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PICOPHYTOPLANKTON: ECOLOGICAL AND PHYSIOLOGICAL STUDIES IN CULTURE AND IN NATURAL COASTAL AND ESTUARINE WATERS

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ABSTRACT

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PICOPHYTOPLANKTON: ECOLOGICAL AND PHYSIOLOGICAL STUDIES IN CULTURE AND IN NATURAL COASTAL AND ESTUARINE WATERS

by Arantza Iriarte

The distribution and abundance of picophytoplankton and its impact in overall pelagic community chlorophyll a biomass and productivity in two temperate regions, the coastal waters of the southern and central North Sea and the estuarine waters of Southampton Water have been investigated. The study in the North Sea was conducted during researh cruises in March and June/July 1989. The study in Southampton Water involved a ten month seasonal survey during 1990 and included investigation of both temporal and spatial trends of variation.

In North Sea waters phycoerythrin (PE)-containing picocyanobacteria cell numbers ranged between 6×10^5 and 4.4×10^7 cells ml^{-1}. Vertical distribution profiles showed no evidence of a preferential accumulation deep in the euphotic zone, thus calling into question the possible genotypic definition of previous findings of picophytoplankton as shade adapted phytoplankton. In summer, the smaller than 3μm size fraction was estimated to contribute to a mean of 20% of the plankton community chlorophyll a biomass and to more than 80% of the plankton community respiration rate.

In Southampton Water two stations were routinely sampled (i.e. mid and outer estuary). The cycle of total chlorophyll a biomass in mid-estuary was characterized by both, a spring and a more intense summer peak. The timing of the spring phytoplankton bloom is suggested to be primarily controlled by the estuarine flushing rate. In the outer estuary, chlorophyll a maximum ocurred in spring and large phytoplankton are hypothesized to be phosphate limited in summer at this station. PE-containing picocyanobacteria and eukaryotic picophytoplankton cell numbers, and chlorophyll a biomass and carbon fixation rate by the <1μm size-fraction, all showed a positive correlation with temperature, and peaked during the summer at both stations. Possible factors leading to an imbalance between growth rates and grazing rates of picophytoplankton during summer are discussed. Along the longitudinal axis of the estuary, highest numbers of PE-containing picocyanobacteria were recorded at the seward end, and an exponential relationship with salinity was shown. The 1-3(5)μm and <1μm size fractions contributed to around 14 and 6% of the estimated annual rate of depth integrated plankton community primary production respectively. These results suggest the impact of the picophytoplankton in the overall phytoplankton community diminishes from offshore (>50%) to coastal and estuarine waters (<20%). It is suggested that factors limiting growth and accumulation of larger phytoplankton are primarily responsible for an increase in the relative significance of small phytoplankton.

The growth physiology of the oceanic chlorophycean picophytoplankter Pycnococcus provasolii (clone Ω48-23) was also investigated in response to varying conditions of temperature, irradiance and photoperiod. Results suggested this organism to be well suited to growth at low irradiance, and it may well prove a significant member of the phytoplankton assemblages deep in the euphotic zone of oceanic stratified waters.

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PREFACE

Anecdotic reports of the occurrence of phytoplankton in the picoplankton size fraction (i.e greater than 0.2μm, but less than 2.0μm in diameter, *sensu* Sieburth *et al.*, 1978) have been noted as far back as the turn of the century (Lohman, 1911). It is only comparatively recently, however, that marine ecologists have become aware of their abundance and widespread distribution in the sea, and that data on their quantitative impact on the pelagic ecosystem has begun emerging. Initial reports suggested these minute phytoplankters as potential major contributors to oceanic primary production (Li *et al.*, 1983; Platt *et al.*, 1983). These results have far-reaching implications in our conceptualization of the structure of the whole pelagic biota, and have stimulated worldwide research aimed to investigate the global significance of the picophytoplankton as primary producers in the marine environment, their trophic importance, as well as to examine the eco-physiological characteristics that might explain spatio-temporal variations in their distribution and abundance.

The study presented in this thesis aimed to examine the following:

A. Investigation of the abundance, spatio-temporal distribution and impact of the picophytoplankton on the overall pelagic community phytoplankton biomass and primary production in temperate coastal and estuarine waters. These are regions, particularly estuaries, that have been as yet comparatively little studied in relation to picoplankton, and require to be examined for an assesment of the global significance of the picophytoplankton in the marine environment.

B. Examination of the growth physiology of an oceanic eukaryotic picophytoplankton species (*Pycnococcus provasolii* Guillard) in response to variations in irradiance, daylength and temperature.
It has been suggested by several authors that oceanic picophytoplankton show characteristics of low light photoadaption (Li *et al.*, 1983; Platt *et al.*, 1983; Iturriaga and Mitchell, 1986;

Prézelin *et al.*, 1986). The hypothesis has been tested in laboratory-controlled experiments, virtually only with the picocyanobacterium *Synechococcus* spp (Morris and Glover, 1981; Barlow and Alberte, 1985; Kana and Glibert 1987a,b). There are now indications that *P. provasolii* Guillard can be an important component of oceanic picophytoplankton, particularly deep in the euphotic zone (Hooks *et al.*, 1988; Guillard *et al.*, 1991) and it was considered interesting to test the hypothesis of low light adaptation in this species.

The two research components presented in this thesis are not directly related to one another, mainly because the culture work was carried using an oceanic isolate, and was primarily focussed on photoadaptational aspects, whereas the field work only marginally addressed photoadaptational aspects of natural populations of picophytoplankton. This merits an explanation.

The research project originally proposed to be completed during the PhD studies of the author also included field work in which photoadaptational characteristics of natural populations of picoplankton were to be more intensely investigated. This involved examination of depth distribution profiles and photosynthetic parameters of populations of picophytoplankton and larger phytoplankton from various depths in the euphotic zone in the North Sea and in the North Atlantic. These investigations were subject to ship availability.

Unfortunately, participation in an oceanic research cruise could not be arranged during the period of the research, instead participation in two cruises in North Sea waters was made possible. However, budget limitations imposed the use of techniques to measure photosynthesis that proved to be less than optimal for photosynthesis measurements in the picoplankton size fraction (e.g. oxygen technique and ^{14}C technique in combination with cellulose nitrate filters) as discussed in the appropiate section. This resulted in a restricted potential of the data from the North Sea to infer photoadaptational characteristics of picophytoplankton.

CHAPTER ONE

STUDIES ON PICOPLANKTON PRIMARY PRODUCTION AND BIOMASS IN THE NORTH SEA AND SOUTHAMPTON WATER

In this chapter the field work component of the research undertaken is described in five sections: a brief introduction to the study, a literature review covering the main topics under consideration (i.e. ecology and physiology of picophytoplankton, phytoplankton dynamics in estuarine systems and a synopsis of the study areas), a detailed account of the methodology adopted, a section of presentation of results and a concluding discussion of the results.

1.1. INTRODUCTION

The comparatively recent discovery of the ubiquitous distribution of minute chroococcoid cyanobacteria (Johnson and Sieburth, 1979; Waterbury *et al.*, 1979) and eukaryotic algae of similar size (Johnson and Sieburth, 1982; Takahashi and Hori, 1984; Joint and Pipe, 1984; Murphy and Haugen, 1985) in the marine environment prompted speculation on their contribution to the overall dynamics of the marine microflora. It is now well established that the picophytoplankton , i.e. phytoplankters in the size range of 0.2-2μm in diameter (*sensu* Sieburth *et al.*, 1978) make a substantial contribution (>50%) to the water column primary production in oceanic tropical and subtropical waters (Li *et al.*, 1983; Platt *et al.*, 1983; Iturriaga and Mitchell, 1986; Glover *et al.*, 1985). However, as yet comparatively little is known about their significance in other marine ecosystems, particularly in the more eutrophic temperate coastal and estuarine waters and in upwelling regions. Thus to have a full and clearer view of the global impact of marine picophytoplankton a need for more extensive research in these areas has been identified.

There is growing recognition of phytoplankton cell size as a legitimate parameter of physiological and ecological importance (Malone, 1980; Ulanowicz and Platt, 1985) and that the size-structure

of the phytoplankton can have profound implications on the overall community biota, in particular on the trophic interactions (Goldman, 1988). A comprehensive analysis of the spatio-temporal variability of the size distribution of the phytoplankton, in which picoplankton can no longer be overlooked, appears thus as one of the vital steps for the understanding of the coherent structure of the whole pelagic community biota.

The main objective of the investigations presented in this chapter was to examine the distribution and abundance of picophytoplankton and its contribution to overall pelagic community chlorophyll a biomass and primary production in two temperate regions, the coastal waters of the North Sea and the estuarine waters of Southampton Water. The study in the North Sea includes field work conducted during two two-week cruises in areas of the southern and central North Sea during March and June/July 1989. The study in Southampton Water involved a ten month seasonal survey (January to October 1990) and included investigation of both seasonal and spatial trends of variation.

1.2. LITERATURE REVIEW

1.2.1. Picophytoplankton: ecology and physiology

For decades the apparent paucity of cyanobacteria in the world's oceans had puzzled many scientists on account of their ancient marine history and the diversification and success they have achieved in terrestrial and freshwater habitats (Fogg *et al.*, 1973). Advances in the techniques of epifluorescence microscopy revealed in the late 1970's the abundance and widespread distribution of minute chroococcoid cyanobacteria (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979) particularly of the genus *Synechococcus* and *Synechocystis*. These results subsequently led to the utilization of finer size fractionations in phytoplankton biomass and productivity studies and also to a more detailed investigation of the smallest cells in floristic examinations of natural plankton assemblages. It has now become apparent that eukaryotic phytoplankters of similar size range can also be numerically abundant in certain marine environments (Johnson and Sieburth, 1982; Takahashi and Hori, 1984; Joint and Pipe, 1984; Murphy and Haugen, 1985; Hargraves *et al.*, 1989) (see also Table 1.2.2). More recently, therefore, the prokaryotic and eukaryotic picophytoplankton have been studied as an operationally defined group or size class. Accumulated evidence suggests that cell size can have an important bearing on processes such as nutrient uptake, photon absorption, grazing, suspension and sinking (see Malone, 1980 and references therein). Cell size is thus a likely factor influencing the spatial and temporal distribution and abundance of phytoplankton. This has reinforced the consideration of size classes as potential functional groups and justifies, at least from an ecological point of view, the emphasis placed on studying the small photolithotrophic organisms (prokaryotic and eukaryotic) as a functional group.

Operative delimitations of size classes are often difficult to establish and discrepancies over the denomination and boundaries of the size class to be utilized have arisen. Workers in the early 1980's adopted the term picoplankton, for plankton in the range 0.2-2.0μm in diameter (*sensu* Sieburth *et al.*, 1978), originally believed to include almost exclusively the bacterioplankton. Many experimentalists, though, as a result of undertaking size-fractionation studies have

adopted a more functional definition of picoplankton as cells passing a 1μm pore-size sieve (Li *et al.*, 1983) and a re-definition of picoplankton as plankton in the size range of 0.1-1.0μm has been suggested (Sicko-Goad and Stoermer, 1984). Other workers, however, have pointed out that in the open ocean there is a group of small phytoplankters more or less clearly differentiated by their numerical dominance over larger forms which includes many eukaryotes in the range 2-5μm, that is, in between the picoplankton and the smaller nanoplankton (2-20μm) according to the classification of Sieburth *et al.* (1978), and have thus proposed the use of the older term ultraplankton (Murphy and Haugen, 1985), defined as organisms up to 10μm in diameter (Sverdrup *et al.*, 1942). However, it has been argued that ultraplankton is too loose a term and, instead, picoplankton should be used , with sufficient regard for the appropriate particle size (i.e. less than 3μm in diameter) (Stockner and Antia, 1986). Moreover, there are recent indications that, although most picocyanobacteria are smaller than 1μm in diameter, 1μm pore sized nucleation track polycarbonated filters (Nuclepore) can retain a large proportion of these cells during fractionation by filtration (Li, 1986; Craig, 1986). The majority of studies on picophytoplankton involving fractionation by filtration, however, have adopted the definition of phytoplankton cells passing through 1μm pore sized Nuclepore filters and retained in 0.2μm pore sized Nuclepore filters.

With the discovery of the abundance of picophytoplankton evidence has been given to sustain the hypothesis that in many marine environments a large proportion of particles in the picoplankton size fraction are metabolically active intact photolithotrophic cells. In contrast, former views were strongly inclined to consider that particles in this size range were almost exclusively heterotrophic (Sorokin, 1971), and that those containing photosynthetic pigments were essentially fragments of larger cells (Lasker and Holmes, 1957; Gieskes *et al.*, 1978; Herbland and Le Bouteiller, 1981). Furthermore, indications are now that the contribution of the picophytoplankton to overall photosynthetic biomass and primary production in the aquatic environment can be substantial (see Table 1.2.1). Results show that, in general, picophytoplankton are responsible for greater than 50% of the primary production in oceanic oligotrophic waters in the tropics

Table 1.2.1. Summary of the percentage contribution of small phytoplankton to chlorophyll (Chl) and primary production rate (PP) in various oceanographic provinces.

Reference	Location	Size (μm)	Chl %	PP %
Gieskes *et al.*, 1979	Tropical Atlantic	<1	43-53	
Herbland and Le Bouteiller, 1981	Equatorial Atlantic	<3	40	20
Li *et al.*, 1983	Tropical Pacific	<1	25-90	20-80
Platt *et al.*, 1983	Atlantic	<1		60
Takahashi and Bienfang, 1983	Subtrop. off Hawaii	<1		60
Herbland *et al.*, 1985	Equatorial Atlantic	<1	25	
Glover *et al.*, 1986	NW Atlantic	<1	23-50	
Iturriaga and Mitchell, 1986	N Pacific	<1		64
Furnas and Mitchell, 1988	Tropical Coral Sea	<2	59-80	
Glover *et al.*, 1988	Sargasso Sea	<5	53-69	
Odate and Maita, 1988	Subtropical Pacific	<2	84	
	Subarctic Pacific	<2	29-51	
Chávez, 1989	Pacific offshore	<1	11	50
	coastal	<1	36-62	
Larsson and Hagstrom, 1982	Baltic Sea	<3		20-31
Joint and Pomroy, 1983	Celtic Sea	<1		20-30
Bienfang *et al.*, 1984	Coast of Hawaii	<3	60-80	
Magazzú and Hull, 1985	Coast of Madagascar	<3	0-83	20-82
Joint and Pomroy, 1986	Porcupine Sea Bight	<1		15-23
Shim and Kahng, 1986	Yellow Sea	<1	4-18	3-16
Magazzú *et al.*, 1987	Strait of Messina	<1	56-63	24-43
Legendre *et al.*, 1988	Coral reef waters	<2	20-80	
Raimbault *et al.*, 1988	Mediterranean Sea	<1	33-41	
Maita and Odate, 1988	Funka Bay	<2		3-59
Jochem, 1989	Kiel Bight(Baltic)	<3	0.5-23	1-39
Madariaga and Orive, 1989	Gernika Estuary	<3		10
Ray *et al.*, 1989	Chesapeake bay	<2		9-13
Smith *et al.*, 1985	Canadian Arctic	<1		10-25
Legendre *et al.*, 1987	Arctic	<1	≤3	

and subtropics. The contribution of the picophytoplankton appears to diminish at higher latitudes, but it can still account for 20 to 40% of the plankton community rate of carbon fixation in coastal temperate waters during summer (Joint and Pomroy, 1983; Jochem, 1989). The contribution is less significant in polar regions. Smith *et al.* (1985) found the fraction passing 1μm filters to be responsible for 10 to 25%

of total phytoplankton photosynthesis in an eutrophic area of the eastern Canadian Arctic. Legendre *et al.* (1987) reported the fraction smaller than 1μm to contribute only 3% of the chlorophyll *a* biomass of under ice plankton flora in Hudson Bay.

