest if the natural assemblages were nutrient limited or a genotypic shade organism.

The recovery of ^{14}C during the extraction procedure was examined by comparing the activity of the unextracted samples with the sum of the activity of the four macromolecular components. Recovery of radioactive label was generally greater than 90% for the larger size fraction, however for the phytoplankton <1.0μm only about 60% recovery was obtained. Hitchcock, (1983) and Palmisano and Sullivan, (1985) noted that the extraction procedure of Li et al., (1980) was only a crude fractionation of cellular constituents. Improvements in the solvent extraction scheme for isolating the major constituents of cyanobacteria, such as an increase in trichloroacetic acid concentration from 5% to 10% with a concomitant decrease in incubation time (Konopka and Schnur, 1980) would have given a more accurate description of the physiological state of the *Synechococcus* cells under the given conditions. Despite the difference in the efficiency of extraction Li's method (1980) is still a useful method for making relative comparisons among microalgal communities and has been used in previous studies of the photosynthetic picoplankton (Glover et al., 1985a; Glover and Smith, 1988).

Assimilation of ^{14}C into the various fractions increased approximately linearly (Figure 5.4), and when normalised to

chlorophyll *a* concentration the specific rate of incorporation of ^{14}C into all cellular constituents of the phytoplankton <1.0μm was approximately double that of the larger phytoplankton.

Typical results from this study of the phytoplankton <1.0μm in the North Sea show highest incorporation into protein (56.1%) after twenty-four hours. Allocation of ^{14}C into the other cellular components after twenty-four hours were polysaccharide and nucleic acids 16.9%, lipid 19.8% and low molecular weight metabolites 7.7%.

Previous studies on photosynthetic carbon metabolism (Fogg, 1956; Bassham 1971; Lancelot and Mathot, 1985) inferred that labelled ^{14}C is first incorporated into low molecular weight metabolites. If other nutrients are available (eg N and P), these low molecular weight metabolites can be converted into catalytically active biomass, (protein and nucleic acid). This is useful as an indication of the physiological status of the cells and implies relative growth rate (Konopka and Schnur, 1980). If nutrients are not available low molecular weight metabolites may accumulate or be converted into storage compounds including polysaccharides and lipids.

Mann and Carr, (1974) found that the cellular concentrations of ribosomes in *Anacystis nidulans* increased as growth rate increased. Therefore the greater capacity for incorporation of label into protein observed by the phytoplankton grown at high irradiances in the surface waters could be a consequence of faster growth rate. Most of the TCA-soluble fraction (referred to as polysaccharides), from the oceanic *Synechococcus* clone DC-2, growing at high growth rates (approximately fourteen hours doubling time) did not appear to be polysaccharide since it behaved like RNA toward mild alkaline hydrolysis (Cuhel and Waterbury, 1984). Although the composition of

the polysaccharide and nucleic acid fraction was not examined, these previous results and the high percentage incorporation of ^{14}C in the protein fraction suggests that the *Synechococcus* assemblage and the larger phytoplankton form the North Sea were growing at high growth rates. If this is correct then the percentage incorporation of ^{14}C in the polysaccharide and nucleic acid fraction was probably composed of more RNA than reserve product.

The high rate of incorporation into protein could also result from less than optimal nutrient availability (Morris and Skea, 1978), growth at low irradiance (Konopka and Schnur, 1980), or a shift from high to low irradiances (Harding et al., 1985).

Nutrient concentrations were measured throughout the cruise and nitrate values were very low. Using a sensitive chemiluminescence assay, Woodward and Owens (1989) showed that nitrate concentrations were <20 nmol l^{-1} over the whole of the stratified region and only exceeded 50 nmol l^{-1} in the coastal regions. At these low nitrate concentrations, Owens et al., (1989) found that ammonium uptake was always greater than that of nitrate. It was assumed that these low concentrations of available nitrogenous nutrient must constitute a less than optimal nutrient supply for the phytoplankton.

Data from natural populations is often difficult to interpret because water movements and mixing mean that it is impossible to establish the previous light history of the cells. If physiological adjustment by the phytoplankton is faster than changes in environmental conditions, the cells continuously adjust their metabolic activities to the new conditions (Vincent, 1980), if not the cells can only adjust to mean environmental conditions (Savidge, 1979; Falkowski, 1980).

The irradiance received by the phytoplankton at 10 metres was estimated according to the Lambert-Beer Law: $I_d = I_o e^{-kd}$, where d is depth, I_o is the incident radiation upon the surface of the water (full sunlight = $1700\mu Em^{-2}s^{-1}$; Richardson et al., 1983), I_d is the photon flux density at depth, d, and k is an extinction coefficient. Typically, clear temperate coastal waters exhibits a k for photosynthetically active radiation, 400 to 700nm, of about 0.15 (Richardson et al., 1983). An estimate of the irradiance at 10 metres in the North Sea was $380\mu Em^{-2}s^{-1}$. At this irradiance phytoplankton was not expected to be light limited, particularly the cyanobacteria which have previously been shown to saturate growth and photosynthesis at much lower irradiances (Morris and Glover, 1981).

The intracellular ^{14}C allocation in this study resembled the pattern obtained for light limited cultures of a diatom at high and low growth rates (Smith and Geider, 1985). However in *Synechococcus* species most assimilated carbon is directed into proteins (Glover et al., 1985a; Glover and Smith, 1988) and this characteristic pattern of carbon fixation is not altered by either photoinhibitory or limiting levels of illumination (Glover et al., 1985a). A different pattern of ^{14}C incorporation might be expected for a different species, however it appears that some additional factor other than light limitation was effecting the physiological state of the phytoplankton.

Konopka and Schnur, (1980) found that in nutrient sufficient cultures incorporation into protein increased as the irradiance decreased, however different allocation patterns were found with different nutrient limitations. If the irradiance used for the incubations was lower than previously experienced by the

phytoplankton an increase in the allocation of ^{14}C label into protein (Harding et al., 1985) resulted. This was probably a consequence of the faster growth rate of the phytoplankton.

The incubation irradiance was approximately 160$\mu Em^{-2}s^{-1}$ therefore it seems likely that the high value of percentage incorporation into protein was also an effect of decreased irradiance. Morris and Skea, (1978) noted however that phytoplankton from nutrient depleted water responded less dramatically to reduced irradiances, therefore it is inferred that the major influence on the ^{14}C allocation pattern in this study was the low available nutrients.

If most of the polysaccharide fraction was assumed to be RNA, then the major storage product was lipid. Li and Platt, (1982) found that the percentage ^{14}C incorporate into lipids was about 18% of total ^{14}C fixed, which is very similar to the average percentage fixed in this study. McConville et al., (1985), noted that the carbon allocation required for the essential or structural forms of lipid, such as membrane phospholipid would be expected to display relatively conservative allocation patterns, therefore additional synthesis of lipid is for storage products.

The high calorific value of lipid suggests storage of high energy products available for survival during adverse conditions for the cell. High lipid content is a temporal difference associated with seasons, bloom conditions or different phases of growth of the cells. So as phytoplankton proceed from logarithmic to stationary phase, cellular composition is often altered by an accumulation of reserve materials such as lipids of carbohydrates.

Station 6 was the exception to the general representation produced from the mean values of the fractions at each time interval.

Percentage incorporation of ^{14}C into lipid was highest at 36.8% compared to 30.4% in the protein fraction after twenty-four hours. There are other published examples of high amounts of label in the lipid fraction. Smith and Morris (1980), found up to 80% of the label in the lipid fraction of phytoplankton from the Southern Ocean under conditions of low temperature -0.2°C to -1.8°C, and low irradiance. Low-light adapted algae incorporated significantly less ^{14}C into proteins and more into low molecular weight metabolites and lipids than the same species isolated from a high light environment (Rivkin and Voytek, 1987). Harding et al., (1985) working with cultures of a chrysophyte, dinoflagellate and diatom demonstrated a decrease in label into protein and increased assimilation into lipid following movement into low light. However this increase did not exceed the percentage of label in the protein fraction as in this study.

A reduced allocation of ^{14}C label into protein and increased allocation into storage products such as carbohydrates and lipids, might imply a persistent nutrient deficiency, however as only the phytoplankton <1.0μm at this station was effected this was probably not the case.