The picocyanobacteria cell concentration typically ranges between 10^6 and 10^8cells l^{-1} (Table 1.2.2). They appear to be less abundant in polar seas (10^5-10^6cells l^{-1}) and in some brackish waters (see for example Vaulot and Xiuren, 1988). Eukaryotic picophytoplankters have less frequently been enumerated. There are few reports of total cell counts of eukaryotic picoplankters, usually defined as phytoplankton smaller than 2 or 3μm in diameter. These show numbers to be typically one order of magnitude lower than for picocyanobacteria (10^5 to 10^7cells l^{-1}). In other studies abundance of individual species are reported. In Narragansett Bay (USA), observations on standing stocks of autotrophic picoplankton from 1985 to 1988 showed that *Chaetoceros tenuissimus* can reach a maximum of 10^3cells ml^{-1}, *Chlorella* sp., and *Minutocellus polymorphus* can have peaks of the order of 10^4cells ml^{-1} and the Chrysophyceae *Aureococcus anophagefferens* can form major blooms of cell concentrations higher than 10^6cells ml^{-1} (Hargraves *et al.*, 1989).

All identified picocyanobacteria have been assigned to the order Chroococcales : unicellular species that divide by binary fission in one or more planes at right angles to one another. The genus *Synechococcus* appears to be the most prominent and almost all isolates have been assigned to *Synechococcus* and *Synechocystis*. Their pigment composition has been very important in their discovery, since the strong and characteristic autofluorescence distinguishes them from most eukaryotic phytoplankton and makes it very easy to recognize them under an epifluorescence microscope. They posses the phycobilliproteins, i.e. phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC), as major accessory photosynthetic pigments. Among the different strains of *Synechococcus*, variations in the composition and organization of the photosynthetic apparatus have been observed. There are PE lacking clones, containing PC and APC (absorption maxima at 620 and 650 nm respectively), as for example strains WH5701 and WH8018; and PE containing clones, where all three PE, PC and APC can be found, as in strains WH7803 and WH8007.

According to Wood (1985), among the latter it can be distinguished between those in which PE contains phycoerythrobilin (PEB) chromophores (absorption maxima at 550nm) and those in which PE contains PEB and PUB (phycourobilin) chromophores. The latter has an absorption maxima at 492nm and its presence enhances the light harvesting ability at blue wavelengths.

Table 1.2.2. Summary of cell abundance of phycoerythrin-rich chroococcoid picocyanobacteria (Cyan.) and eukaryotic picophytoplankton (Eukar.) for various oceanographic provinces.

Reference	Location	Cyan. (cells $l^{-1} \times 10^7$)	Eukar.
Johnson and Sieburth, 1979	Sargasso Sea	0.1-0.2	
	Caribbean Sea	0.06-0.6	
Waterbury *et al.*, 1979	Gulf Stream and Woodshole harbour	0.1-36	
Li *et al.*, 1983	Tropical Pacific	1-150	
Platt *et al.*, 1983	Mid Atlantic ridge	0.4-1.7	
Glover *et al.*, 1985	Gulf of Maine	1.5-11.7	0.06-4.4
Glover *et al.*, 1986	NW Atlantic	0.9-29	
Murphy and Haugen, 1985	Atlantic oceanic	0.28-22	0.35-2.8
	slope	2.1-4.3	0.01-0.6
	coastal	0.94-34	0.20-4.2
Geider, 1988	North Sea	0.01-0.56	0.01-0.9
Howard and Joint, 1989	North Sea	0.2-17	
Takahashi and Bienfang, 1983	Subtrop Hawaiian waters		*ca.* 0.1
Landry *et al.*, 1984	Kaneohe Bay	9.8-11	
Moriarty *et al.*, 1985	Great Barrier Reef	0.1-20	
Krempin and Sullivan, 1981	California coastal waters	0.1-7	
Hag and Fogg, 1986	Irish Sea and Menai Strait	0.1-10	
Magazzú *et al.*, 1987	Strait of Messina	*ca.* 1	
Kuosa, 1988	Gulf of Finland	0.2-24	0.1-0.3
Jochem, 1988	Kiel Bight	<1.3-26	
Campbell *et al.*, 1983	Great South Bay	0.8-30	
Shapiro and Haugen, 1988	Boothbay harbour	0.1-16	
Vaulot and Xiuren, 1988	Dilution zone of the Yangtze River	0.01-20	
Odate, 1989	Funka Bay	0.002-4*	0.1-6*
Hargraves *et al.*, 1989	Narragansett Bay	10**	>100*
Ray *et al.*, 1989	Chesapeake Bay	*ca.* 18	
Smith *et al.*, 1985	Arctic	0.09-0.21	
Walker and Marchant, 1989	Antarctic	0-0.01	

*Values given are yearly maxima
**Value also includes PE-lacking strains.

It has been suggested that besides serving as an energy collector for photosynthesis, phycoerythrin may play a significant role as a nitrogen storage as well in marine *Synechococcus* (Wyman *et al.*, 1985; Barlow and Alberte, 1985). Kana and Glibert (1987a), however, found that PE concentrations in high light grown *Synechococcus* strain WH7803 were so low as to be a relatively ineffective nitrogen reserve. Glibert *et al.* (1986) observed clonal differences in the behavior upon nitrogen depletion and clone WH8018 did utilize cellular reserves of PE to maintain growth. Barlow and Alberte (1987) found that in high light adapted cells of *Synechococcus* (WH7803) an increase of *in vivo* autofluorescence of PE was correlated with a decrease in photoinhibition at high irradiances and suggested a possible photoprotective role for PE.

The eukaryotic component of the picophytoplankton comprises a more diverse group of algae than the picocyanobacteria. Specimens smaller than 5μm in diameter have been identified in the majority of algal classes: Prasinophyceae (Micromonadophyceae), Prymnesiophyceae, Chlorophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae and Chrysophyceae. Specimens in the 0.2 to 2μm in diameter appear to be restricted to the Bacillariophyceae, Prasinophyceae and Chlorophyceae (Thomsen, 1986). Many of these forms have as yet to be precisely classified taxonomically. Thomsen (1986) provides a detailed description and an approach to the taxonomy of the different forms of the smallest eukaryotic phytoplankton identified thus far in the marine environment.

The Prasinophyceae (*sensu latu*) are one of the major component groups of the oceanic eukaryotic picophytolankton. There is a large number of small isolated coccoid prasinophytes as yet poorly identified. Recently Hooks *et al.*(1988) have attempted to classify them into subgroups according to the presence or absence and relative abundances of chlorophylls and carotenoids. Hooks *et al.*(1988) have suggested that the pigments can serve as biomarkers, given that chromatic adaption does not occur in these organisms. An interesting feature is the relative abundance of chlorophyll b, with higher b/a ratios than found in higher plants. Very often many of these strains are referred to as the *Micromonas* group, and they are possibly the most abundant forms of oceanic prasinophytes. Recently some of these

strains (e.g Ω48-23, 1201-2, 1326, BT-5) have been positioned taxonomically in a new genus and species (i.e.*Pycnococcus provasolii*) and included in the class Micromonadophyceae (Guillard *et al.*, 1991). Also important is the unidentified, ubiquitous "scaled prasinophyte" described by Johnson and Sieburth (1982). In the Chlorophyceae, "Chlorella-like" cells have been described from the open ocean (Joint and Pipe, 1984). Many other forms observed in the marine environment have yet to be isolated and brought into culture; as a consequence, very little is known about their physiology.

An area in which great emphasis has been placed is in establishing the spatial and temporal distribution pattern of the picophytoplankton in the marine environment and its possible relationship to physiological characteristics determined by the cell size. With regard to the vertical structure, in oceanic oligotrophic areas, where the surface water is well stratified with an almost permanent pycnocline, maxima of abundance have been recorded deep in the euphotic zone, i.e. in low light areas (Li *et al.*, 1983; Takahashi and Hori, 1984; Murphy and Haugen, 1985; Glover *et al.*, 1986; Furnas and Mitchell, 1988; Legendre *et al.*, 1988). This has led investigations to center on the photophysiology of the picoplankton. Early works, describing size-fractionation studies of natural samples and laboratory experiments with *Synechococcus*, suggested a positive photoadaption to low irradiance and the picophytoplankton were included as an important component of the so called "shade-flora". Morris and Glover (1981) working with two strains of *Synechococcus* measured relatively low photon flux densities for saturation of growth, 55 and 45$\mu Em^{-2}s^{-1}$, which yielded minimum generation times of 11 and 13 hours respectively for strain DC-2 (WH7803) and SYN. Barlow and Alberte (1985) in a study conducted with *Synechococcus* (strains WH7803 and WH8018) reported maximum rates of growth at 25$\mu Em^{-2}s^{-1}$ and increased efficiency of utilization of light (α) and light saturated rates of photosynthesis (P_{max}) in cells grown under low light conditions (10-50$\mu Em^{-2}s^{-1}$) in relation to those grown at high photon flux densities.

Field studies have undertaken basically two different, though complementary approaches; the investigation of picoplankton as shade adapted phytoplankton, by examining if subsurface populations have

enhanced photosynthesis at low photon flux densities in comparison to populations from the near surface; and to investigate if picoplankton are better adapted to growing at low light than larger phytoplankton. Platt *et al.* (1983) found in natural oceanic populations of phytoplankton from the subsurface chlorophyll maximum of the Atlantic that chlorophyll specific α and P_{max} were higher for the smaller than 1μm size fraction than for the larger than 1μm size fraction and picoplankton also showed strong photoinhibition of photosynthesis. Glover *et al.* (1985) observed that *Synechococcus* spp. dominated size-fractions made a more significant contribution to primary production under conditions of low photon flux density. Prézelin *et al.* (1986) from a study in the Northwest Atlantic ocean concluded that the 0.6-1μm fraction had lower requirements for light saturated photosynthesis (I_k) than the larger than 5μm fraction and although P_{max} expressed in terms of chlorophyll , i.e. assimilation number, for picoplankton was highest at the surface, α normalized to chlorophyll concentration increased with depth. Iturriaga and Mitchell (1986) reported from a study in the North Pacific ocean that populations of picoplankton (passing through 1μm filters) from the subsurface chlorophyll maximum showed higher rates of photosynthesis at low photon flux densities (up to $200\mu Em^{-2}s^{-1}$) than picoplankton populations from the surface.

Picoplankton are not restricted to low light environments, however. Abundant populations of *Synechococcus* spp. (Waterbury *et al.*, 1979; Krempin and Sullivan, 1981; Hag and Fogg, 1986; Howard and Joint, 1989) having high rates of growth (Bienfang *et al.*, 1984; Landry *et al.*, 1984) and photosynthesis, and showing no apparent degree of photoinhibition (Iturriaga and Mitchell, 1986; Furnas and Mitchell, 1988; Howard and Joint, 1989) have also been found near the surface. It appears that in well mixed waters numbers of picocyanobacteria are relatively uniform with depth within the euphotic zone (Murphy and Haugen, 1985; Jochem, 1989). There are also reports of picoplankton populations less well adapted to low light than some larger forms. Lower efficiency of utilization of low light for the fraction smaller than 1μm fraction than for the fraction larger than 1μm has been reported by Magazzú *et al.* (1987) in populations from the top 10m of the Strait of Messina (Mediterranean Sea) and also by Legendre *et al.*

(1987) for under ice populations sampled in the Canadian Arctic. Howard and Joint (1989) found that the chlorophyll normalized initial slope and assimilation number were greater for the picoplankton size fraction than for larger phytoplankters in surface populations from the North Sea, but argued that this pattern could be the result not only of adaptation to shade conditions but also of nutrient limitation. They further observed a large incorporation of carbon into protein and interpreted this as an indication of a possible nutrient limitation. Furthermore, in this study no evidence of any significant degree of photoinhibition was found in either the picoplankton or the larger phytoplankton and thus it was not possible to conclude that picoplankton were low light adapted. Furnas and Mitchell (1988) also found near surface populations of picophytoplankton from coastal and oceanic regions of the tropical Coral Sea to photosynthesize at near maximum rates at irradiances of up to $1300\mu Em^{-2}s^{-1}$. Surface picophytoplankton populations showed significant enhancement of assimilation number over deep-living populations and there was no evidence that α was greater for the subsurface picophytoplankton, concluding that picoplankton from tropical oceans can adapt to a wide range of light levels. Recently, Glover *et al.* (1988) have reported a surface bloom of *Synechococcus* in Sargasso Sea waters, with characteristics of typical high-light adapted phytoplankton populations. Joint and Pomroy (1986), comparing two locations, an oceanic station and a temperate shelf water mass concluded that there was not enough evidence to support the hypothesis that picophytoplankton from either of the two areas were significantly more adapted to low light conditions than larger phytoplankton, because the major difference in photosynthetic parameters between picoplankton from the bottom of the euphotic zone and the near surface waters was a decrease in assimilation number in the former in comparison to the latter.

It is as yet unclear whether these differences in light response are a result of the capacity of the picophytoplankton, as a size class, to acclimate to a wide range of light levels in response to changes in environmental conditions, or rather a reflection of differences in the floristic composition of the picoplankton (including variations in strains or races of *Synechococcus*) and the

basic physiology of the various species. Laboratory studies with pure cultures provide a very useful basis for investigating the capacity for acclimating to different illumination conditions. Few investigations of this type have been carried out, and these have been restricted almost exclusively to strains of *Synechococcus*. Results are contradictory. Barlow and Alberte (1985) reported enhanced efficiency of utilization of low light and light saturated rates of photosynthesis for low light (10-50$\mu Em^{-2}s^{-1}$) grown *Synechococcus* strain WH7803. Saturation of growth occurred at photon flux densities of 25$\mu Em^{-2}s^{-1}$ and cells exposed to 250$\mu Em^{-2}s^{-1}$ showed no growth. Low light grown cells showed a 20 to 30% decrease in the rates of photosynthesis at photon flux densities higher than 150$\mu Em^{-2}s^{-1}$ and were able to maintain these rates up to irradiances of 1000$\mu Em^{-2}s^{-1}$. Even at these levels of irradiance low light grown cells showed higher rates of photosynthesis than cells grown at photon flux densities of 250$\mu Em^{-2}s^{-1}$. Kana and Glibert (1987b) for the same strain also found greater α and P_{max} values in low light grown cells, with photoinhibition of photosynthesis at irradiances higher than 250$\mu Em^{-2}s^{-1}$. Photoinhibition in this study appeared to be more severe than that reported by Barlow and Alberte (1985). High light (up to 2000$\mu Em^{-2}s^{-1}$) grown cells, however, did not experience photoinhibition and, in contrast to Barlow and Alberte's findings, could photosynthesize at near maximal rates at high photon flux densities (up to 2000$\mu Em^{-2}s^{-1}$). The main methodological difference between the two studies was that Kana and Glibert pre-acclimated the cells gradually to high irradiances over several weeks. It has also been pointed out that pure cultures may be subject to an intense artificial selection in the laboratory (Joint, 1986).

Although, in general, picophytoplankton seem capable of efficiently utilizing low light levels, the growth of picoplankton populations at sites of low irradiance is probably not the result of the photosynthetic characteristics exclusively, but other factors such as the nutrient availability might also play a major role. It has been suggested that the vertical distribution of picoplankton in the sea is more related to the nitracline than to the thermocline (Herbland *et al.*, 1985). Peaks of abundance of picophytoplankton at subsurface depths appear to be typical of populations from the tropics and

subtropics, i.e. areas of almost permanent water column stratification and low nutrient concentration, where living at sites of low light i.e. deep in the euphotic zone, may have the advantage of living at the forefront of the moderate nutrient influx through advection from below the pycnocline. However, in surface mixed layers and areas of high nutrient concentration (e.g. coastal embayments and estuarine areas) picoplankton seem capable of developing under conditions of high irradiance.

It has been suggested that eukaryotic picophytoplankton show maxima below the peak of the picocyanobacteria in the water column (Murphy and Haugen, 1985). This pattern has been related to differences in the response to light quality, in turn, associated with differences in the photosynthetic pigment composition (Glover *et al.*, 1985). In laboratory experiments with unialgal species eukaryotes have been found to show greater photosynthetic efficiency in blue-violet and blue light, whereas *Synechococcus* performed best at green wavelengths (Wood, 1985; Glover *et al.*, 1987). To what extent it can be generalized that eukaryotic picoplankton peak below the picocyanobacteria is as yet unclear and further investigations are needed.

The latitudinal distribution of picoplankton shows that they are most abundant in the tropics and subtropics and least abundant in the polar seas (Table 1.2.2). Murphy and Haugen (1985) found a strong correlation between latitude and numbers of chroococcoid picocyanobacteria and related this pattern mainly to temperature.

Most studies also reveal a marked spatial variability in inshore-offshore gradients. It is apparent that picophytoplankton are more important in relation to larger forms in offshore areas than in areas close to shore (Chavez, 1989). Studies seem to suggest a similar distribution pattern even within smaller scale spatial gradients, such as in estuarine systems.

Throndsen (1978) found that in the Oslo fjord system (Norway) phytoplankton concentration and production was higher in the inner Fjord, but whilst the contribution of the nanoplankton to overall phytoplankton community was also markedly higher in the inner Fjord, the ultraplankton size fraction was relatively more important in the outer Fjord. Jochem (1989) similarly reported that in the Kiel Bight

(FRG), both nanoplankton and picoplankton biomasses were higher towards the inner part, the Kiel Fjord, where there was generally a higher concentration of nutrients. Nanoplankton contribution to overall phytoplankton community chlorophyll *a* and primary production also increased towards the more eutrophicated inner area, but the relative contribution of the picoplankton size fraction increased in the opposite direction, towards the outer open Bight. Larsson and Hagstrom (1982) also found picoplankton to be less important in relation to total phytoplankton biomass (10%) in an inner eutrophicated area in the southern archipelago of Stockholm (Sweden) than in an area in the southern part, open to the Baltic (25%). Kuosa (1988) estimated that in the Gulf of Finland the picoplankton accounted for 7.3% of total phytoplankton biomass in the outer archipelago and open sea area, as against 1.7% in the inner archipelago. Numbers of picoplankters, however, also increased towards the outer areas of the transect in this study. Similarly, Vaulot and Xiuren (1988) found numbers of *Synechococcus* to increase in the offshore direction in the dilution zone of the Changjang river (Yangtze), China.

Although there is agreement among the various studies on the lower contribution of picoplankton in relation to larger size classes at more productive regions, the actual concentration of picoplanktonic cells and primary production is not always greater in the more eutrophic areas (Larsson and Hagstrom, 1982; Glover *et al.*, 1985; Throndsen, 1978; Kuosa, 1988).

In the longitudinal axis of an estuarine system two opposite gradients can be found: a salinity gradient increasing towards the seaward end and a nutrient/pollution/turbidity gradient increasing towards the riverine head of the estuary. In a study in the dilution zone of the Changjang river (Vaulot and Xiuren, 1988) a strong salinity gradient was found and PE-rich cyanobacteria were more abundant at higher salinities. Shapiro and Haugen (1988), however, failed to find a clear positive correlation between PE-rich *Synechococcus* numbers and salinity in Boothbay Harbour. In the studies in areas of the Baltic Sea, salinity varied little longitudinally, however, strong nutrient and possibly also pollution gradients, arising mainly from anthropogenic inputs from highly urbanized

regions, were found. The effect of pollutants or turbidity on cell size distribution of phytoplankton has not yet been addressed in any detail, but nutrients seem to have an important bearing on the relative abundance of large and small phytoplankters, high nutrient environments typically favouring larger cells (Takahashi and Hori, 1984; Maita and Odate, 1988).

In relation to the temporal variation, picophytoplankton abundance seems to show little seasonal change in tropical offshore waters. In coastal and estuarine areas, marked seasonal variations have been found in temperate regions. The seasonal variation in the abundance of chroococcoid cyanobacteria has been assessed in several coastal and estuarine areas, e.g. southern California (USA) coastal waters (Krempin and Sullivan, 1981), the Irish Sea and the Menai Strait, UK (Hag and Fogg, 1986), Woodshole Harbor, USA (Waterbury *et al.*, 1986), Boothbay Harbor, USA (Shapiro and Haugen, 1988), Funka Bay, Japan (Odate, 1989) and the dilution zone of the Changjang river, China (Vaulot and Xiuren, 1988). In all these studies winter levels were low and peaks of abundance were recorded in summer. Exception was in the study in Boothbay Harbor, USA (Shapiro and Haugen, 1988), where the concentration of *Synechococcus* also showed a peak in mid-winter. Eukaryotic picophytoplankton abundance also peaked in late summer in Funka Bay, Japan (Odate, 1989). Joint *et al.* (1986) have reported values for the seasonal contribution of different phytoplankton size-fractions to overall primary production in the temperate shelf waters of the Celtic Sea. The picoplankton contributed to 22.4% of the total annual primary production and peaked just following the spring diatom bloom and again in August, both periods coinciding with low ambient nutrient levels. In studies conducted in Narragansett Bay (USA), Oslo Fjord (Norway) and Kiel Bight (FRG), winter-spring and autumn blooms were found to be dominated by microplankton, whereas during summer periods nanoplankton was the most significant fraction. (Durbin *et al.*, 1975; Throndsen, 1978; Shapiro and Haugen, 1988; Jochem, 1989). Ultra/picoplankton appeared to peak in mid-late summer (Throndsen, 1978; Jochem, 1989).

Despite their recognition as major primary producers in the worlds oceans, there is very little information on the fate of picophytoplankton primary production. There seems to be strong

evidence to suggest that picoplankton populations are controlled by predation, rather than by sinking (Takahashi and Bienfang, 1983). Because of their similar size, picophytoplankton have been assumed to be grazed by the same organisms that feed on heterotrophic bacteria. In the microbial loop model proposed by Azam *et al.* (1983), microflagellates are the primary grazers of bacteria. Joint (1986) and Sherr and Sherr (1984) suggested they could also be grazers of picoalgae. Joint and Williams (1985) further argued that bacterial biomass in the open ocean might not be sufficient to support the microflagellate populations and that picophytoplankton could serve as an additional source of food to meet their carbon requirements. There is now much evidence of ingestion and assimilation of picocyanobacteria and other picoalgae by microflagellates, both from freshwater and marine waters (Johnson *et al.*, 1982; Caron *et al.*, 1985; Fahnenstiel *et al.*, 1986; Parslow *et al.*, 1986; Sherr *et al.*, 1991). It is worthwhile noting that microflagellates have been found to feed on larger diatoms as well (Suttle *et al.*, 1986). More recently, ciliates have been shown to be able to feed on bacteria (Turley *et al.*, 1986) and picoalgae (Iturriaga and Mitchell, 1986; Kudoh *et al.*, 1990) as well. There seems to be a general consensus in acknowledging that bacterial and picoplankton biomass in the sea is too small to entirely satisfy the ciliate population food requirements (Fenchel, 1980; Joint, 1986). It has also been shown that the small particle size and the low density of picoplankton are a constrain for an efficient capture by the filtering systems of ciliates (Banse, 1982; Jørgensen, 1983), and that, consequently, other food sources must be more important to ciliates. However, there is some evidence to suggest that the clearance by ciliates can account for an important fraction of the total grazing on picoplankton (Kudoh *et al.*, 1990). Thus, opinion is growing in favor of including the picophytoplankton and the small nanophytoplankton in the microbial loop model. As to other grazers, some dinoflagellates have been found to graze picophytoplankters (Kimor, 1981) and the mucous net-feeders, believed to efficiently ingest particles less than 3μm, have been suggested as potential grazers of picophytoplankton (Joint, 1986). The quantitative importance of these processes and that of the efficiency of transference of energy from the microbial loop, that should now

include the picophytoplankton, into the larger planktonic organisms remains to be further investigated.

1.2.2. Phytoplankton primary production in estuarine systems

The estuarine ecosystem is made up of a group of habitats of a heterogeneous physiographical nature. As a result, some estuaries are highly unproductive, others sustain large populations of commercial fish and invertebrates, which, in turn are based on high rates of primary production (see Ketchum, 1983). In some estuaries the degree of eutrophication is such that they bear serious problems arising from nuisance phytoplankton blooms. Furthermore, the spatio-temporal variations within a single estuary are generally very large and phytoplankton dynamics are of great complexity.

Boynton *et al.*, (1982) attempted to determine the parameters that control spatio-temporal variations in primary production and phytoplankton biomass in estuaries. From the intercomparison of 63 estuarine systems the authors concluded that the correlation with a number of physical and chemical parameters was low. The only possible generalization was that production and biomass were high during the warm periods of the year. This is a good illustration of the large inter and intra-estuarine variability. A diagrammatic representation of factors influencing phytoplankton production rates is presented below (from Boynton *et al.*, 1982) (Figure 1.2.1).

Light energy can be a primary limiting factor in estuarine systems. Phytoplankton need a threshold surface mixed layer photon flux density estimated to be *ca.* 40ly d^{-1} (196W m^{-2}) for a pronounced increase in growth rate (Riley, 1967). This is attained when the mixed layer primary production sufficiently exceeds respiration. The critical depth concept (Sverdrup, 1953) is thus applicable to estuarine systems as well as coastal waters (Cole and Cloern, 1984). Hitchcock and Smayda (1977) explained a retarded winter-spring diatom bloom in Narragansett Bay in 1973 in terms of exceptionally low light levels and subsequent delay in the achievement of the critical photon flux density. Stratification of the water column can help reduce the depth of the surface mixed layer and, in that sense, the mean critical

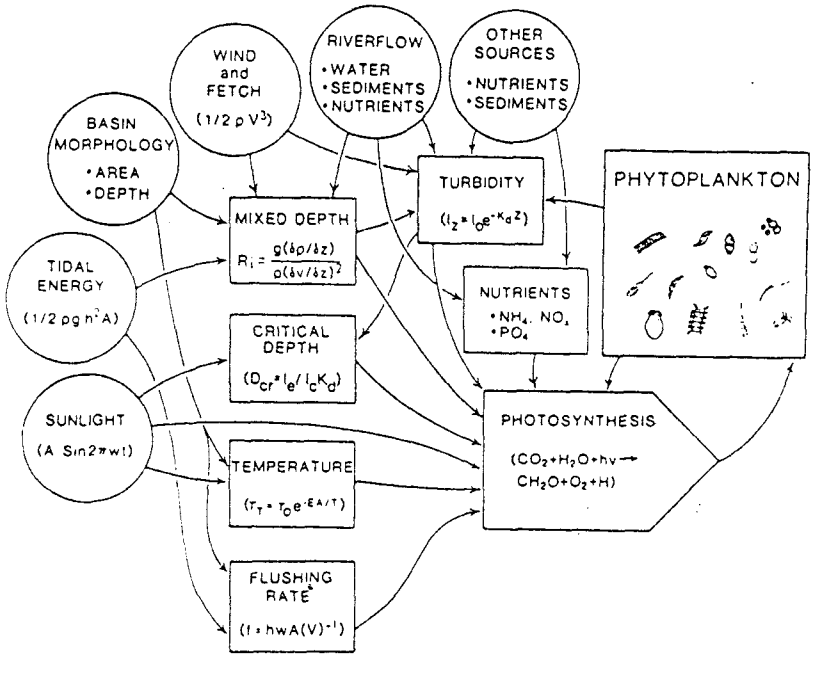

Figure 1.2.1. A conceptual model indicating sources of materials and mecanisms influencing phytoplankton production in estuarine ecosystems (from Figure 1 in Boynton *et al.*, 1982).

irradiance for the mixed layer is attained earlier in the year. The onset of the spring bloom has been found to be triggered by thermal and/or haline stratification in a number of estuaries (Hitchcock and Smayda, 1977; Tett and Wallis, 1978; Radach and Moll, 1990).