Increased incorporation into storage products is also found in stationary phase or scenescent cells and a high proportion of scenescent cells in the water column implies that the cells were not being grazed effectively. The number and type of organisms present in the water column were not examined, but there was no apparent reason for a lack of *Synechococcus* grazers.

It was concluded that there was a less than optimal nutrient availability for both phytoplankton fractions, but that the available resources were utilised to maintain the growth rate. The marginal

differences between the allocation into polysaccharides and nucleic acids, and lipids may reflect a more severe nutrient limitation in the >1μm fraction, where increased allocation of label into polysaccharides and nucleic acids could indicate more storage compounds and less RNA. The difference in the allocation to reserve products may also reflect a species difference.

5.2.3 Photosynthetic parameters of natural assemblages

The importance of environmental factors in regulating the photosynthetic rate of natural phytoplankton assemblages can be assessed through their effect on the parameters describing the light-saturation curve (Jassby and Platt, 1976; Platt and Jassby, 1976; Platt et al., 1980; Côté and Platt, 1983).

Adaptation to low light is one of the best known physiological responses that produces changes in photosynthesis-irradiance curves (Harding et al., 1987). It can be expressed as an improvement of the cell's efficiency of photosynthesis in low light and is measured as increased α expressed on a per cell basis or decreased I_k (Falkowski, 1980; Yentsch, 1980; Prézelin, 1981).

The physiological processes of phytoplankton are adapted to a fluctuating environment. Phytoplankton grow in a dynamic photic regime where changes in the light environment bought about by vertical mixing, changes in sunlight intensity and water clarity can alter the physiological characteristics of the phytoplankton. If physiological adjustment by the phytoplankton is faster than changes in environmental conditions, the cells continuously adjust their metabolic activities to the new conditions (Vincent, 1980); if not

the cells can only adjust to mean environmental conditions (Savidge, 1979; Falkowski, 1980). Since the mean light intensity is determined by the degree of vertical mixing in the mixed surface layer, increased vertical mixing will result in a lower P_m^B (Demers and Legendre, 1982).

Taguchi (1976) showed that α^B and P_m^B were inversely related to cell size, therefore variations in α^B and P_m^B of phytoplankton populations could reflect the size distribution of cells (Platt and Jassby, 1976). The size-dependency of the photosynthetic parameters is however modified by environmental factors as has been demonstrated for temperature (Eppley, 1972), light intensity (Yentsch and Lee, 1966; Beardall and Morris, 1976) and nutrient limitation (McAllister et al., 1964; Thomas and Dodson, 1972).

Rates of photosynthesis, cell division and the mechanisms and time courses of photoadaptation are all species specific (Harris, 1978; Rivkin and Voytek, 1987). Both laboratory and field reports have shown that cyanobacteria are photosynthetically efficient at low irradiances (Morris and Glover, 1981). Platt et al., (1983) suggested that the magnitude of parameters such as the assimilation number (P_m^B), the initial slope (α^B) and the adaptation parameter (I_k) indicated that phytoplankton <1μm, from the sub-surface chlorophyll maximum of the sub-tropical Atlantic were more adapted to low light than larger phytoplankton from the same assemblage since both P_m^B and α^B were higher and I_k lower for the cyanobacterial fraction. However, in the surface mixed layer of a shelf sea, Joint and Pomroy, (1986) found little evidence to support the hypothesis that picoplankton assemblages from the surface mixed layer were significantly more adapted to low irradiances than the larger phytoplankton.

Other authors have shown that neritic and oceanic populations of photoautotrophic picoplankton in temperate and subtropical waters photosynthesise rapidly at near surface irradiance levels (Putt and Prézelin, 1985; Iturriaga and Mitchell, 1986; Glover et al., 1986a; Prézelin et al., 1986; Gieskes and Kraay, 1986; Kana and Glibert, 1987a; Furnas and Mitchell, 1988).

The data obtained in this study in the North Sea show that values of both P_m^B and α^B were significantly greater for the phytoplankton <1.0μm than for the larger phytoplankton assemblages. Mean values of P_m^B were 6.01 mgC (mg chl a)$^{-1}h^{-1}$ for the cyanobacterial fraction and 2.89 for the >1μm fraction, based on 20 determinations. Similarly, the mean value of α^B was 0.20 [mgC (mg chl a)$^{-1}h^{-1}$][μEm$^{-2}s^{-1}$]$^{-1}$ for the <1.0μm fractions and 0.046 [mgC (mg chl a)$^{-1}h^{-1}$][μEm$^{-2}s^{-1}$]$^{-1}$ for the >1μm phytoplankton fraction. However there is uncertainty about some of the values of α^B since they are greater than the upper limit for alpha of 0.115 [mgC (mg chl a)$^{-1}h^{-1}$][μEm$^{-2}s^{-1}$]$^{-1}$, calculated by Platt and Jassby, (1976).

This upper limit was based on a maximum quantum yield of 0.12 mol C E^{-1}. Alpha values in the >1.0μm fraction are much as expected and compare favourably with those found elsewhere (Platt et al., 1983). This suggests that the incubation was not the problem, but that the extraction of chlorophyll a was incomplete in the cyanobacterial fraction. For the >1.0μm phytoplankton the acetone extraction was apparently sufficient, however if the extraction was incomplete in the <1.0μm fraction, a low concentration of chlorophyll a (μg l^{-1}) would be estimated, and the corresponding value of alpha would be high as observed in this study. Stauffer et al., (1979)

reported that acetone (90%) extraction of several limnatic cyanobacteria *Microcystis*, *Oscillatoria*, *Aphanizamenon* and *Anabaena*, yielded erratic recoveries of chlorophyll *a*: they recommended methanol (100%) or acetone-dimethyl sulfoxide for chlorophyll extraction from cyanobacteria.

A maximum assimilation number of about 25 mgC (mg chla)$^{-1}$h^{-1} was calculated by Falkowski, (1981). This value was estimated from photosynthetic turnover times and the number of photosynthetic units.

Assimilation numbers reported for temperate phytoplankton typically fall between 2 and 10 mgC (mg chl *a*)$^{-1}$ h^{-1} (Falkowski, 1981). This was generally found to be true in this study (Table 5.7). Only one exceptionally high assimilation number was obtained, 11 mgC (mg chl *a*)$^{-1}$ h^{-1} at station 11 near Flamborough Head on the UK coast. Chlorophyll *a* concentration was not low compared to other chlorophyll *a* concentrations in the <1.0μm fraction, therefore it is suggested that the hydrographic regime associated with the Flamborough Front had created conditions, perhaps increased nitrate concentrations which increased the assimilation number.

Photoinhibition occurred at only 7 stations in the <1.0μm fraction and once in the larger phytoplankton. In no case was there any indication of strong photoinhibition such as that observed by Platt et al., (1983) in natural assemblages and in laboratory cultures (Barlow and Alberte, 1985). Kana and Glibert, (1987a) have achieved growth of a *Synechococcus* species in the laboratory up to 2000μEm^{-2}s^{-1} without the cultures showing signs of photoinhibition. Near-surface picoplankton in the Coral Sea were only inhibited at irradiances greater than 2200μEm^{-2}s^{-1} (Furnas and Mitchell, 1988), whereas deep-living picoplankters became photoinhibited at 200 to

$400\mu Em^{-2}s^{-1}$.

The maximum irradiance experienced by samples in the incubator in this study was approximately $1200\mu Em^{-2}s^{-1}$. Since photoinhibition was found on only a few occasions it was inferred that the <1.0μm phytoplankton assemblages, sampled from the surface mixed layer of the North Sea were generally not shade-adapted.

The occurrence of photoinhibition was patchy and may have been a result of increased mixing of the water column bringing shade-adapted phytoplankton <1.0μm into the surface mixed layer. Alternatively the observed photoinhibition may be caused by a different species of *Synechococcus* being more predominant in the sample. As reported by Glibert et al., (1986) different clones of *Synechococcus* react differently to environmental conditions.

In addition to previous light history the probable effect of nutrient depletion on the photosynthetic apparatus must be considered. It has been known for many years that nitrogen limitation results in the partial loss of chlorophyll from all plants, including marine phytoplankton (Yentsch and Vaccaro, 1958), in addition to the loss of pigment there is a decrease in the size and number of photosynthetic units (Perry et al., 1981). The photosynthetic parameters P_m and α are normalised to chlorophyll *a* concentration (P_m^B and α^B) and the value of these parameters will be a consequence of the nutrient status of the phytoplankton cells and will change in response to nutrient limitation as well as to light history. If the interpretation of the ^{14}C cellular fractionation experiments is correct, and if these picophytoplankton were nutrient limited changes in P_m^B and α^B cannot prove unequivocally that the cells were adapted to grow at low irradiance.

McAllister et al., (1964) and Curl and Small, (1965) suggested that for the net and nanoplankton assimilation ratios below 3 mgC (mg chl a)$^{-1}h^{-1}$ were indicative of a nutrient deficiency, while values above 5 indicated an adequate nutrient supply. Using this definition the assimilation numbers for the >1μm fraction in the North Sea inferred nutrient deficiency, whereas the <1.0μm fraction was generally nutrient sufficient. However as mentioned previously, chlorophyll extraction in the <1μm fraction was probably incomplete, and as a consequence assimilation numbers in this fraction were artificially high. A reduction in P_m^B in this fraction would imply that some nutrient limitation occurred.

Frequency distribution diagrams (Figure 5.11 (a), (b) and (c)) show that considerably more variation was found in the values of the photosynthetic parameters α^B and P_m^B in the <1.0μm phytoplankton fraction compared with the larger phytoplankton. The high α^B values of 0.630 and 0.458 were not included in the frequency distribution diagrams as the remaining data was sufficient to show the wide scatter in the α^B values. The wide variation is most probably a result of the incomplete extraction of chlorophyll *a* from the cyanobacterial cells, which makes estimates of α^B and P_m^B artificially high.

Alpha values for the phytoplankton >1μm were generally clustered together, whereas the α^B estimates for the phytoplankton <1.0μm were not found in any groups, but scattered up to high values. The frequency distribution diagram of P_m^B shows that in the >1μm phytoplankton a low and closely clustered set of values was estimated. Assimilation numbers, in the phytoplankton <1.0μm fraction, appear to have two or possibly three ranges of values.

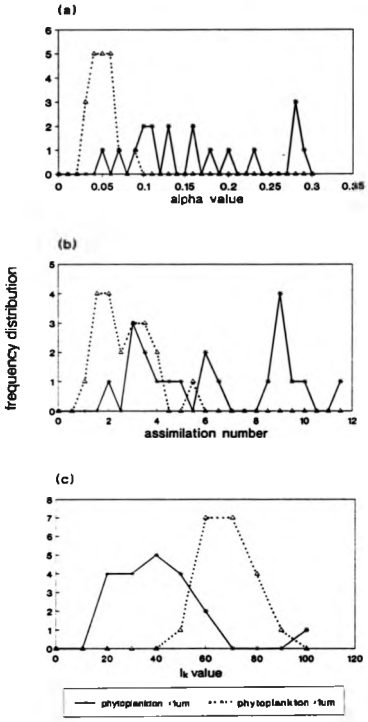

Fig. 5.11. Relative frequency distributions of (a) initial slope (α^B), (b) assimilation number (P^B_m) and (c) I_k value, in the phytoplankton < 1μm and phytoplankton > 1μm.

These may correspond to different *Synechococcus* clones being dominant in the water column. By studying assimilation numbers for the phytoplankton <1.0μm in Table 5.7 in conjunction with the cruise track (Figure 5.1), it would appear that the higher P_m^B corresponded to stations in the mid northern North Sea. The high value at station 11, of 11 mgC (mg chl a)$^{-1}$ h^{-1} may have been a result of increased upwelling of nutrients at the frontal boundary.

In contrast to the widely ranging values of α^B and P_m^B, I_k values for the phytoplankton <1.0μm were clustered together with a mean of 36.57 [μEm^{-2}s^{-1}]. There was one outlier, at station 11 which was high due to the high assimilation number. I_k values in the >1μm fraction were also grouped together, but at higher irradiances with a mean of 62.24 [μEm^{-2}s^{-1}]. I_k values are often used to indicate shade-adaptation in phytoplankton assemblages (Falkowski, 1980), however in this study it appears more likely that the low I_k number was a result of nutrient limitation of the phytoplankton.

Low assimilation numbers and constant standing crop size, suggest that an algal-bloom may have already reached stationary phase (Palmisano et al., 1985) and cells that are in stationary phase may be less physiologically responsive to changes in irradiance. The photosynthetic parameters estimated for the >1.0μm phytoplankton were low through out the study and may have been approaching stationary phase, hence differences in P_m^B and α^B cannot prove unequivocally that the cells were adapted to grow at low irradiance.

CHAPTER SIX

NORTH SEA CRUISE 1988

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NORTH SEA CRUISE 1988

6.1 RESULTS

6.1.1 Study Area and sampling programme.

The study was conducted in the North Sea onboard the RRS Challenger from 3rd to 22nd April 1988. At this time the whole of the study area was vertically well mixed, as stratification in the area north of the Flamborough Front (Pingree et al., 1978) had not yet occurred.

Figure 6.1 shows the cruise track with the positions of the sampling stations indicated. Station 1 was sampled off the South coast of the UK, near to Plymouth. This was the only station not in the North Sea but was included in the results as it was still in shelf waters. Severe weather on 4th April 1988 and busy traffic lanes in the Channel on 5th April 1988 limited the sampling to surface waters; therefore, only chlorophyll *a* measurements and counts of *Synechococcus* cells were carried out on these days. Sampling was confined to the surface between 9th and 10th April 1988 (stations 3 to 4) due to severe weather in the vicinity of the Orkney Isles. The period 15th to 19th April 1988 was spent in the Firth of Forth recovering lost equipment.

Cell counts were discontinued from 22nd April 1988, at 1745 GMT (station 13) as there was much detritus and sediment in the collected samples. Prior filtration of the samples to remove excess detritus

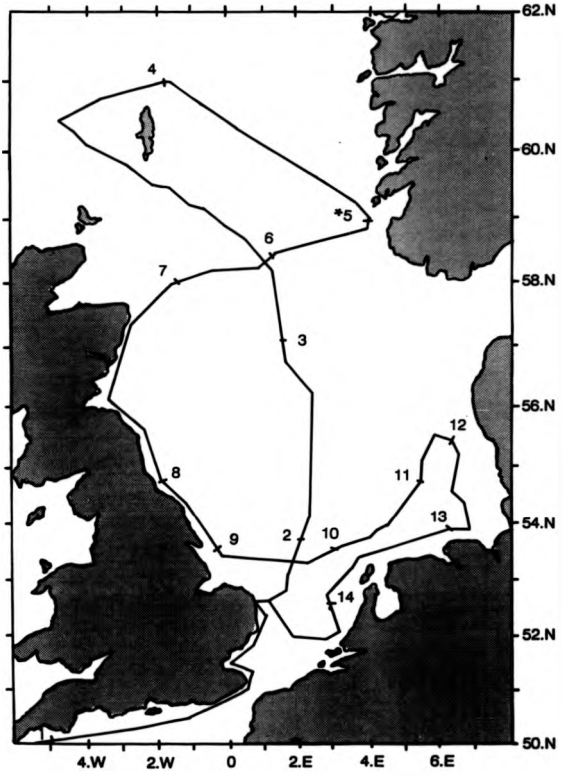

Fig. 6.1. Station positions (1 - 14) in the North Sea during April 1988. The cruise track (————) and the occurrence of photoinhibition (*) are indicated.

was attempted but this did not prove successful - the number of *Synechococcus* cells retained within the detritus on the prefilter could not be ascertained.

6.1.2 Distribution and vertical profiles in the North Sea

The numbers of cyanobacteria found in the surface water are shown in Figure 6.2. Cell numbers in the North Sea ranged from 7.1×10^5 to 9.9×10^6 cells l^{-1}, but the highest cell numbers (1.3×10^7 cells l^{-1}) were found at station 1 in Channel waters.

Water temperatures ranged from 4.6°C to 9.0°C. The numbers of *Synechococcus* found at these temperatures are comparable to those found by Murphy and Haughen (1985) in the North Atlantic during the early summer.