In estuarine systems or specific areas within an estuary with substantial riverine and anthropogenic inputs, the concentration of suspended particles can be high and availability of light is restricted by the turbidity of the water column. Examples of turbid estuaries and differences in water column integrated primary production between the turbid zones and outer clearer areas are presented in Table 1.2.3.

Table 1.2.3. Comparison between the rate of primary production (PP in $gCm^{-2}yr^{-1}$) in inner turbid areas (T) and outer clearer zones (C) in selected estuaries. Photic zone depths (Z_p) are given in units of meters.

Estuary	Z_p		PP		Reference
	T	C	T	C	
Bristol Channel	0.5	10	7	165	Joint and Pomroy (1981)
Forleague Bay	0.5	1	196	439	Randall and Day (1987)
Delaware Estuary	-	-	70	392	Pennock and Sharp (1986)
San Francisco Bay	<0.5	5	80	210	Cloern (1987)
Ems-Dollard	<1	5	61	381	Colijn (1983)

Light attenuation in most estuaries appears to be primarily due to suspended sediments (Lively *et al.*, 1983 ; Brylinski and Daborn, 1987; Cloern, 1987), in contrast to the open ocean, where phytoplankton biomass has a more significant impact on the light extinction coefficient (Smith and Baker, 1978). In some estuaries colloidal and dissolved compounds rather than suspended particles have been identified as primary contributors to the light attenuation in the water column (Randall and Day, 1987).

In turbid estuaries production tends to peak in summer, i.e. at times of reduced rainfall and maxima of insolation (e.g. Fourleague Bay (Randall and Day,1987), Ems-Dollard (Colijn *et al.*, 1987), Delaware Estuary (Pennock and Sharp, 1986), Cornwallis Estuary

(Brylinsky and Daborn, 1987), lower Hudson Estuary (Malone, 1977), Great South Bay, New York (Lively *et al.*, 1983), Bristol Channel (Joint and Pomroy, 1981), San Francisco Bay (Cole and Cloern, 1984) etc.). Increased turbidity can also be the result of resuspension of bottom sediments by turbulence originated from tidal currents and/or wind driven waves (Ward, 1985).

The mixing of varying volumes of seawater and freshwater and the pattern of water circulation determine the horizontal and vertical distribution of salinity. Phytoplankton may be exposed in the course of hours to large variations in salinity due to movements along the longitudinal axis with tidal and other current movements, and so, subjected to varying conditions of osmotic stress. Estuarine species are the most euryhaline amongst the phytoplankton (Brand, 1984). However, estuaries can receive allochthonous populations from the river and from offshore. Coastal species have some degree of tolerance for low salinity (Brand, 1984). Some freshwater species show some degree of halotolerance (Foerster, 1973; Rice and Ferguson, 1975; Jackson *et al.*, 1987), but massive death and disintegration of freshwater algae at the head of the estuary, at the beginning of the brackish water section, has also been observed (Blanc *et al.*, 1969) and increases in the ratio of phaeophytin to chlorophyll from the freshwater areas to the initial part of the mesohalinic waters have been reported (Fast and Kies, 1990).

Simple thermodynamic considerations show that temperature affects the rate of activity of a chemical reaction and as such is an influencing factor in the physiology of the community biota. Eppley (1972) found that temperature sets an upper limit to the maximum rate of growth of phytoplankton in the sea. Phytoplankton assimilation numbers have also frequently been found to be correlated with water temperature (Malone, 1977a; Li, 1980; Joint and Pomroy, 1981; Cole and Cloern, 1984; Pennock and Sharp, 1986). However, how much control temperature exerts on phytoplankton growth in relation to other parameters in estuarine habitats is not clear. Since irradiance and temperature co-vary in time, it is often difficult to separate the effects of these two parameters. In shallow, clear, temperate estuaries and embayments temperature has been identified as a major controlling factor for phytoplankton production (Steemann Nielsen,

1958; Vatova, 1961; Williams and Murdoch, 1966). Peaks of production occur in summer and the main explanation put forward is a more rapid regeneration of nutrients in warm periods, as a result of enhanced bacterial activity at higher temperatures (Williams and Murdoch, 1966).

Estuaries are systems of high external nutrient loading from rivers and anthropogenic sources. Excessive nutrient concentration can give rise to situations of eutrophication. Examples of estuarine systems with different external nutrient loadings (taken from Jaworski's review (1981) are presented below (Table 1.2.4).

Table 1.2.4. Yearly nitrogen (N) and phosphorus (P) loadings and the classification in relation to the degree of eutrophication in selected estuaries taken from Jaworski (1981).

Estuary	P ($gm^{-2}yr^{-1}$)	N ($gm^{-2}yr^{-1}$)	Classification
Potomac Estuary (1969-1971)	4.30	20.2	Hyper eutrophicated
Delaware Estuary (1976)	18.90	100.0	Highly industrialized
Thames Estuary (1971)	109.80	462.5	Highly industrialized
Archipelago-Stockholm (1971)	3.48	28.5	Eutrophic
Narragansett Bay (1977)	1.82	7.1	Non-eutrophic
Albemarle Sound (1974)	0.80	4.9	Non-eutrophic

As it can be seen, in some estuaries a close relationship between nutrient loading and the degree of eutrophication has been demonstrated. In other estuaries the river flow is large and the residence time is short e.g. Thames and Delaware estuaries, hindering large accumulations of nutrients or plankton biomass. This is also the case in many fjords (see Sinclair *et al.*, 1981). In other estuaries, since high nutrient concentrations are normally accompanied by high levels of suspended matter, light, restricted by turbidity, becomes limiting, and there is an inverse correlation between nutrient concentration and primary production, with phytoplankton production peaking in summer and seawards down the zone of highest nutrient concentration, which normally coincides with the zone of high turbidity (Joint and Pomroy, 1981; Cole and Cloern, 1984).

Kemp and Boynton (1984) elaborated a theory for late blooming estuaries by which part of the nutrients entered 3 to 5 months earlier (winter-spring) could be available to the summer phytoplankton. According to these authors, annual inputs of dissolved inorganic nutrients from river discharge are transformed into particulate forms by flocculation, sorption and some assimilation by phytoplankton in the low salinity, high turbidity zone of the estuary and are transported seaward through cycles of deposition and resuspension. In summer, when the water temperature rises, the decomposition of these detrital particulates by benthic bacteria is enhanced, and the dissolved inorganic nutrients produced diffuse into the euphotic zone, where phytoplankton receive sufficient light to maintain substantial production rates. Nixon (1981) argues that a rapid return of recycled nutrients into the water column is eased in shallow systems, such as in many estuaries and this facilitates the coupling between decomposition and production. Even in estuaries in which the major bloom takes place during the winter-spring transition, a series of minor blooms have been reported in summer periods. Many of them have been ascribed to entrainment of bottom nutrients not just by diffusion, but by turbulent mixing resulting from wind events (Iverson *et al.*, 1974; Walsh *et al.*, 1978; Winter *et al.*, 1975; Takahashi *et al.*, 1977). Similarly, Ward and Twilley (1986) found a strong influence of allochthonous inputs on the nutrient distribution in spring and a marked effect of internal processes in summer and fall in an estuary tributary to Chesapeake Bay.

Estuarine phytoplankton are, in general, non-nutrient limited (Williams, 1972), i.e. nutrients are reported in excess to phytoplankton demands almost all year round. Nixon (1981) argues that this characteristic is the result not only of the riverine and anthropogenic inputs, but also of nutrient recycling processes and other sources, such as the contribution from marshes, the incorporation of nutrients brought in by the nutrient rich deep water from offshore into the euphotic zone, and the trapping of nutrients in the counter current freshward flow that sink from the upper seaward flow of freshwater. Exceptions can be found for example in spring blooming areas with a deep water column, where the entrainment of regenerated nutrients into the euphotic zone can be less important,

such as the Strait of Georgia, with a mean depth of 156m, where nutrients have been reported limiting in summer (Stockner *et al.*, 1979).

High rates of water column respiration can cause an oxygen sag in estuaries, particularly in areas of maximum turbidity, favouring denitrification processes, which can subsequently lead to marked depletions of nitrate (Meybeck *et al.*, 1988). In turbid estuaries oxygen minima can also be the result of intense nitrification, particularly during summer (Relexans *et al.*, 1988; Kausch, 1990).

Primary production and phytoplankton biomass are interdependent and factors affecting the accumulation of algal biomass directly influence the total primary production rate in the water column. The accumulation of phytoplankton biomass depends largely on the residence time of the water in the estuary. Phytoplankton biomass accumulation in estuaries can only occur when the population residence time is long enough for a sufficient number of cell divisions to occur (Ketchum, 1954). Studies in the lower Hudson estuary showed that phytoplankton were washed out of the estuary by surface layer flow during autumn, winter and spring when growth rates were low relative to surface dilution rates, and tended to accumulate during the summer when growth rates exceeded dilution rates (Malone, 1977). In this estuary netplankton growth rates were too low to allow the development of blooms at any time of the year. In the Duwammish estuary (USA) the flushing time of riverine water in the upper part of the estuary is less than a day, and hence insufficient for large accumulations of phytoplankton biomass (Welch *et al.*, 1972). The Bristol Channel (UK) is an example of an estuary where the long flushing time (250 days) (see Joint and Pomroy, 1981) is not detrimental to phytoplankton biomass accumulation. Stratification during summer periods precludes accumulation of species with high sinking rates, e.g. net diatoms (Smayda, 1970). Mixing events due to wind and tide originated turbulence can also influence the standing stock of phytoplankton through resuspension of "seed cells" from the benthos that can make use of the nutrients entrained in the same manner, giving rise to an increased phytoplankton growth.

Limitation of phytoplankton growth by grazing is a well documented feature in estuaries (Martin, 1970; Malone and Chervin,

1979; Deason and Smayda, 1982). A summer bloom of *Skeletonema costatum* in Narragansett Bay coincided with a release in grazing pressure on phytoplankton through the presence of the carnivorous ctenophore *Mnemiopsis leydi* that grazed effectively on herbivorous zooplankton. The bloom declined with the depletion of the ctenophore and subsequent increase in the population of herbivorous zooplankton (Deason and Smayda, 1982).

An important feature steming from the above considerations is that besides the long term climatic variations, short term variations of meteorological and other physical events are important in regulating the phytoplankton dynamics in estuaries (Takahashi *et al.*, 1977; Sinclair *et al.*, 1981; Litaker *et al.*, 1987; Madariaga and Orive, 1989). It is becoming apparent that high frequency processes, i.e. processes with periods approximating phytoplankton division times, must be taken into account for a comprehensive analysis of the coherent structure of estuarine dynamics (Litaker *et al.*, 1987).

1.2.3. Study areas

1.2.3.1. The North Sea

The North Sea is a continental shelf area in the northwest of Europe semi-enclosed by land masses with highly industrialized human populations. It extends from latitude 51°N to 61°N and longitude 40°W to 9°E. It is supplied by two inflows of water from the Atlantic ocean : a major one from the north, between Scotland and Norway and a smaller from the south through the Straits of Dover. It is also connected with the Baltic Sea through the Skagerrak, receiving water of low salinity. Other minor inflows are from rivers such as the Ems, Elbe, Maas, Humber, Thames and the Rhine. The North Sea covers an area of 575000km^2 and it is classically divided into three subareas, the southern bight, that extends between latitude 51°N to 54°N, with water depths typically of less than 40m; the Central North Sea extending from latitude 54°N to 57°N with water depths of 40 to 100m, with exceptions such as the area of the Dogger Bank and the coast of Denmark; the northern North Sea, extending north of 57°N with water

depths up to 150m.

The stratification pattern of the water column also varies geographically. The southern North Sea and also the British coast is shallow and is characterized by strong tidal currents (1 to 3 knots mean spring tidal velocity, from Eisma, 1973, cited in Zijlstra, 1988), as a result of which the water column is permanently well mixed (homohaline, homothermal). Areas of lower tidal current velocity are the central North Sea and northwestern North Sea. The water column in these areas is homohaline, but undergoes thermal stratification in the warmer seasons, in certain areas the thermal stratification being permanent. In the northeastern North Sea, in and near the Skagerrak inflow, the less saline, less dense water entering from the Baltic Sea leads to a permanent, or sometimes seasonal, halocline (see Zijlstra, 1988).

Surface water temperatures show a seasonal variation, more pronounced in the southern areas (a range of 14 to 15°C) and less marked (a range of 5°C) in the northern areas. Bottom temperatures show similar ranges, but in the thermally stratified northern regions there is very little seasonal variation (0 to 2°C) (Anon. 1962).

Surface salinities are typically around 35ppt in the northern areas and between 34 to 35ppt in the central and southern areas, except in coastal areas with considerable input of riverine water, and near the Skagerrak inflow of the less saline water of the Baltic Sea (Anon. 1962). Although the main nutrient input to the North Sea is from the Atlantic Ocean (*ca.*232000t of phosphorus and 1724000t of nitrogen annual import, see Gerlach, 1987), in the southern North Sea riverine inputs have in the last three decades increased progressively and are now of considerable importance. In the southern North Sea, river loads, mainly derived from agricultural fertilizers, were estimated to contribute 34 to 36% of the phosphorus and nitrogen concentrations in 1980 (see Gerlach, 1987). The high river discharge of nutrients and other contaminants, such as toxic chemicals, heavy metals and oil products derived from waste water emissions and sewage sludge dumping, have lead to highly polluted coastal areas.