As in the previous cruise in July 1987, (Chapter 5) distribution was patchy, but in the approach to the Channel and to the north of Scotland, numbers of *Synechococcus* cells were similar and usually in the range $3\text{-}4 \times 10^6$ cells l^{-1}. This consistency might be related to the severe weather which was encountered in these areas. The water column was well mixed and the numbers therefore correspond to an average number for the whole of the mixed zone. No vertical profiles were sampled in these areas of the North Sea, but it is likely that they would have demonstrated similar numbers of *Synechococcus* cells throughout the water column. In addition the mixing of the water column may have removed concentrations of grazers from the immediate vicinity of the cyanobacteria, therefore allowing numbers of cyanobacteria to increase temporarily.

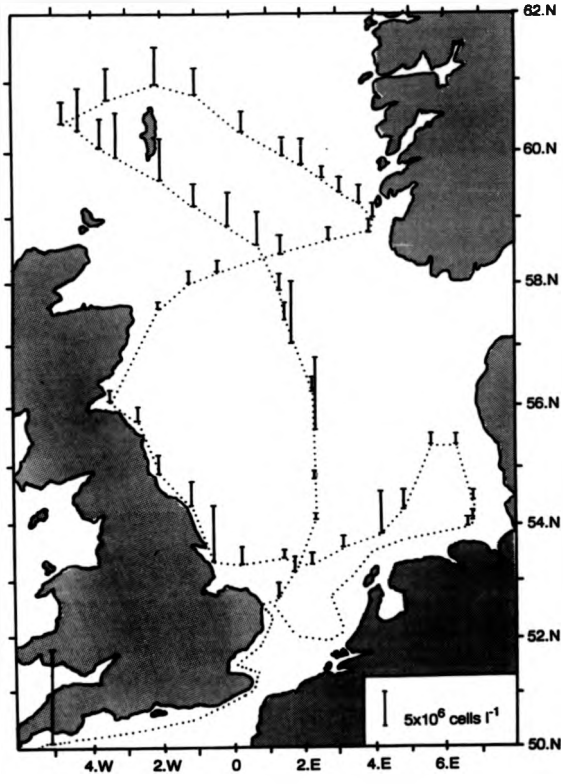

Fig. 6.2. The abundance and distribution of cyanobacteria in the North Sea during April 1988. The cruise track (·····) is indicated.

Only three vertical profiles were made during the cruise. The first was between stations 2 and 3 on 7th April 1988 at 1815 GMT and is shown in Figure 6.3 (a). Maximum numbers of cyanobacteria were found at 10m depth with 8.4×10^6 cells l^{-1}, whereas the count at the surface was 6.9×10^6 cells l^{-1}. Chlorophyll *a* concentrations in the phytoplankton >1μm showed a gradual increase with increasing depth, 1.85μg l^{-1} to 10.2μg l^{-1}, whereas in the phytoplankton <1μm, chlorophyll *a* concentrations decreased from 0.07μg l^{-1} at the surface to 0.04μg l^{-1} at 25m.

The second vertical profile (Fig. 6.3 (b)) was at station 6, the only 24 hour station to be occupied. Cyanobacterial numbers were highest at the surface 3.7×10^6 cells l^{-1}, decreased to 3.2×10^6 cells l^{-1} at 10 metres and then increased gradually to 3.6×10^6 cells l^{-1} at the bottom, at 80 metres. Chlorophyll *a* concentrations in the <1.0μm fraction also demonstrated a decrease in value from the surface at 0.08μg l^{-1} to 0.07μg l^{-1} at 10 metres. However beyond a depth of 40 metres the chlorophyll *a* in the <1μm fraction decreased fairly rapidly whereas cyanobacterial numbers did not. Chlorophyll *a* in the >1μm phytoplankton showed a peak of 0.50μg l^{-1} at a depth of 40 metres but at all other depths remained at a value of about 0.43μg l^{-1}.

Figure 6.3 (c) shows the vertical profiles obtained at 17.40 GMT on 21st April 1988 (station 9). There was little variation in cyanobacterial numbers over the depth sampled, although a slightly lower number, 3.7×10^6 cell l^{-1} were found at 10 metres compared to the surface (4.3×10^6 cells l^{-1}) or 25 metres (4.7×10^6 cells l^{-1}). Chlorophyll *a* >1μm also demonstrated a minimum value at 10 metres with 2.8μg l^{-1} compared 6.9μg l^{-1} at the surface and 5.1μg l^{-1} at

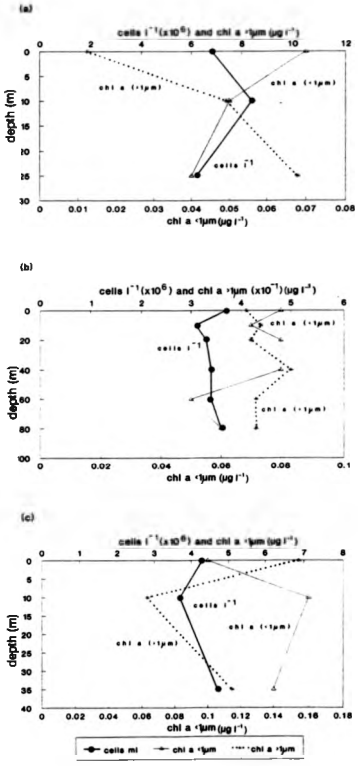

Fig. 6.3. Numbers of cyanobacteria and vertical distribution of chlorophyll *a* (> 1μm) and chlorophyll *a* (< 1μm) at (a) 18.15 GMT on 7th April 1988, (b) 08.00 GMT on 13th April 1988 and 17.40 GMT on 21st April 1988.

35 metres. The chlorophyll *a* concentrations in the phytoplankton <1.0μm however showed a rapid increase from the surface value of 0.1μg l^{-1} to 0.16μg l^{-1} at 10 metres and then remained at this higher concentration with increasing depth.

6.1.3 Photosynthesis-irradiance curves

A similar approach was taken to investigating the physiology of the picoplankton assemblages as in July 1987, and the photosynthetic parameters of samples taken from 10m depth were determined. Most samples were taken shortly after dawn between 0730 and 0930 GMT, but stations 9, 11 and 13 were sampled between 1730 and 1830 GMT, just before dusk.

Figure 6.4 shows typical examples of the fitted photosynthesis-irradiance curves for both fractions. Curves of best-fit were plotted through the points by a computer package (LSFITS) written by M. Carr (PML), using the equation of Platt et al., (1980). The projector used in the incubation box was adapted in this study in an attempt to lessen the scatter in the data points which was observed in previous studies at the lowest irradiances. Considerably more scatter of the data points between approximately 200μE$m^{-2}s^{-1}$ and the maximum irradiance of 1500μE$m^{-2}s^{-1}$ was observed than from the minimum irradiance of 5μE$m^{-2}s^{-1}$ to 200μE$m^{-2}s^{-1}$. This initially casts doubt over the accuracy of P_m^B, however standard errors of the assimilation numbers were all less than 10% and most were less than 5% of the computed value, hence the values of P_m^B calculated from the LSFITS programme were recorded.

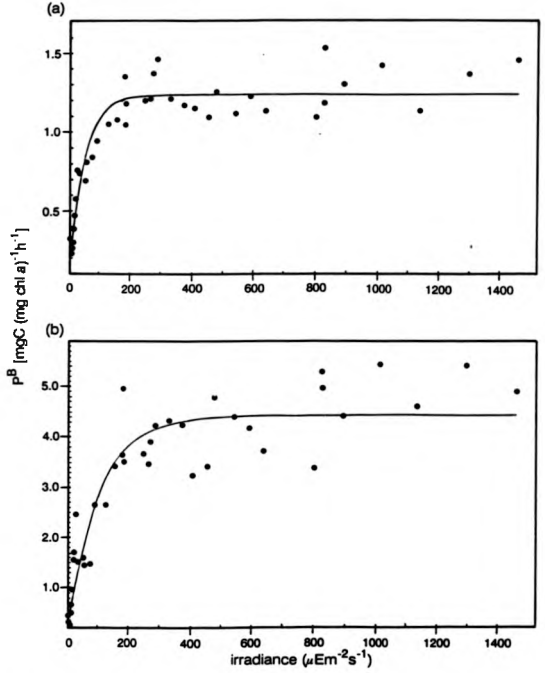

Fig. 6.4. Typical photosynthesis–irradiance curves obtained during the cruise for (a) phytoplankton <1μm and (b) phytoplankton >1μm, at station 12 on 22 April 1988.

The photosynthetic parameters describing the initial slope α^B, the plateau of the curve P_m^B and the derivative I_k (P_m^B/α^B) are shown in Table 6.1.

Alpha values at low irradiance were initially calculated from the curve fitting equation of Platt et al., (1980) as part of the LSFITS programme. However an improved α^B value was found by fitting the data to a linear regression function on 13 values of the curve. These were data points 2-14; data point one was omitted as it did not fit the linear part of the curve. The range of irradiances which these data points spanned was from 5 to 70$\mu Em^{-2}s^{-1}$. All α^B values, except one (station 12, >1.0μm fraction) calculated in this way gave F values that were significantly above the 0.1% level; even station 12 was significant at the 1.0% level. The values of α^B in Table 6.1 were calculated by this separate linear regression function on the data points.

Values of α^B in the phytoplankton >1.0μm fraction ranged from 0.006 to 0.055 with a mean of 0.023 [mgC (mg chl a)$^{-1}$ h^{-1}] [$\mu Em^{-2}s^{-1}$]$^{-1}$, this compares with a range of 0.005 to 0.096 and a mean of 0.024 [mgC (mg chl a)$^{-1}h^{-1}$][$\mu Em^{-2}s^{-1}$]$^{-1}$ for the <1.0μm fraction. Alpha values were higher in the >1μm phytoplankton except at stations 2, 7, 8 and 9, which were positioned in the central North Sea and along the East coast of the UK.

Values of P_m^B in the phytoplankton >1.0μm fraction ranged from 0.85 to 8.44 with a mean of 2.97 [mgC (mg chl a)$^{-1}h^{-1}$]. This compares with the phytoplankton <1.0μm fraction which varied from 0.66 to 11.19, with a mean of 2.80 [mgC [mg chl a)$^{-1}h^{-1}$]. P_m^B values were higher in the phytoplankton >1.0μm than the phytoplankton <1.0μm except at stations 2,7,8 and 9.

Table 6.1. Photosynthetic parameters derived from photosynthesis–irradiance curves for phytoplankton<1µm and phytoplankton >1µm: initial slope (α^B) [mgC (mg chl a)$^{-1}$h^{-1}][µEm^{-2}s^{-1}]; P_m^B [mgC (mg chl a)$^{-1}$h^{-1}]; I_k [µEm^{-2}s^{-1}].

	phytoplankton <1µm			phytoplankton >1µm		
station	α^B	P_m^B	I_k	α^B	P_m^B	I_k
1	0.045	6.15	136.40	0.055	8.44	153.47
2	0.012	1.83	152.50	0.008	0.85	106.50
3	0.023	1.74	75.52	0.026	3.25	124.96
4	0.019	1.68	88.26	0.029	3.41	117.55
5	* 0.025	* 2.76	* 110.25	* 0.046	* 6.11	* 132.91
6	0.013	1.42	108.92	0.022	3.29	149.41
7	0.020	3.30	162.05	0.009	1.23	136.33
8	0.096	11.19	116.55	0.010	1.25	124.50
9	0.019	2.14	112.47	0.006	0.73	121.17
10	0.014	2.05	146.71	0.015	2.04	136.00
11	0.019	1.17	61.63	0.021	2.80	133.19
12	0.010	1.24	123.70	0.017	4.42	259.71
13	0.015	1.76	117.60	0.029	1.34	46.28
14	0.005	0.66	131.80	0.023	2.40	104.48
mean	0.024	2.79	117.45	0.023	2.97	131.89

* occurrence of photoinhibition

These were the same stations as the exceptions for the α^B values. Both parameters at station 8 were very high compared to the α^B and P_m^B values determined at the other stations in this study. The irradiance at which light saturation first occurred is given by the value I_k (Table 6.1). I_k values ranged from 46.3 to 259.7, with a mean of 131.9$\mu Em^{-2}s^{-1}$ in the phytoplankton >1.0μm, compared to a range of 61.6 to 162.1, and a mean of 117.5$\mu Em^{-2}s^{-1}$ in the <1.0μm fraction. I_k values were generally higher in the >1.0μm fraction but there was usually only a small difference in the I_k from both fractions which is indicated by the closeness of the means.

Figure 6.5 and 6.6 express the α^B, P_m^B and I_k values in Table 6.1 and the chlorophyll *a* concentrations obtained for the two size-fractions as histograms. The α^B and P_m^B values at station 8 in the <1.0μm fraction were considerably larger than the mean value determined at any other station. Large values of photosynthetic parameters might result from an underestimation of chlorophyll *a* concentration. Examination of the chlorophyll a concentration ($\mu g\ l^{-1}$) in Fig. 6.6 (c) for the phytoplankton 1.0μm at station 8, showed that although chlorophyll a concentration was low, values for stations 7 and 9 were even lower. These stations did not show the high photosynthetic parameters of station 8 and it is unlikely that the high values obtained at station 8 resulted from an underestimation of chlorophyll a concentration.

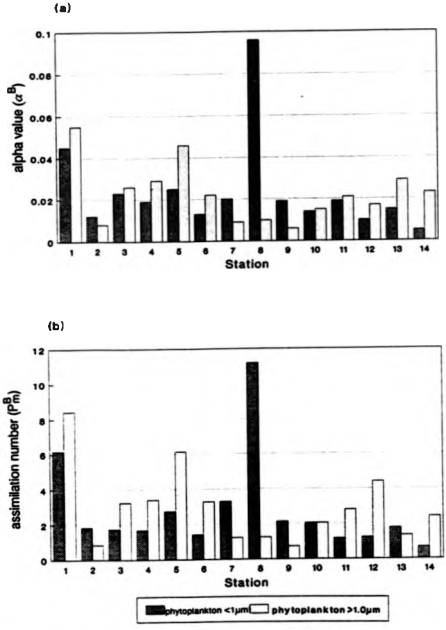

Fig. 6.5 Comparison of (a) alpha values (α^B), [mgC (mg chl *a*)$^{-1}$h^{-1}][μEm^{-2}s^{-1}]$^{-1}$ and (b) assimilation numbers (P^B_m), [mgC (mg chl *a*)$^{-1}$h^{-1}], calculated for the phytoplankton < 1μm and the phytoplankton > 1μm at 14 stations in the North Sea during April 1988.

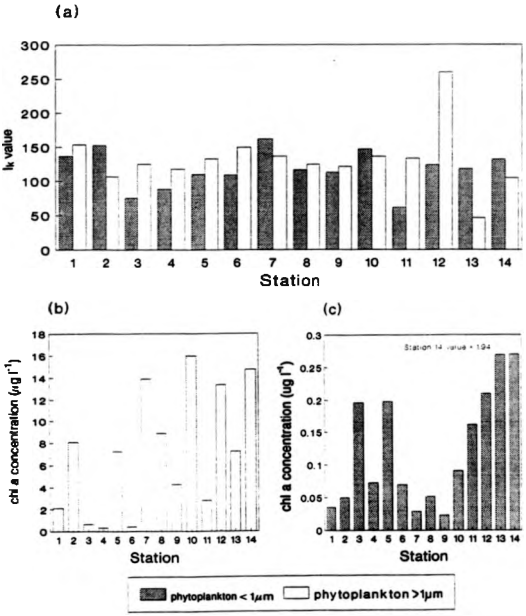

Fig. 6.6. Comparison of (a) I_k values, (b) chlorophyll *a* concentrations in the phytoplankton > 1µm and (c) chlorophyll *a* concentrations in the phytoplankton < 1µm at 14 stations in the North Sea during April 1988.

The high values of the photosynthetic parameters at station 8 influence significantly the calculated mean values. If station 8 data were omitted, values of P_m^B in the phytoplankton <1.0μm, ranged from 0.66 to 6.15 with a mean of 2.12 [mgC (mg chl a)$^{-1}$h^{-1}], and α^B values ranged between 0.005 and 0.045 with a mean of 0.018 [mgC (mg chl a)$^{-1}$ h^{-1}][μEm^{-2}s^{-1}]$^{-1}$.