Parallel to the increase in nutrient discharge total phytoplankton biomass in the southern area also increased from 9μg C l^{-1} in 1962 to 37μg C l^{-1} in 1984 (see Gerlach, 1987). The pattern,

however, seems to have started to reverse since 1984 in Dutch coastal waters due to a reduction in Rhine river outflow (Reid *et al.*, 1990). The diatom population, which is the dominant fraction of the net plankton, seems to have remained constant or if anything has decreased slightly. This appears to be due to silicate limitation, through a reduction of silicate loading from the river discharges (Radach and Berg, 1986 cited in Gerlach, 1987). Flagellates have become important particularly in the microplankton and nanoplankton size fractions and organisms such as the prymnesiophyte *Phaeocystis* spp. recurrently form dense blooms of up to 50mgchl m^{-3} (Gieskes and Kraay, 1977). Blooms of mucilaginous colonial *Phaeocystis* sp. have been reported during spring in the well mixed waters of the southern North Sea, following the spring diatom bloom, which has been suggested to collapse due to silica limitations. One of the consequences of the blooms of *Phaeocystis* spp. is the observed banks of foam and froth on coastal european beaches (Bäjte and Michaelis, 1986; Lancelot *et al.*, 1987). The diatoms and *Phaeocystis* spp. contribute to 20 and 30% of the spring bloom in Belgian coastal waters. Flagellates are important during summer months. Species of the dinoflagellate *Ceratium* are common bloom-forming phytoplankters in summer stratified waters of the central and northern North Sea and they are reported as dominant in the thermocline. Because of its size (250μm), even if numbers are not high, the contribution to the biomass can be significant. Gieskes and Kraay (1984) estimated that during July in the Oyster Ground, 50% of the biomass was comprised by *Ceratium* and the other half was comprised by microflagellates smaller than 5μm in diameter. Blooms of coccolithophorids have also been reported. *Emiliania huxleyi* blooms in Norwegian coastal waters during summer and produces quite spectacular milky discolourations of the water (Tangen, 1979). Blooms of other dinoflagellates and diatoms are also common, some of them with harmful effects for fish and invertebrates; about one third of the blooms are estimated to be toxic (Reid *et al.*, 1990).

Although the complete North Sea may be considered as a more or less eutrophic sea (Beukema, 1987 cited in Duursma *et al.*, 1987), eutrophication through anthropogenic loading of nutrients affects only 5 to 10% of the North Sea (see Duursma *et al.*, 1987), mainly nearshore and estuarine areas and there is no conclusive evidence of

an increasing trend in the frequency of blooms (Mommaerts, 1985, 1986a,b).

In a survey during October 1986 in eastern areas of the southern and central North Sea the nanoplankton (2-20μm) biomass was estimated to be equal to that of the microplankton (20-200μm) (Geider, 1988). 80% of the nanoplankton was included in the 2-4μm size fraction and was mainly comprised of small flagellates and monads (Geider, 1988). Phycoerythrin-rich cyanobacteria have been reported at levels between $2.5x10^6$ and $1.7x10^8$ cells l^{-1} during summer months, with lowest numbers occurring in near-shore waters of the British coast (Howard and Joint, 1989).

Calanoid copepods constitute about 80% of the zooplankton biomass (Evans, 1973; Steele, 1974) in the North Sea. The dominant species include *Temora longicornis, Pseudocalanus elongatus, Acartia clausi, Centrophages hamatus* and *Paracalanus parvus* (Daro and Gijsegem, 1984). Copepod food ingestion seems to be insignificant in spring and autumn, when the major algal production suitable for their diet occurs. Peaks of density of copepods occur in summer, when the food source in terms of concentration, particle size and edibility is less adequate (Fransz and Gieskes, 1984). In summer months grazing rate is highest and it apparently matches the primary production. The delay between copepod production and primary production suggests about 75% of the primary production is not directly eaten by copepods. Therefore much of the photoassimilated carbon must be assumed sinks to the sea floor or is exported from the southern North Sea or is consumed by protozoa and bacteria.

The North Sea is an important fishing area, with highly developed fisheries which contribute about 5% to the world's fisheries, with herring and mackerel constituting the two most abundantly fished species (see Zijlstra, 1988). Fish production has been estimated to be around 0.85gC $m^{-2}yr^{-1}$ (Steele, 1974).

1.2.3.2. Southampton Water

Southampton Water is a typical coastal plain estuary, situated in the south coast of the United Kingdom. It is an approximately linear body of water, 10km long and 2km wide, with a dredged central

shipping channel of about 13m below mean tide. It receives water from two main tributaries at its head, the river Test on the western side and the river Itchen on the eastern side. Both these rivers contribute to 45% of the total inflow to the Solent and to 1/75 of the mean tidal prism of Southampton Water, which is estimated to be 108 and $54m^3$ at spring and neap tides respectively. Typical discharges from the Test are estimated as $25m^3s^{-1}$ in mid winter, diminishing exponentially throughout the summer to about $6m^3s^{-1}$, and the inflows from the Itchen are about half of these quantities (Webber, 1980). A third river, the Hamble, discharges nearer to the mouth and is less important. The estuary also receives sewage effluent from the urbanized area of Southampton and major industrial/chemical sites located on the west bank are the Power Station at Fawley and the Esso oil refinery.

The sediment bed is mainly mud or sandy mud, most of it entering from the sea with the tides, and extensive mudflats become exposed at low tide.

Southampton Water has been defined as a partially mixed estuary (Dyer, 1970), although its salinity structure depends greatly on the tidal stage, and to a lesser extent on the seasonal cycle of freshwater flow. At high tide, seaward from around mid-estuary the water column is almost homogeneous, with salinity increasing slowly and gradually towards the sea. Upstream, towards the head of the estuary, stratification increases. At low water, the estuary is stratified throughout. At Redbridge salinities can range from 0-25ppt during a tidal cycle. At Marchwood surface salinities can occasionally fall below 20ppt, but bottom salinities are rarely below 30ppt at high water. At Dockhead salinity is normally higher than 30ppt at the surface during summer and autumn, and can reach 35ppt at Calshot. During low water surface salinity at Calshot can fall below 30ppt during periods of high rainfall (Dyer, 1970; Phillips, 1980).

The tidal regime is described as a double high water, followed by a fast 4 hour ebb. The flood tide flows in two periods, from low water to about 3.5 hours before high water and from about 2.5 hours before high water until high water, separated by a young flood stand (Dyer, 1970). Westwood and Webber (1977) estimated that during the 13hour neap tide new water entering the estuary constituted 32% of the flood tidal prism, which reflects a reasonable degree of flushing.

Several studies have been carried out to investigate the composition and distribution of the biota of pelagic and benthic communities. Reports on phytoplankton populations show that diatoms dominate during spring (Savage, 1965; Burkill, 1978; Kifle, 1989). In summer, blooms of the phototrophic ciliate *Mesodinium rubrum* are a recurrent feature (Rees and Williams, 1982; Soulsby *et al.*, 1984; Whitfield, 1985; Wilde, 1988; Kifle, 1989; Antai, 1990; Crawford, 1991). Kifle (1989) also noted an extraordinary large abundance of microflagellates, which made up for a larger proportion of total phytoplankton biomass during the winter months.

Seasonal variations in chlorophyll *a* levels have been shown to broadly follow the seasonal cycle of temperature (Kifle, 1989), with a series of phytoplankton blooms occurring in spring followed by larger peaks during summer at the time of the red water events (Bryan, 1979; Antai, 1990; Kifle, 1989), reaching maximum values averaged for the water column of 54.3 and 19.8mg m^{-3} at Northwest Netley and Calshot Spit respectively during the year 1988. On the longitudinal axis chlorophyll *a* levels have been found to decrease from the mid part of the estuary towards the mouth (Bryan, 1979; Antai, 1990; Kifle, 1989). Bryan (1979) estimated an estuarine average annual primary production of 123gC $m^{-2}y^{-1}$ for Southampton Water, with a maximum of 450gC $m^{-2}y^{-1}$ at a station located in Marchwood Channel during a survey in 1974-1975.

The herbivorous zooplankton community is largely composed of calanoid copepods and cirripede larvae, which appear to show peaks of biomass abundance in early spring and autumn (Zinger, 1989; Lucas, 1991). During the summer, gelatinous predators, coelenterates and ctenophores, are conspicuous and possibly responsible for the decline in herbivorous zooplankton numbers (Williams, *et al.*, in prep., cited in Lucas, 1991).

Studies on the microheterotrophic community have shown that bacterial abundance in the estuary correlates with water temperature and chlorophyll *a* concentration and that the microflagellate population follows fairly closely the distribution of the bacteria, suggesting a direct trophic interaction (Antai, 1990). Aloricate ciliates have been found to be the most abundant forms of heterotrophic ciliates (Leakey, 1989). Their peaks of abundance

broadly followed those of the bacteria and microflagellates, which in turn, were highly correlated with the abundance of the phototrophic ciliate *Mesodinium rubrum*.

1.3.MATERIAL AND METHODS

1.3.1.Studies in the North Sea

1.3.1.1.Sampling protocol and sample manipulation

The study was conducted opportunistically during two cruises on board the RRV Challenger as part of the Natural Environmental Research Council's (NERC) North Sea Project. The first cruise was in March 1989 and sampling sites were located in the southern North Sea. The second cruise was in June/July 1989 and was one of the monthly series of survey cruises, including sampling sites both in southern and central North Sea waters. The sampling locations of this study are shown in Figure 1.3.1.

Water samples were collected generally at dawn from near the surface (top 10meters) with 10 liter Niskin bottles attached to a CTD rosette. Subsamples were then taken for primary production rate, respiration rate and biomass (i.e. chlorophyll *a* and cell counts) measurements in the total plankton community (unfractionated sample) and in the smaller than 3μm size fraction.

For biomass determinations in the smaller than 3μm fraction seawater was fractionated by reverse filtration (Williams, 1981). The filtration system consisted of two perspex cylinders that fitted one inside the other (Figure 1.3.2.). The diameter of the inner cylinder was 142mm and a 3μm pore sized Nuclepore filter was cemented to the perspex wall. The water sample was decanted into the outer cylinder and the inner cylinder was placed on top and allowed to settle down by gravity. The filtrate was removed by siphoning with a silicone rubber tube.

For chorophyll a measurements 100 and 300ml duplicate subsamples for the total community and for the fraction passing through 3μm filters respectively were filtered on to Whatman 25mm diameter GF/F filters, folded in half, and stored frozen in small plastic bags at -20°C. From the smaller than 3μm fraction sample a further 200ml subsample was preserved in 1% glutaraldehyde for picocyanobacteria cell counts. During the June/July cruise a Zeiss epifluorescence microscope was taken on board, and some of the cell counts (i.e. when the vessel was steady) made within a few hours of

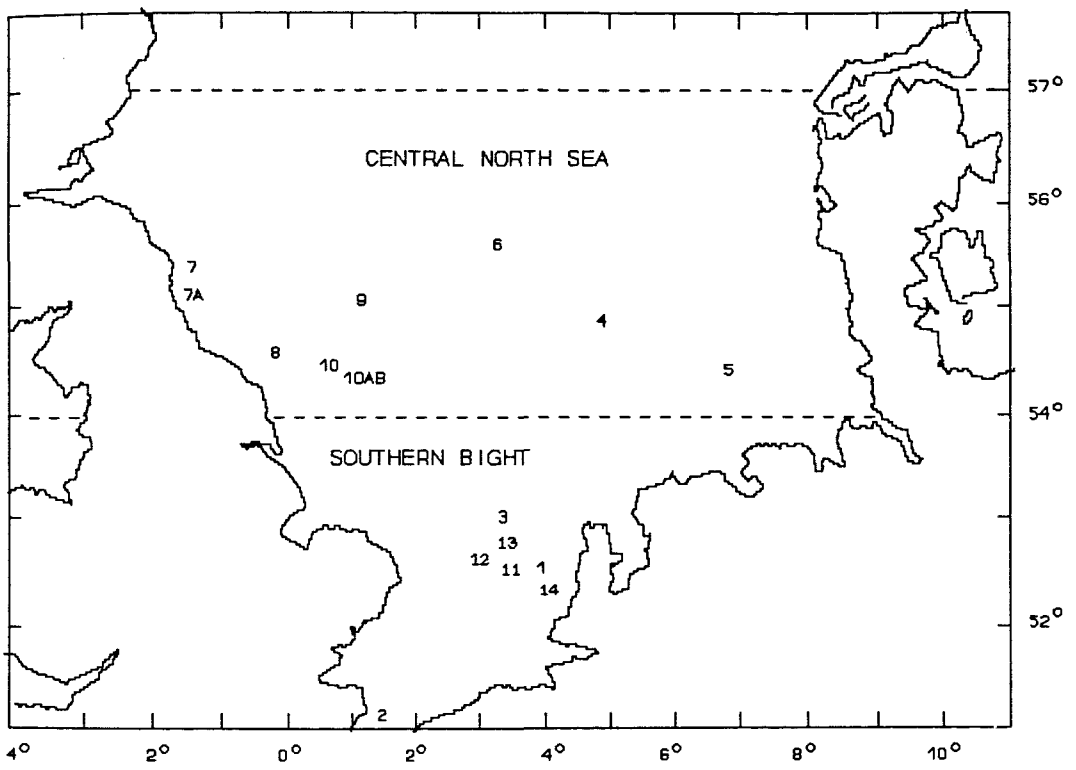

Figure 1.3.1. Map of the North Sea, showing sampling sites during studies in June/July (1 to 10AB) and in March 1989 (11 to 14).

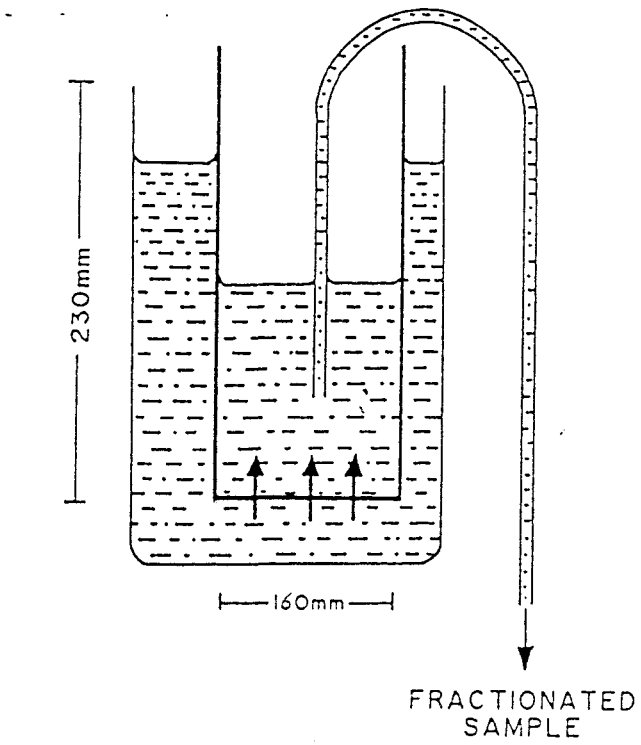

Figure 1.3.2. Diagrammatic presentation of the reverse filtration system used for size fractionation during the studies in the North Sea (from Figure 2.4 in Antai, 1990).

sampling. Chlorophyll *a* analysis and the remaining cell counts were made on returning to the shore laboratory (i.e. cell counts within three days and chorophyll a determination within two months).

i) Physico-chemical variables

Vertical profiles of temperature, salinity, and downwelling irradiance were obtained using a Neil Brown CTD system. These data have been accessed through the NERC North Sea Project Data Base. Light extinction coefficients have been estimated as the slope of the regression line (calculated from least squares regression analysis) of the natural log of the downwelling irradiance plotted against depth.

Incident irradiance was recorded continuously with two solarimeters (Kipp and Zonen) positioned on top of the bridge. Data have also been accessed through the NERC North Sea Project Data Base where values are logged as 10 minutes integrations.

Vertical profiles of nutrient (silicate, nitrate, nitrite, ammonium, phosphate) concentrations were also obtained from the NERC North Sea Project Data Base.

1.3.1.2. Chlorophyll a analysis

Chlorophyll *a* concentration was measured fluorometrically. Filters were homogenized in 90% acetone and solutions were centrifuged at 4000rpm for 5 minutes. Fluorescence was measured in an Aminco fluorocolorimeter before and after acidification and chlorophyll *a* and phaeopigment concentrations were determined according to equations given in Parsons *et al.* (1984a). The concentration of chlorophyll *a* in the standard chlorophyll *a* solution (from *Anacystis nidulans*, Sigma Ltd.) was determined spectrophotometrically in a Pye Unicam SP6 following Lorenzen's (1967) equations.

1.3.1.3. Picocyanobacteria cell enumeration

Phycoerythrin-containing chroococcoid cyanobacterial cell enumeration was carried out using a Zeiss epifluorescence microscope. A known volume of sample (10 to 50 ml) was filtered through a 25mm diameter, 0.2μm pore sized polycarbonate Nuclepore filter (a 0.45μm

Whatman cellulose nitrate membrane filter was placed underneath to act as a backing filter) at low vacuum pressure(less than 10cm Hg). The filter was then placed on a slide which was previously coated with a thin film of low fluorescing oil (Gurr Univert immersion oil) and covered with a coverslip with a drop of oil on the side facing the filter. The coverslip was gently pressed against the filter to remove excess oil, thus preventing cells from moving about. A further drop of oil was placed on top of the coverslip for objective immersion purposes.

A Zeiss microscope provided with a super pressure mercury lamp HO-200W, blue excitation filters (BG12 467889 and BG3 467888) and a plan apochromat x100 oil objective, yielding an overall magnification of x1000 was used. 30 to 100 non-overlapping fields of an eye piece counting grid (of not less than 100 cells) were counted per filter. A test was carried out to determine the replicability of the cell counts. Repeated mean counts of the same sample differed by less than 10% and were not statistically different from one another when compared (ANOVA $p<0.001$). Cell concentration was calculated from the following equation:

$$C = cF(fv)^{-1}$$

where

C= cell concentration in the sample (cell ml^{-1})

c= mean cell number per field

F= internal filter area (μm^2)

f= field area (μm^2)

v= volume of sample filtered (ml)

The internal diameter of the filter tower used was 16.62mm, which gives an internal filter area (πr^2) of $2.169 \times 10^8 \mu m^2$. The field area was determined by measuring the dimensions of the grid with an eyepiece ocular micrometer calibrated against a stage slide micrometer. For a magnification of x1000 the field area was $1.02 \times 10^4 \mu m^2$.

1.3.1.4. Primary production measurements

i) On deck incubations

Primary production was measured in on deck incubators at six light levels. These were plastic containers interconnected with a system of tubing through which surface seawater was pumped continuously to maintain the temperature at close to *in situ* levels (±2°C). Incubation bottles were fixed in the incubators by using terriclips attached to the bottom of the container. A range of natural light levels of 100, 58, 28, 19, 10 and 5.2% surface incident solar irradiance was achieved using various layers of neutral density screen (mosquito netting). Incubations were carried out from dawn to dusk.

Total plankton community primary production and respiration rate measurements were undertaken using the light and dark oxygen technique. Samples were incubated in 125ml Pyrex bottles. Five replicate samples were incubated at each light level and five were incubated in the dark. A further five bottles were fixed with Winkler reagents at the beginning of the incubation. Rates of respiration were also measured in the smaller than 3μm size fraction and fractionation was carried out by reverse filtration (Figure 1.3.2) prior to incubation. Samples were incubated in 60ml BOD bottles.

In parallel to the incubations for oxygen evolution measurements, samples were incubated for size fractionated carbon uptake measurements using the ^{14}C technique. Total plankton community samples (unfractionated) were incubated in 125ml Pyrex bottles. Two replicate samples were incubated in the light, one in the dark and one was used as a zero time control. Fractionation was carried out post-incubation by serial filtration.

A set of measurements of primary production in the smaller than 3μm size fraction was also carried out using the oxygen technique and fractionating the sample prior to incubation. These sets of measurements were performed in a light gradient box and were planned to derive photosynthetic parameters that would enable to infer possible photoadaptaive characteristics. However, the oxygen technique proved not sensitive enough to detect small changes in photosynthetic rates with relatively small changes in irradiance in this fraction and the data obtained are considered unreliable. These data have not been

included in this thesis.

ii) Oxygen evolution measurements: the Winkler titration technique

Incubation bottle volume calibration

The nominal volume of each individual bottle was determined to the nearest 0.001 ml by weighing each bottle empty and then full of distilled water. The water temperature was recorded after each weighing and all volumes normalized to 25°C according to the following equation defining the expansivity of glass with temperature:

$$V_{t1} = V_{t0}(1+3\alpha(t_1-t_0)$$

where

V_{t1} = the volume (ml) at the incubation temperature t_1 (°C)
V_{t0} = the volume (ml) at 25°C (t_0)
α = the coefficient of expansivity (3.2×10^{-6} for pyrex)

To minimize variations in temperature weighing was carried out in a constant temperature room, where the distilled water was allowed to stand for a few hours prior to weighing.

Filling and fixing bottles

Bottles were filled with seawater by siphoning through a silicone rubber tube, allowing each to overflow at least twice the bottle volume and avoiding the introduction of any bubbles. Care was taken to flush all bottles evenly to obtain a uniform distribution of the algal population.

Samples were fixed by successive additions of 1 or 0.5ml (depending on whether 125ml or 60ml bottles were used) of manganous chloride and an alkaline iodide (solutions were prepared according to Carrit and Carpenter, 1966). Bottles were then carefully stoppered to avoid air bubbles being trapped and contents mixed immediately by inverting. The precipitate of manganese hydroxide thus formed was allowed to settle to at least half way down the height of the bottle before titration. Bottles were stored under water prior to titrating to avoid air drying out the stoppers.

Winkler titration

Immediately before titrating each sample 1 or 0.5 ml (depending on the bottle volume) of 10N sulphuric acid was added to liberate the tri-iodide ion. A small magnetic stirring bar was used to mix the contents. The procedure followed was based on a whole bottle titration and a photometric end point detection, based on the disappearance of the tri-iodide ion and subsequent increase in the transmission of light in the near ultraviolet band as the acidified sample is titrated against a solution of thiosulphate (Bryan *et al.*, 1976). Thiosulphate was added with an automated microburette (Dosimat 655) controlled by a Hewlett Packard 85 microprocessor (Williams and Jenkinson, 1982).

Standardization of thiosulphate

Since sodium thiosulphate is not a primary standard, calibration of the thiosulphate solution was carried out regularly. A set of five 60ml BOD bottles were half filled with distilled water. To each bottle *ca.* 10ml of a solution of 0.01N potassium iodate was added. The exact volume of iodate added was determined by weight. Pre-weighed samples were prepared prior to going to sea. 0.5 ml of sulphuric acid followed by a further 0.5ml of alkaline iodide were then added to each bottle followed by mixing the contents by gentle swirling between each addition. The bottles were stoppered and stored in the dark for 15min to allow the iodine to be released. The bottles were then filled to the neck with distilled water, carefully rinsing the stoppers into the bottles. The titration procedure was as described for the samples. The normality of the thiosulphate solution was calculated from the following expression :

$$N_T = (V_I\ N_I)/V_T$$

where

N_T = the normality of the thiosulphate solution (N)

V_I = the volume of iodate (ml)

N_I = the normality of the iodate solution (N)

V_T = the volume of thiosulphate added (ml)

Calculation of oxygen concentration

Oxygen concentration was calculated with the aid of a computer program from the following expression :

$$O = 10^6(V_T\ N_T)/(4(V-V_R)$$

where

O = the oxygen concentration (μM)

V_T = the volume of thiosulphate added (ml)

N_T = the normality of the thiosulphate solution (N)

V = the bottle volume (ml)

V_R = the volume of reagents (manganous chloride and alkaline iodide) added (ml)

The bottle volume was corrected for the expansivity of glass at the incubation temperature with the computer programme.

Calculation of gross, net photosynthesis and respiration

With the oxygen technique gross photosynthesis was estimated as the difference between the mean oxygen concentration in the light and the mean oxygen concentration in the dark incubated bottles. Dark respiration was calculated as the difference between the mean zero-time fixed and the mean dark incubated bottle oxygen concentrations. The difference between gross photosynthesis and dark respiration is referred to as net photosynthesis.

iii) Carbon uptake determination: the ^{14}C technique

The method used was based on that introduced by Steemann Nielsen (1952) and details of the protocol adopted are given below.

Outline of protocol

After filling the bottles, 1ml of sample was removed from each bottle in order that no overflow would occur when adding the ^{14}C solutions. Immediately before beginning the incubation, 0.2ml of a stock solution of ^{14}C-sodium bicarbonate (10μCi ml^{-1}) was added to each bottle . At the end of the incubation period the contents of the bottles were filtered at low vacuum pressure (less than 10cmHg) and filters were rinsed with a small volume (*ca.* 5ml) of filtered

seawater. Filters were then stapled onto record cards and acid fumed by placing them in a desiccator containing a small beaker with concentrated hydrochloric acid for 2 minutes, in order to remove any remaining inorganic radiolabelled carbon. Filters were left in a fume cupboard to dry and then transfered to scintillation minivials and stored in a dessicator until returning to the university laboratory. For radioactivity counting, 5.5ml of a scintillation cocktail (Unisolve 1, Koch Light Ltd.) was added and radioactivity was measured on a Beckman L.S. 3100 series scintillation counter. Counting efficiency was determined by the external standards channel ratio method. The quench correction curve used was produced by Garcia (1989).

Duplicate zero-time blanks were determined by filtering samples immediately after adding the ^{14}C -sodium bicarbonate solution.

Fractionation procedure for ^{14}C measurements

Samples were filtered through 47mm, 3μm Whatman cellulose-nitrate filters (in measurements undertaken during the March cruise and on experiments 1 and 2 during the June/July cruise polycarbonate Nuclepore filters were used). Filtrates were collected in a beaker and re-filtered through a 0.2μm polycarbonate Nuclepore filter.

Preparation of stock ^{14}C-bicarbonate solution

An ampoule of $Na_2{}^{14}CO_3$ (Amersham International) was diluted in a solution containing 3.5% w/v NaCl, 0.3g l^{-1} anhydrous sodium carbonate and one pellet of sodium hydroxide in deionized water, to a final specific activity of 10μCi ml^{-1} (Garcia, 1989). The solution was then transferred into glass ampoules that were sealed by heating under a flame torch. After cooling, ampoules were sterilized by autoclaving them and sealing was checked by placing the ampoules in a beaker containing a strong solution of the dye methylene blue during autoclaving. Ampoules which contents became coloured were discarded. The sealed vials were then irradiated overnight under UV light in order to remove any possible radiolabelled organic carbon present (Williams *et al.*, 1972).

Calculation of carbon uptake rates

Rates of carbon fixation into particulate material were estimated from the following expression:

$$C = (A_F W) / (A_T t) \qquad \text{Eq (1)}$$

where

C = the carbon uptake rate (μmol C $l^{-1}h^{-1}$)

A_F = the activity in the filter corrected for blank (dpm)

W = the total inorganic carbon present in the seawater (μM) and was estimated from the salinity (S) expressed in units of ppt following Parsons *et al.* (1984a) as $W = 0.96\ (0.067S-0.05)\ 10^3$

A_T = the total activity in the bottle (dpm)

t = the incubation time (hours)

^{14}C excretion rates

The rate of excretion was determined as the rate of extracellular release of radiolabelled organic carbon. After filtering the contents of the bottles through 0.2μm filters, approximately 20ml duplicate samples of each filtrate solution were placed in scintillation vials and frozen at -20°C. On return to the departmental laboratory, samples were thawed and acidified to pH 2 with 37% phosphoric acid to transform all inorganic carbon to carbon dioxide and this then purged by bubbling with air for 2 hours. After bubbling, 5ml subsample of the solution was mixed with 15ml of the scintillation cocktail and radioactivity was counted as for the filters. Rates of excretion were calculated by substituting A_F in equation (1) for the activity (dpm) in the filtrate.

iv) P v I curve fitting: parameter calculation

Rates of photosynthesis (i.e. gross oxygen production rate for oxygen data and carbon fixation rate for ^{14}C data) at the corresponding photon flux densities were fitted by non-linear least square regression analysis, using a modified Marquardt procedure for minimization of sum of squares (Nash, 1979), to a curve defined by the following mathematical expression (Platt *et al.*, 1980):

$$P = P_s\ (1-e^{-\alpha I/Ps})e^{-\beta I/Ps} \qquad \text{Eq (2)}$$

where

P = the rate of gross photosynthesis (μmol(O_2 or C) $l^{-1}h^{-1}$) at a given light intensity (I in $\mu Em^{-2}s^{-1}$)

P_s = the potential light saturated rate of gross photosynthesis (μmol(O_2 or C) $l^{-1}h^{-1}$)

α = the initial linear light-limited slope (μmol(O_2 or C) $l^{-1}h^{-1}(\mu Em^{-2}s^{-1})^{-1}$)

β = the negative slope at high irradiance levels (μmol(O_2 or C) $l^{-1}h^{-1}(\mu Em^{-2}s^{-1})^{-1}$)

The parameter P_s only represents the actual maximum rate of photosynthesis (P_{max}) when there is no photoinhibition, i.e. the parameter β equals 0. P_{max} (in units of μmol(O_2 or C) $l^{-1}h^{-1}$) is calculated from the following expression (Platt *et al.*, 1980):

$$P_{max} = P_s \left(\frac{\alpha}{\alpha+\beta}\right) \left(\frac{\beta}{\alpha+\beta}\right)^{\beta/\alpha}$$

Other useful parameters to describe the *P v I* curves were also derived:

I_k, the irradiance for saturation of photosynthesis ($\mu Em^{-2}s^{-1}$), was calculated as

$$I_k = P_{max}/\alpha$$

I_c, the compensation irradiance for photosynthesis ($\mu Em^{-2}s^{-1}$), defined as the photon flux density at which net photosynthetic rate equals zero (i.e. gross photosynthetic rate = respiration rate) was determined as

$$I_c = R/\alpha$$

where

R = the respiration rate (μmolO_2 $l^{-1}h^{-1}$)

I_m, the irradiance at which maximum rate of photosynthesis is achieved ($\mu Em^{-2}s^{-1}$) was calculated as

$$I_m = \frac{Ps}{\alpha \log_e \left(\frac{\alpha+\beta}{\beta} \right)}$$

I_b, the irradiance ($\mu Em^{-2}s^{-1}$) at which the rate of photosynthesis is reduced to 0.37Ps was used as an index of photoinhibition.