Figure 6.7 shows the frequency distribution of each of the parameters in both fractions. The adjustment of the mean value appears to be justified when these diagrams are considered as both of the station 8 parameters appear as outliers. As both parameter values are high the derivative I_k at station 8 does not appear to be significantly different from the majority of the other calculated I_k values. In addition the main cluster of I_k values in the phytoplankton <1.0μm is lower than that of the phytoplankton >1μm.

There was only one occurrence of photoinhibition. This was at station 5 (* in Table 6.1 and Figure 6.1), from a water sample taken off the coast of Norway. Figure 6.8 shows the photoinhibited curves. Both an inhibited and a non-inhibited curve could be fitted to the data points using the equation of Platt et al., (1980); however the standard errors of α^B and P_m^B were less for the photoinhibited curve, and therefore the parameters quoted (Table 6.1) were from this inhibited curve.

The problem of sediment in the sample was also encountered at station 9 on 20th April 1988. A sample from 10 metres was collected and a ^{14}C incubation to determine photosynthetic parameters was carried out. Only 20 bottles were used as the light reaching the final bottle in the high light side of the incubator was less than 1μEm^{-2}s^{-1}.

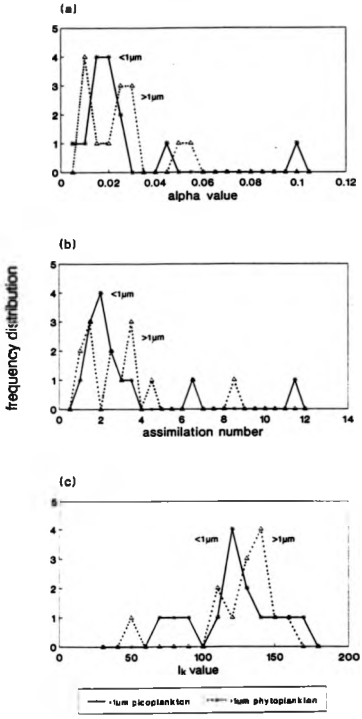

Fig. 6.7. Relative frequency distributions of (a) initial slope (α^B), (b) assimilation number (P^B_m), and (c) I_k value, in the phytoplankton < 1μm and phytoplankton > 1μm.

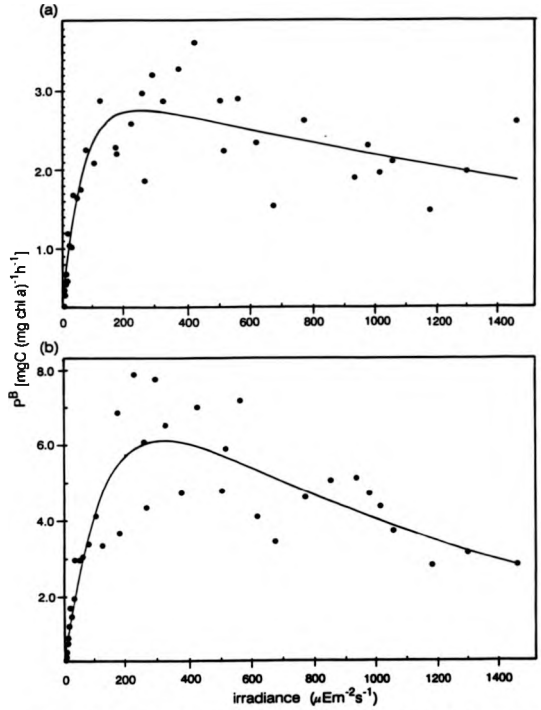

Fig. 6.8. Photosynthesis-irradiance curves obtained during the cruise showing photoinhibition of photosynthesis at high irradiances at station 5 in (a) the phytoplankton $< 1\mu$m and (b) the phytoplankton $> 1\mu$m.

Although irradiances in the incubator were reduced, they were sufficient for the phytoplankton to reach the photosynthetic maximum. Despite the reduced irradiance that the natural assemblage received in the sea there was no evidence of inhibition by the light gradient in the incubator.

6.2 DISCUSSION

6.2.1 Abundance and distribution of cyanobacteria in the North Sea

Seasonal variations in the abundance of phycoerythrin-rich cyanobacteria have been reported in coastal waters off Southern California (Krempin and Sullivan, 1981), in surface waters of Woods Hole Harbour (Waterbury et al., 1986), in surface samples obtained from the Menai Straits and Western Irish Sea (El Hag and Fogg, 1986) and samples collected from the Baltic Sea, Kattegat and Skagerrak (Schmaljohann, 1984). Maximum numbers of cyanobacteria were typically observed from June to August with high abundances being maintained until November (Waterbury et al., 1986). Minimum numbers of approximately 10^5 to 10^6 cells l^{-1} were found during February and March, although during these months no unicellular cyanobacteria were observed in samples collected from the Baltic Sea, Kattegat or Skagerrak (Schmaljohann, 1984).

In addition to seasonal variations there is a general decline in the abundance of cyanobacteria with northerly increasing latitude and with decreasing temperature (Murphy and Haugen, 1985; El Hag and Fogg, 1986; Joint, 1986; Waterbury et al., 1986). *Synechococcus* species are rarely found in polar waters where temperatures remain below 5°C throughout the year, although Smith et al., (1985) observed cyanobacteria in concentrations of 10^6 cells l^{-1} in Arctic waters where water temperatures were only slightly above 0°C.

The onset of the Spring bloom occurs at the beginning of April when the water temperature reaches 6°C (Waterbury et al., 1986). Water temperatures in the surface waters of the North Sea during the cruise were generally just above 6°C (personal communication Dr. N

Owens), hence concentrations of *Synechococcus* cells above 10^3 ml^{-1} might indicate the beginning of the Spring bloom in these waters. The distribution of phycoerythrin-rich *Synechococcus* species was patchy (Figure 6.2) ranging from 7.1×10^5 to 9.9×10^6 cells l^{-1}. These densities resembled those observed in the early summer at similar latitudes and temperatures in the North Atlantic (Murphy and Haugen, 1985) and suggest that a *Synechococcus* bloom may have begun. The distribution of the numbers of cyanobacteria were more variable in the southern Bight of the North Sea and in coastal regions. In this study only the phycoerythrin-rich *Synechococcus* species were counted as phycocyanin-rich species were not recognisable using the chosen microscope set-up. Strains with phycocyanin predominating seem to be only a minor component in both coastal (Campbell, 1983) and oceanic waters (Murphy and Haugen, 1985) however Glover (1985) reported that phycocyanin-rich *Synechococcus* are only abundant in coastal waters, particularly in estuaries.

The variation in the numbers of *Synechococcus* species, particularly in coastal regions may have been a result of a different species distribution, with phycocyanin-rich *Synechococcus* species being a major component of the *Synechococcus* population and yet remaining unrecognised.

Cell numbers were highest at Station 1 with 1.3×10^7 cells l^{-1}. This station was located in Channel water off the Devon coast and may well have been influenced by increased water temperatures.

In well mixed water, cell numbers are relatively constant with depth within the euphotic zone (Murphy and Haugen, 1985), whereas in stratified waters they reach a maximum on the thermocline (Fogg, 1987). During the cruise no stratification of the water column was

observed and although only a few depths were sampled for the determination of vertical profiles, it would seem that the numbers of *Synechococcus* species did not vary greatly with depth.

6.2.2 Photosynthetic parameters of natural assemblages

The variation during April 1988, in the parameters describing the photosynthetic-irradiance curves of the phytoplankton <1.0μm and >1μm, from the North Sea were examined. Changes in the P-I curve parameters are generally associated with changes in the physiological state of the phytoplankton (Harding et al., 1987). However environmental factors also modify the P-I response (Platt et al., 1980: Côté and Platt, 1983).

Physiological adaptation occurs when the time required for adaptation is significantly shorter than the residence time (Falkowski, 1983). The natural assemblages sampled in this study were from well-mixed waters and the phytoplankton were unlikely to have sufficient time in which to adapt to the rapidly changing environmental conditions; thus the photosynthetic parameters were probably an averaged response to the fluctuating conditions.

In addition seasonal differences in the values of the photosynthetic parameters may occur. Variations in the initial slope α^B and P^B_m of up to a 5-fold difference have been reported for phytoplankton off the coast of Nova Scotia (Platt and Jassby, 1976). This varied with depth and at a given depth.