Because the value of β is scaled to the value of Ps, I_b was considered a more suitable photoinhibition index for intercomparison purposes.

v) Estimation of water column integrated rates of primary production

Gross oxygen production and carbon fixation rates obtained in the on deck incubations at the corresponding irradiances were fitted to equation 2 (Platt *et al.*, 1980) as described in subsection iv) in this section. The parameters Ps, α and β thus obtained were used to estimate gross photosynthetic rate (Pzt) at the irradiance at a given depth and time of the day (Izt) from the expression

$$Pzt = Ps\ (1-e^{(-\alpha Izt/Ps)})\ e^{(-\beta Izt/Ps)}$$

Izt was calculated from the expression

$$Izt = Iot\ e^{-kz}$$

where

Iot = the incident irradiance (PAR) at the sea surface at a given time ($\mu Em^{-2}s^{-1}$)

k = the light (PAR) extinction coefficient (m^{-1})

z = the depth in the water column (m)

Integrated daily rates of primary production and respiration were calculated only for the portion of the water column that was well mixed, i.e. in which the plankton community is likely to have been roughly homogeneously distributed. In most locations, in the southern Bight, the mixed layer includes the entire water column.

Daily mixed layer integrated rates of gross primary production were determined from the expression

$$P_g = \int_{z=0}^{z=m} \int_{t=0}^{t=d} P_{zt}\, dz\, dt$$

where

P_g = surface mixed layer integrated daily rate of gross photosynthetic rate (mmol(O_2 or C) $m^{-2}d^{-1}$)

z = the depth in the water column (m)

m = the depth of the mixed layer (m)

t = time (30 minute intervals)

d = the illuminated period of the day (sum of 30 minute intervals)

P_{zt} = gross photosynthetic rate (μmol(O_2 or C) $l^{-1}h^{-1}$)

From the oxygen evolution data, the mixed layer integrated daily rate of respiration was estimated assuming dark respiration rate to be constant throughout the entire day and over the whole mixed layer as follows:

$$R_d = 24mR$$

where

R_d = mixed layer integrated daily rate of respiration (mmolO_2 $m^{-2}d^{-1}$)

m = the depth of the mixed layer (m)

R = respiration rate (μmolO_2 $l^{-1}h^{-1}$)

Daily mixed layer integrated rates of net primary production (P_n in mmolO_2 $m^{-2}d^{-1}$) were determined as

$$P_n = P_g - R_d$$

1.3.2. Studies in Southampton Water

1.3.2.1. Sampling protocol and sample manipulation

i) Seasonal sampling

Sampling was performed from January to October 1990 twice a month (unless hindered by bad weather conditions) on board of the departmental vessel *Labrax* at two selected stations, Northwest Netley buoy, located approximately in the middle of the estuary, 1.5km south-east of the confluence of the Test and Itchen sub-estuaries and Calshot Spit buoy, situated at the mouth of Southampton Water (approximately 8km south-east of NW Netley), in the mid-Solent (Figure 1.3.3). Water samples were collected at high tide with a Van Dorn type bottle from three depths (1, 4, 6m at NW Netley and 1,4,8m at Calshot Spit).

For each station, water collected from the three different depths was mixed in a 25 litre Nalgene carboy. This composite sample was then siphoned into 125ml Pyrex bottles and, on return to shore, bottles were incubated for size-fractionated carbon uptake measurements in the on deck incubators described in section 1.3.1.4, placed on the pier of the marina, for a period of four hours. The incubators were supplied with surface seawater at close to *in situ* temperature.

Size fractionated primary production was measured with the ^{14}C technique. Two replicate samples for each light level were incubated and zero time controls were used as blanks. Fractionation was carried out post-incubation by serial filtration.

From the same three depth composite sample duplicate subsamples of 100ml were filtered onto 25mm GF/F filters and stored frozen at -20°C for chlorophyll *a* determination in the total plankton community (i.e. unfractionated sample). Filtrates were stored frozen at -20°C in polyethylene bottles for later phosphate and nitrate plus nitrite analysis. From the depth composite samples further 200 to 500ml duplicate subsamples were stored in a fridge for later filtration (through a series of polycarbonate Nuclepore filters) in the laboratory for size fractionated chlorophyll *a* determination. A further 200ml subsample was preserved in 1% glutaraldehyde for

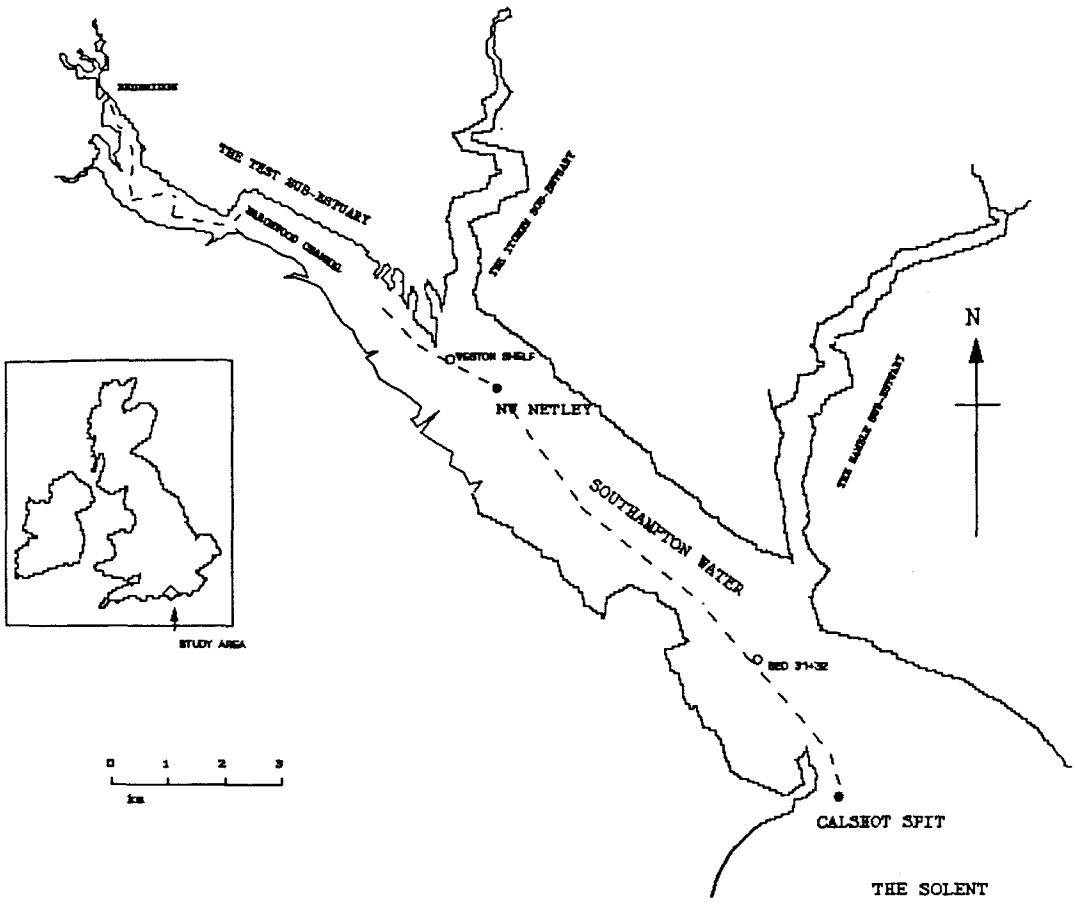

Figure 1.3.3. The area of Southampton Water. NW Netley and Calshot Spit were the routine seasonal sampling sites. The dashed line indicates the route followed during sampling on September 3rd 1990. Two of the navigation buoys sampled in the transect (Weston Shelf and Bed 31+33) are also shown.

picoplankton cell counts. After the incubation, productivity bottles were also transported to the university laboratory for the size fractionated filtration.

Physical variables

Vertical profiles of temperature and salinity were obtained with a digital field salinometer (model MK1) provided with a temperature/salinity probe. The probe was lowered attached to the sampling bottle. Secchi disk depths were also recorded routinely except during samplings in January, February and March, when light extinction coefficients were estimated from downwelling irradiance data obtained with a submersible irradiance sensor and kindly provided by Mr. B.Booty.

ii) Sampling along a longitudinal axis of the estuary

On one occasion on September 3rd 1990 sampling was performed along a longitudinal axis of the estuary from Redbridge (in the Test estuary) to Calshot Spit (The Solent) (Figure 1.3.3). Seawater was pumped continuously from just below the surface into a plastic beaker in which the temperature/salinity probe was immersed. The overflow from the beaker was collected in opaque Duran glass bottles. Samples were taken for prokaryotic and eukaryotic picophytoplankton cell counts, chlorophyll *a* determinations (both, for the total plankton community (unfractionated sample) and for various size fractions) and phosphate, nitrate and nitrite analysis following the protocol described for the routine seasonal sampling.

1.3.2.2. Insolation

Incident solar irradiation was measured during productivity incubation periods with a Kipp and Zonen solarimeter placed on the roof of a porta-cabinet at the university shore-side laboratory (ca.100m from the incubation site) attached to a chart recorder. On three occasions measurements were made with a Macam radiometer/photometer, on dates when the solarimeter was unavailable. The solarimeter and the Macam sensor were intercalibrated. Total photon flux density for the incubation period was calculated by

integrating the light data recorded every 2.5 minutes.

Data on total daily incident solar irradiation in the area for the whole year (1990) was obtained from the Meteorological Office. These were values recorded at a meteorological station based at Lymington (approximately 15km west of the sampling area).

1.3.2.3. Nutrient analysis

The concentration of soluble reactive phosphorus was measured following the method by Murphy and Riley (1962), as detailed in Parsons *et al.* (1984a). A standard solution of phosphate made up in deionized water was used to produce a calibration curve, from which the concentration in the samples could be estimated. Absorbance was measured at 885nm in a PYE Unicam SP6-350 spectrophotometer. The detection limit of the method was 0.03μM. This analysis was performed by Mr. D. Kifle.

Nitrate plus nitrite concentration was determined using a FIA (Flow Injection Analysis) system (Johnson and Petty, 1983). The method is based on the reduction of nitrate to nitrite by running the sample through a copper-cadmium reduction column (Wood *et al.*, 1967). Nitrite is measured by diazotizing with sulphanilamide and coupling with N-(1-naphtyl)-ethylenediamine to form an azo dye that can be detected spectrophotometrically at 520nm (Bendscheneider and Robinson, 1952). A series of standard solutions of nitrate were made up to produce a calibration curve from which the concentration in the samples could be estimated. To avoid problems related to the refractive index arising from variations in salinity, the standard solutions of nitrate were made up in 0.7M sodium chloride (prepared in MilliQ water). To check for the efficiency of the reduction column, standard solutions of nitrite of the same molarity as nitrate were run regularly. The system used was a flow injection analyser from Chemlab Instruments Ltd. attached to a chart recorder. This system was capable of measuring nitrite concentration between around 0.5 to 25μM. When samples contained nitrate concentrations greater than 25μM, appropriate dilutions with the 0.7M sodium chloride solution were prepared.

1.3.2.4.Chlorophyll a analysis

Chlorophyll *a* was determined fluorometrically from 90% acetone extracts. GF/F filters were processed as described in section 1.3.1.2. Chlorophyll *a* from polycarbonate filters was extracted by leaving filters in 90% acetone for 24h in the fridge. The rest of the procedure was as for the GF/F filters (see section 1.3.1.2)

1.3.2.5.Picophytoplankton cell enumeration

Glutaraldehyde preserved samples were filtered through 3μm (occasionally 5μm) polycarbonate Nuclepore filters. From the filtrates, orange fluorescing picocyanobacteria and red fluorescing eukaryotic cell counts were performed according to the procedure described in section 1.3.1.3. Counts were made within 24 to 30h after sampling. The term picophytoplankton is used in its broadest sense in this study and phytoplankters passing through up to a 5μm filter are included.

1.3.2.6.Carbon uptake and excretion measurements

The method used was based on the ^{14}C technique and details of the technique are given in section 1.3.1.4. For this set of measurements a new biodegradable scintillation cocktail was used, Optiphase HiSafe 3 (Pharmacia Ltd.) and a new quench curve was derived (see subsection ii) below). The size fractions investigated and the fractionation procedure used were slightly modified.

i) Serial filtration system

The filtration system consisted of three filtration units fitted in a cascade, one above the other by clamping them to a retort stand. Each filtration unit consisted of a filter holder for 47mm diameter filters mounted onto a separating funnel provided with an inlet for pumping requirements. From top to bottom, 3 (occasionally 5), 1 and 0.2μm polycarbonate Nuclepore (on two occasions Isopore) filters were used. Each filtration unit was operated with a different pump. Filtration pressure for the 3(5) and 1μm filters never exceeded 2cmHg and for the 0.2μm it was never more than 10-15cmHg. A 20ml

subsample from the filtrate was stored frozen for the determination of excretion rate of the total plankton community.

The system was used both for chlorophyll *a* and ^{14}C size fractionated filtration.

ii) Quench correction curve

A quench correction curve was produced based on the method of Reunanen and Soini (1974). A commercially prepared standard kit of capsules containing solid pellets of ^{14}C labelled sucrose was obtained from LKB Wallac. The capsules contained a known amount of radioactivity (cpm). Each capsule was dispensed into a scintillation minivial and 5.5 ml of scintillation cocktail Optiphase Hisafe 3 (Pharmacia Ltd.) were added. Increasing amounts of a chemical quenching agent, carbon-tetrachloride, were then added, so as to obtain a range of external standard channels ratios (ESCR) normally found in experimental samples, i.e. between 0.35 and 0.55. The contents of the vials were well mixed by vigorous shaking and when the sucrose was fully dissolved, radioactivity was counted (cpm) in a Beckman L.S. 3100 series counter.