The data obtained in this study in the North Sea show that values of α^B and P^B_m were greater for the >1μm phytoplankton than for

the phytoplankton <1.0μm. Mean values of α^B were 0.018 [mgC (mg chl a)$^{-1}$h^{-1}][μEm^{-2}s^{-1}]$^{-1}$ based on 13 determinations and 0.023 [mgC (mg chl a)$^{-1}$ h^{-1}] [μEm^{-2}s^{-1}]$^{-1}$ for the >1μm phytoplankton based on 14 determinations. Similarly the mean value of P_m^B was 2.15 mgC (mg chl a)$^{-1}$h^{-1} for the <1.0μm fraction based on thirteen determinations and 2.97 mgC (mg chl a)$^{-1}$h^{-1} for the >1μm phytoplankton based on fourteen determinations.

In general the photosynthetic parameters α^B and P_m^B were similar to each other and low in both size fractions. High assimilation numbers are associated with cells of low volume and fast growth rates (Eppley, 1972). Platt et al., (1983) demonstrated that picoplankton from the tropical North Atlantic had higher P_m^B than the other phytoplankton and Glover et al., (1985a) reported higher cellular rates of photosynthesis in the picoplankton fraction. There was no great difference between the means for the photosynthetic maximum, despite occasional high values which occurred in both fractions, and this was shown more clearly in the frequency distribution diagram Fig.6.6 (b). The data here shows that cell size and volume had little influence on the photosynthetic parameters, suggesting that the physiological state of the cells remained the same. The low P_m^B values also infer a slow growth rate for cells from both size fractions.

The mean values of the initial slopes (α^B) in each fraction demonstrated only a small difference, with the phytoplankton >1.0μm obtaining a slightly higher value. The frequency distribution diagram Fig.6.8 (a) showed the positive skew of the phytoplankton >1.0μm mean and some scatter of α^B values in both fractions. Less variability in α^B values compared to P_m^B values might indicate that the major

variable influencing these parameters was temperature (Malone and Neale, 1981). No significant oscillations in light-limited rate of photosynthesis (α^B) or the assimilation number (P_m^B) were detected, implying again that the phytoplankton <1.0μm observed in this study were an over-Wintering population with low growth rates: ie, the mean temperature was insufficient to promote rapid growth of the *Synechococcus* species.

The values for the initial slope α^B in both assemblages were below the theoretical upper limit of 0.115 [mgC (mg chl a)$^{-1}$h^{-1}] [μEm^{-2}s^{-1}]$^{-1}$ and the P_m^B values were always lower than the theoretical limit of 25 mgC (mg chl a)$^{-1}$h^{-1}. The relatively low values reported here for both size-fractions which were probably bought about by a low water temperature, suggest that the seasonal variation is one of reduced photosynthetic parameters. These values compare well with values obtained off the coast off Nova Scotia at the same time of year (Platt and Jassby, 1976). The turbulence of the water and consequent low mean irradiance in the surface mixed layer in the Winter months, in addition to the effect of low water temperature probably contributes to the lack of photoinhibition observed.

At station 8, situated off the North East coast of the UK, α^B and P_m^B values in the <1.0μm fraction were much higher than the respective calculated means for the whole study, however the I_k value was similar to other I_k values obtained during the study. No anomalies such as a reduced chlorophyll *a* extraction were apparent and the photosynthetic parameters calculated for the larger size-fraction were within the estimated standard errors (2SE). During the previous study in July 1987, an unusually high P_m^B value was reported at a station off the East coast of the UK. Although the stations were

some distance apart and during April 1988 no frontal system was detected, the high photosynthetic parameters may be caused by the same hydrographic effect. This may have been increased nutrient concentrations which are often found at the frontal interface (Fogg et al., 1985; Pingree et al., 1978) or perhaps an increased ability to absorb nutrients by the phytoplankton <1.0μm compared to the phytoplankton >1.0μm.

Higher values of α^B and P_m^B were found in the <1.0μm fraction at four stations, 2, 7, 8 and 9. These stations did not appear to have any common hydrographic features. Station 2 was situated in the mid-southern North Sea, station 7 in the mid-northern North Sea and stations 8 and 9 were located down the East coast of the UK. As noted previously the values of the photosynthetic parameters at station 8 were omitted during calculation of means due to their abnormally high values. It was noticed (Table 6.1 and Fig 6.5 (a) and (b)) that, where a high value for α^B and P_m^B was obtained for the phytoplankton <1.0μm, a below average value was obtained for the phytoplankton >1.0μm. The increased rate of photosynthesis inferred from the higher photosynthetic parameters may have occurred because conditions had become beneficial for the phytoplankton <1.0μm and the size difference may have enabled them to utilise the available nutrients more efficiently (Taguchi, 1976). The numbers of phycoerythrin-rich cyanobacteria at these four stations were not particularly high, ranging from $2x10^6$ to $5x10^6$ cells l^{-1} so, although a higher photosynthetic rate was obtained, no increase in growth rate was implied.

Leakage of sample onto the lower filter of the size-fractionation apparatus (Fig 2.3) may also give these sort of results

ie: low values for the larger fraction and high values for the smaller size-fraction.

Values of the derived parameter I_k in the phytoplankton <1.0μm and phytoplankton >1.0μm assemblages showed little variation within each size fraction over the area studied. In addition the mean values obtained, 117.52μEm^{-2}s^{-1} for the phytoplankton <1.0μm and 131.89μEm^{-2}s^{-1} for the phytoplankton >1.0μm, did not vary substantially from each other although the value for the phytoplankton >1.0μm was slightly higher. Adaptation to low light can be observed as an improvement of the cells efficiency of photosynthesis in low light or a decreased I_k value (Harding et al., 1987). No photoadaptation was inferred from the data in this study as the I_k and α^B values obtained from each size-fraction were typically very close in value. Taguchi (1976) suggested that smaller cells might demonstrate higher photosynthetic parameters compared to larger cells and this was supported by Platt et al., (1983) who reported picoplankton with higher photosynthetic rates than other phytoplankton within the assemblage. As the photosynthetic parameters obtained in this study for the phytoplankton <1.0μm were lower than for the larger size-fraction it was suggested that the mean irradiance for the turbulent surface-mixed layer was insufficient to evoke photoadaptation. The effect of seasonal variation overrides the effect of small size and any photoadaptation which may occur in *Synechococcus* species.

One unusually low value in the phytoplankton >1.0μm fraction occurred at station 13 which was the result of a low P^B_m. This may have been a result of reduced irradiance in these waters (southern North Sea, Rhine Estuary), where it was noted that filtering was

abandoned due to the increased volume of detritus present in the water samples.

The seasonal effect is one of reduced photosynthetic activity, as demonstrated by the generally low α^B and P^B_m values obtained and the I_k values which were similar for both size-fractions. Rather than an indication of adaptation to low irradiances the seasonal effect overrides the possibility of any light adaptation being demonstrated from the photosynthesis-irradiance curves.

The phytoplankton assemblage studied on this cruise appeared to consist of a small number of cells maintained at the minimum growth rate for survival, whilst conditions were unfavourable ie: low temperature. However at some stations, signs of improved conditions were indicated (increased P^B_m and α^B), and the small population can be thought of as seeding the Spring Bloom.

GENERAL DISCUSSION

Much of the research was laboratory based and although some of the techniques were directly applicable to field investigation, others provided information to compliment the field data, enabling a better understanding of what may occur in the natural environment.

In this study all laboratory experiments were carried out with batch or continuous cultures, grown under a continuous light regime. Natural assemblages experience a light/dark cycle, thus although the laboratory data gave a representation of what may be occurring in the natural environment the cells may be under some stress and therefore cannot be presumed to be growing under optimum conditions.

Respiratory rates of *Synechococcus* WH7803 obtained in the laboratory were relatively low compared with marine heterotrophs, at 15µl O_2 h^{-1} (mg dry weight)$^{-1}$, but were in the same range as reported by other authors for freshwater cyanobacteria.

Three inhibitors were investigated for their ability to eliminate heterotrophic activity from natural assemblages and thus enable the respiratory activity of the cyanobacteria to be determined. Laboratory investigations on axenic cultures of *Synechococcus* WH7803 and a marine heterotroph *Alteromonas haloplanktis* proved unconvincing, as inhibition of the marine heterotroph was incomplete and did not occur rapidly. As both rapid and total inhibition of heterotrophs were important requirements for this approach to be viable, no further fieldwork was carried out.

The cellular composition of *Synechococcus* cells in batch culture, and in the <1µm phytoplankton fraction obtained from the North Sea and coastal waters near to Plymouth, Devon was

investigated. In the laboratory cultures, growing exponentially and at low irradiance, most ^{14}C was incorporated into proteins. As irradiance increased the proportion of label found in the polysaccharides and nucleic acids fraction increased. Cuhel and Waterbury, (1984) reported that there was little evidence for polymeric carbohydrate in the hot-acid soluble fraction (polysaccharides and nucleic acids) of rapidly growing cultures of *Synechococcus* species and that most of the label was incorporated into RNA. Although no such test was carried out in this study, if it were true, then the label incorporated into the polysaccharides and nucleic acids of the exponentially growing cultures was probably RNA. High incorporation of ^{14}C label into the polysaccharide and nucleic acid fraction and a corresponding low incorporation of label into the protein fraction, in cells that were not growing exponentially, was probably due to carbohydrate storage products; therefore this pattern of ^{14}C incorporation is an indication of nutrient limitation. Low assimilation of ^{14}C (@ 10%) into lipid and the low molecular weight metabolites was found in the laboratory cultures.

Natural assemblages of *Synechococcus* species typically incorporated most label into proteins, least into low molecular weight metabolites and almost equal amounts into lipids and polysaccharides and nucleic acids (@20%). The high incorporation into lipids in the natural assemblages was interpreted as the normal situation for the phytoplankton <1μm and the low value obtained for the laboratory cultures was a result of growing *Synechococcus* in continuous irradiance conditions. The samples obtained from natural assemblages were from the surface-mixed layer and as such were likely to experience much higher irradiance than the highest irradiance

tested in the laboratory. A high incorporation of label into polysaccharide and nucleic acids, however was not seen. If compared with the laboratory investigation this could imply that the cells were growing exponentially, but not at a high irradiance, as the percentage label incorporated into the polysaccharides and nucleic acids was relatively low; however a high rate of incorporation into protein can result from a less than optimal nutrient availability (Morris and Skea, 1978). Nitrate concentrations in the North Sea during the 1987 cruise were low and therefore the incorporation pattern may reflect nutrient deficiency to some degree, whereby available nitrogen is utilised to maintain the growth rate at the maximum possible under the prevailing conditions.

Photosynthetic parameters were measured on both research cruises on the North Sea and for cells of *Synechococcus* WH7803 grown in a continuous culture system (turbidostat) in the laboratory. In the laboratory, growth rates were increased by increasing the incident irradiance upon the culture. The photosynthetic maximum measured from these cultures demonstrated a gradual increase with increasing growth rate, whereas the initial slope showed no apparent change or perhaps a slight decrease with increasing irradiance. Pigment concentrations in these cultures indicated an increase in the number and size of the photosynthetic unit with decreasing irradiance, and a corresponding increase in the efficiency might therefore be expected. Wyman et al., (1985) reported uncoupling of the phycoerythrin to the photosynthetic apparatus, with a corresponding increase in the autofluorescence of the cells at low irradiances. This mechanism could explain the apparent lack of change in the initial slope with increasing irradiance.

Photosynthetic parameters were measured in two size fractions of phytoplankton (<1μm and >1μm) in July 1987 and April 1988. The photosynthetic maximum (P^B_m) and the initial slope (α^B) in the phytoplankton <1.0μm appeared to exhibit a seasonal pattern similar to the effect of increased irradiance on cultures grown in the laboratory; i.e. with the longer days and increased intensity of sunlight during the summer (July 1987) values increased by comparison with those determined during the early spring (April 1988). However, the prevailing conditions in the early spring were unfavourable for the growth of *Synechococcus* species, in particular the temperature was apparently too low to sustain high growth rates. This was shown by the reduced photosynthetic activity and reduced number of cells present. Hence a seasonal effect was demonstrated rather than the effect of different irradiances on a natural assemblage of phytoplankton <1μm.

CONCLUSIONS

CONCLUSIONS

Until recently the cyanobacteria present in the ocean were thought to make very little contribution to open ocean productivity. However in 1979 the widespread occurrence of very small (0.5-1.0μm) cyanobacteria assigned to the genus *Synechococcus* was reported. These have been subsequently been shown to contribute significantly to marine productivity.

This investigation was carried out in order to understand the contribution of *Synechococcus* species to the carbon flow in marine waters and to look at some of the physiological characteristics of the *Synechococcus* clone WH7803.

From this study it was concluded that:-

1. Growth of *Synechococcus* WH7803, in batch culture saturated between 55 and 59$\mu Em^{-2}s^{-1}$.

2. The respiratory rate of batch cultures of *Synechococcus* WH7803 was approximately 15μl O_2 h^{-1} (mg dry weight)$^{-1}$. This relatively low value compares quite well with values obtained for other cyanobacteria, in the literature and this study. However the available values were for freshwater cyanobacteria and typically the values reported were slightly lower than for the marine *Synechococcus* species. It was suggested that the slightly higher respiratory rate of *Synechococcus* WH7803 compared to the other freshwater cyanobacteria may be due to a necessity for a sodium extrusion system, which would require increased level of adenosine tri-phosphate (ATP) or due to synthesis of an osmoregulatory compound.

3. The heterotrophic potential of *Synechococcus* WH7803 was investigated using a variety of different substrates. No stimulation of the respiratory rate, in the dark was observed, and although *Synechococcus* WH7803 has been shown to assimilate some compounds by other authors, it was concluded from this study that this organism was not a heterotroph.

4. Changes in pigment concentrations and the corresponding changes in the photosynthetic parameters of cultures of *Synechococcus* WH7803, grown in turbidostats at different specific growth rates, suggested mechanisms by which the efficiency of light capture and energy transfer was maximised. The photosynthetic maximum (P_{max}) increased with increasing specific growth rate. At low specific growth rates ($<0.025h^{-1}$), obtained in this study by growing at low irradiances, increasing P_{max} was accompanied by a decrease in the number of photosynthetic units. As the specific growth rates increased above $0.025h^{-1}$, i.e. the irradiance increased, the increasing P_{max} was accompanied by a decrease in the size of the photosynthetic unit. Values of the initial slope remained relatively constant, or demonstrated only a slight decrease with increases in the specific growth rate. It was inferred that some of the absorbed light energy was lost and that this may be due to uncoupling of the phycoerythrin from the photosynthetic apparatus, as has been shown to occur by other authors.

5. The numbers of *Synechococcus* observed in the temperate waters of the North Sea demonstrated a seasonal distribution. In July 1987 (summer) numbers ranged from $2.5x10^6$ to $1.7x10^8$ cells l^{-1}, and in

April 1988 (winter/early spring) they ranged from 7.1×10^5 to 9.9×10^6 cells l^{-1}.

6. In actively growing laboratory cultures of *Synechococcus* WH7803, the percentage incorporation of ^{14}C into protein remained high at high and low irradiances, whereas incorporation into the polysaccharide and nucleic acid fraction was typically higher at high irradiances. Allocation of ^{14}C into the low molecular weight metabolites and lipids remained low and fairly constant. In old cultures more ^{14}C was incorporated into the polysaccharide and nucleic acid fraction than into protein. Cellular fractionation of natural assemblages revealed slightly different ^{14}C allocation patterns, reflecting the nutrient status of the cells. Overall, as in laboratory cultures it was found that changes in the percentage incorporation into proteins and polysaccharides and nucleic acids showed the most variation and hence an indication of nutritional status. More generally however, there was always more lipid in samples from natural assemblages. It was suggested that this was due to the culturing methods in the laboratory which did not include a light/dark cycle.

7. Higher values of the photosynthetic parameters α^B and P_m^B were obtained for the phytoplankton <1μm sampled the the surface mixed layer of the North Sea, than for the phytoplankton >1μm. This could infer shade-adaptation of the <1μm phytoplankton, however the samples were not taken from a low light environment and if the nutrient status of the cells was taken into consideration, it could not be proved that the phytoplankton <1μm were shade-adapted

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