From the cpm values the percentage efficiency of counting was calculated. From a linear fit of the plot of % eficiency *versus* ESCR (Figure A.1 in Appendix) by least square regression analysis, a quench correction curve of the type:

$$y = -30.062+142.38x \qquad (r= 0.984,\ n=20,\ p<0.001)$$

where

x= E.S.C.R.

y= efficiency (%)

was obtained. Counting of the same samples was repeated three times with a lapse of a month between the first and last counting and quench correction curves obtained for each one were not statistically different.

iii) Estimation of water column integrated rates of primary production

During the survey in Southampton Water information on daily incident irradiance was only available as an integral value for the

day and so, it was not possible to apply the method used for the North Sea data. Instead the following procedure was adopted. With the light extinction coefficient data the depths of 100, 58, 28, 10, 5.2% incident irradiance were estimated. To obtain values of whole water column production, the carbon fixation rate at the bottom of the water column (10m at Calshot Spit and 8m at NW Netley) was estimated. For this purpose, the equation of Platt *et al.* (1980) was fitted to the P *v* I data as described in section 1.3.1.4 and, from the photosynthetic parameters thus obtained, carbon uptake rate was estimated at the irradiance reaching the bottom of the water column. The irradiance at the bottom of the water column was calculated from the light extinction coefficient values. Total rates of carbon fixation for the experimental incubation time interval were then integrated with depth down to the bottom of the water column by planimetry. Daily integrated rates were calculated by multiplying by a factor expressing the ratio between daily available light and light available during the experimental incubation as recommended by BIOMASS (O'Reilly and Thomas, 1983).

1.4. RESULTS

1.4.1. Seasonal study in Southampton Water

1.4.1.1. Temperature and salinity distribution

The mean water column temperature recorded from January to October varied between 8.4 and 20.8°C, and was always slightly higher at NW Netley than at Calshot Spit.

The mean water column salinity at high tide for the same period ranged from 30.8 to 34.8ppt and followed fairly closely the cycle of temperature, with lowest values being recorded in winter, i.e. at times of most intense rainfall and river runoff. Temperature and salinity were well correlated ($r=0.9$, $n=26$, $p<0.001$).

Vertical profiles of both salinity and temperature showed the water column to be well mixed at high tide throughout the entire sampling period at Calshot Spit (Table A1 and Table A2 in Appendix). At NW Netley, however, a salinity gradient of up to 5.5ppt in the top 4m was consistently found during the winter months, up to the first week of April. Temperature distribution was rather more homogeneous with depth and the vertical gradient was for most of the year not large enough to induce an effective thermal stratification. Exception was during some exceptionally hot days in late July and early August (air temperatures of up to 24.6°C), when a temperature difference of 1.4°C was measured between 1 and 6m.

1.4.1.2. Insolation and water transparency

Irradiance data was obtained from the Meteorological Office. The monthly averaged total daily irradiation for the area ranged from 4.66 to 42.82 $Em^{-2}d^{-1}$ (57 to 523gcal $cm^{-2}d^{-1}$) during the study period (Table 1.4.1.1). Riley (1967) suggested phytoplankton need a threshold surface mixed layer irradiance of *ca.* 40gcal $cm^{-2}d^{-1}$ (*ca.* 3.27 $Em^{-2}d^{-1}$) for a pronounced increase in growth rate. The mean surface mixed layer irradiance was calculated according to the equation given by Sinclair *et al.* (1978). At Calshot Spit, in March this parameter reached values of only 2.37 $Em^{-2}d^{-1}$ (29gcal $cm^{-2}d^{-1}$) and increased to values of 5.16 and 10.15 $Em^{-2}d^{-1}$ (63 and 124gcal $cm^{-2}d^{-1}$) as averaged

for the first and second half of the month of April respectively. At NW Netley, however, because the surface mixed layer was shallower than at Calshot Spit during the winter, due to the salinity gradient, already during the second half of the month of February irradiation reached a mean value of 3.35Em^{-2}d^{-1} (41gcal cm^{-2}d^{-1}) in the top 4m and was in excess to the critical value of 40gcal cm^{-2}d^{-1} (Riley, 1967) by the first half of March, 5.65Em^{-2}d^{-1} (69 gcal cm^{-2}d^{-1}).

Values of Secchi disk depth (Z_S) in units of metres were converted to light extinction coefficients (k) in units of per metre as recommended by Pilgrim (1987), where $k = \frac{1.5}{Zs}$ (Figure 1.4.1.1). At Calshot Spit values of extinction coefficient were highest during winter and autumn and during these periods the water column was less transparent than at NW Netley. NW Netley, however, also exhibited relatively high values during the summer, which were higher than at Calshot Spit. This showed the major influence of abiotic particles, i.e.wind driven resuspended bottom sediments, during winter and autumn, winds being stronger in the more exposed outer area of the estuary represented by Calshot Spit, and the switch during the biologically productive periods of the year, spring and summer, to a stronger dependance of the water transparency on the biotic standing stock (see below for results of phytoplankton biomass at the two stations).

1.4.1.3.Nitrate+nitrite and phosphate concentration

Nitrate+nitrite concentration was measured in duplicate samples and the standard difference of the duplicate values expressed as a percentage of the mean was on average 0.5%. The seasonal distribution of nitrate+nitrite concentration as measured in the three depth composite water samples is presented in Figure 1.4.1.2 and in Table A.3 in Appendix. Values varied between 2.9 and 29.7μM at Calshot Spit and 1.8 and 65.2μM at NW Netley. Both stations were characterized by high winter and lower summer concentrations. A major drop in concentration was recorded from February to March. At NW Netley, minimum values were recorded during the major algal blooms, i.e. the *Phaeocystis* sp. bloom during the second week of May and during the red water event in the first week of August. The low values during the

Table 1.4.1.1. Daily total suface incident irradiation (I_t) averaged for each month and daily total irradiation averaged for the surface mixed layer (I_{Zm}) determined as the two weekly mean ($Em^{-2}d^{-1}$) at Calshot Spit and NW Netley.

Month	I_t	I_{zm} Cal	I_{zm} Net
January	4.83	nd	nd
		0.32	nd
February	9.66	nd	nd
		nd	3.35
March	20.38	2.37	5.65
		nd	nd
April	32.99	5.16	13.02
		10.15	12.93
May	42.82	8.18	8.76
		8.43	9.33
June	30.45	4.17	4.66
		6.79	5.97
July	42.33	7.53	14.65
		nd	nd
August	34.71	8.27	9.99
		4.91	6.38
September	24.64	nd	nd
		2.21	2.94
October	12.44	1.96	3.19
		nd	nd
November	7.45	nd	nd
		nd	nd
December	4.66	nd	nd
		nd	nd

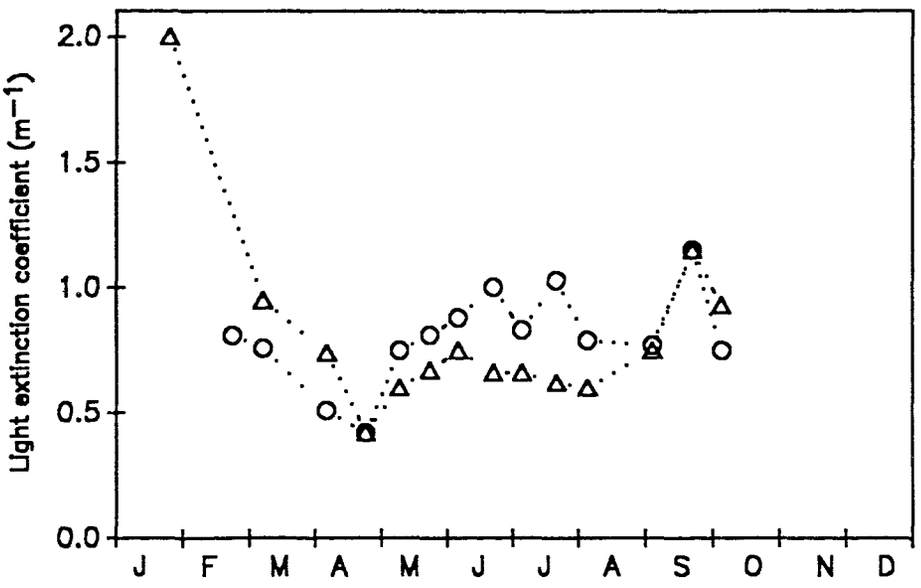

Figure 1.4.1.1. Seasonal distribution of the light extinction coefficient at NW Netley (o) and Calshot Spit (Δ).

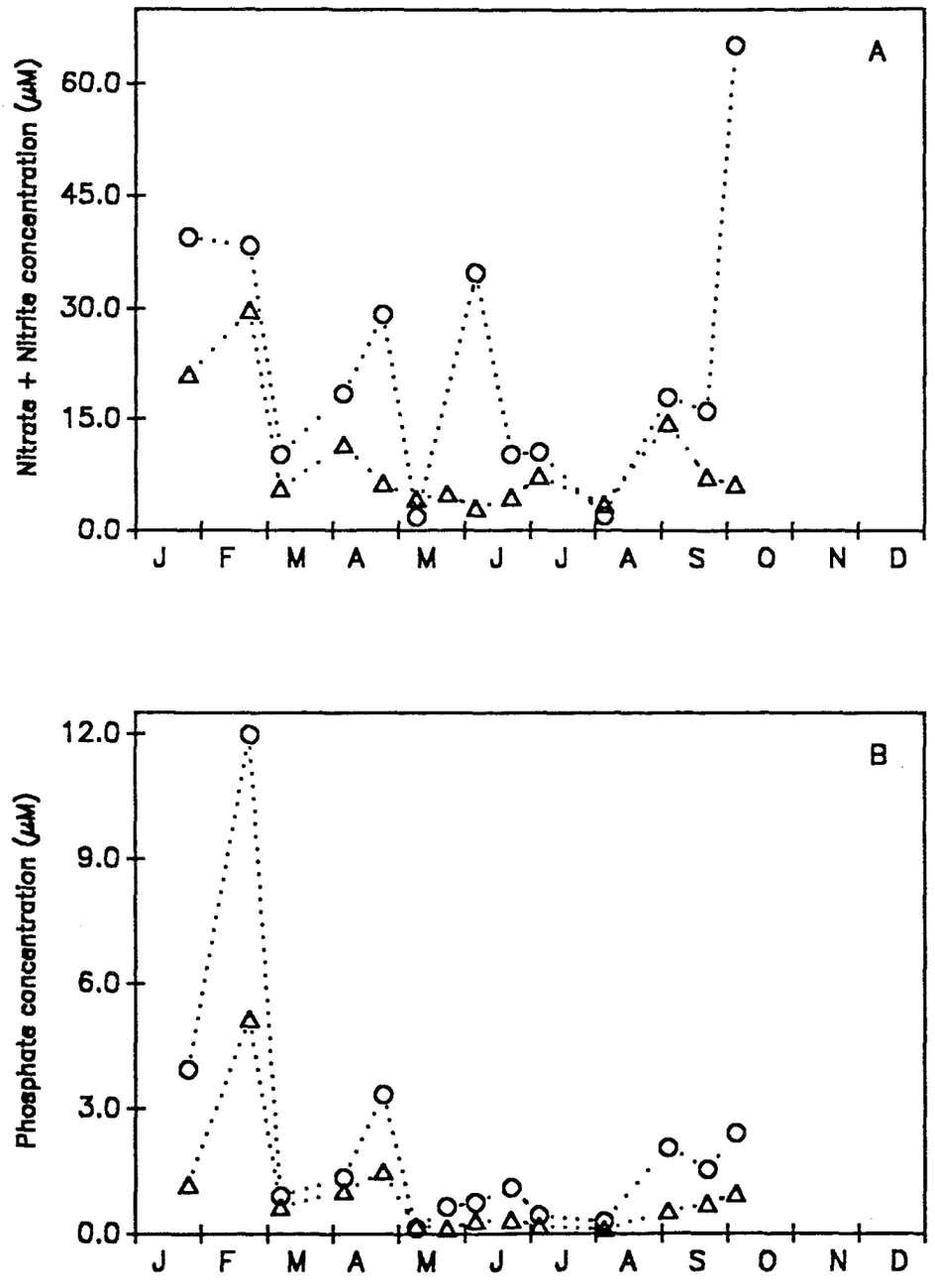

Figure 1.4.1.2. Seasonal distribution of (A) nitrate plus nitrite concentration and (B) phosphate concentration as measured in the three depth composite samples collected at NW Netley (o) and Calshot Spit (Δ).

Phaeocystis sp. bloom were followed by a substantial increase during the first week of June. At Calshot Spit, concentrations showed a smaller increase from April to August. Except on the occassion of the two minima, values at NW Netley were higher than at Calshot Spit.

Figure 1.4.1.2 illustrates the seasonal distribution of phosphate concentration at the two stations. Data are listed in Table A.3 (Appendix). Values ranged from 0.1 to 5.1μM and from 0.1 to 11.9μM at NW Netley and Calshot Spit respectively. Both stations exhibited a very similar pattern of variation in phosphate concentration. As for nitrate+nitrite concentration highest values were recorded in February, followed by a marked decline in March and a subsequent increase, to produce a relatively significant peak, in April. Phosphate concentration was then greatly reduced during the sampling visits in which *Phaeocystis* sp. was in bloom, the last week of April at Calshot Spit and the first week of May at NW Netley. At NW Netley, values exhibited a small recovery during June, but declined markedly again during the red water event in early August. The concentration increased again towards autumn. At Calshot Spit values were low and relatively more constant than at NW Netley from May to August.

1.4.1.4.Total chlorophyll a and phaeopigment concentration

Total chlorophyll *a* concentration was measured on duplicate unfractionated samples collected from January to October at both stations. The standard difference of the duplicate values expressed as a percentage of the mean averaged for all meassurements was 3.8% (SD 4.1). At Calshot Spit the seasonal distribution of chlorophyll *a* in unfractionated samples (Figure 1.4.1.3; Table A.4 in Appendix) showed low winter values followed by a spring peak during the last week of April and intermediate concentrations during summer, beginning to drop to pre spring-bloom levels by October. Although no visible water discolouration was apparent during the spring maximum at Calshot Spit, microscopic examination revealed the spring peak of chlorophyll *a* to be dominated by the prymnesiophyte algae *Phaeocystis* sp. and chain forming diatoms of the genus *Chaetoceros* (D. Kifle, personal communication). At NW Netley winter values were also low and the spring peak was not recorded until the first week of May (Figure

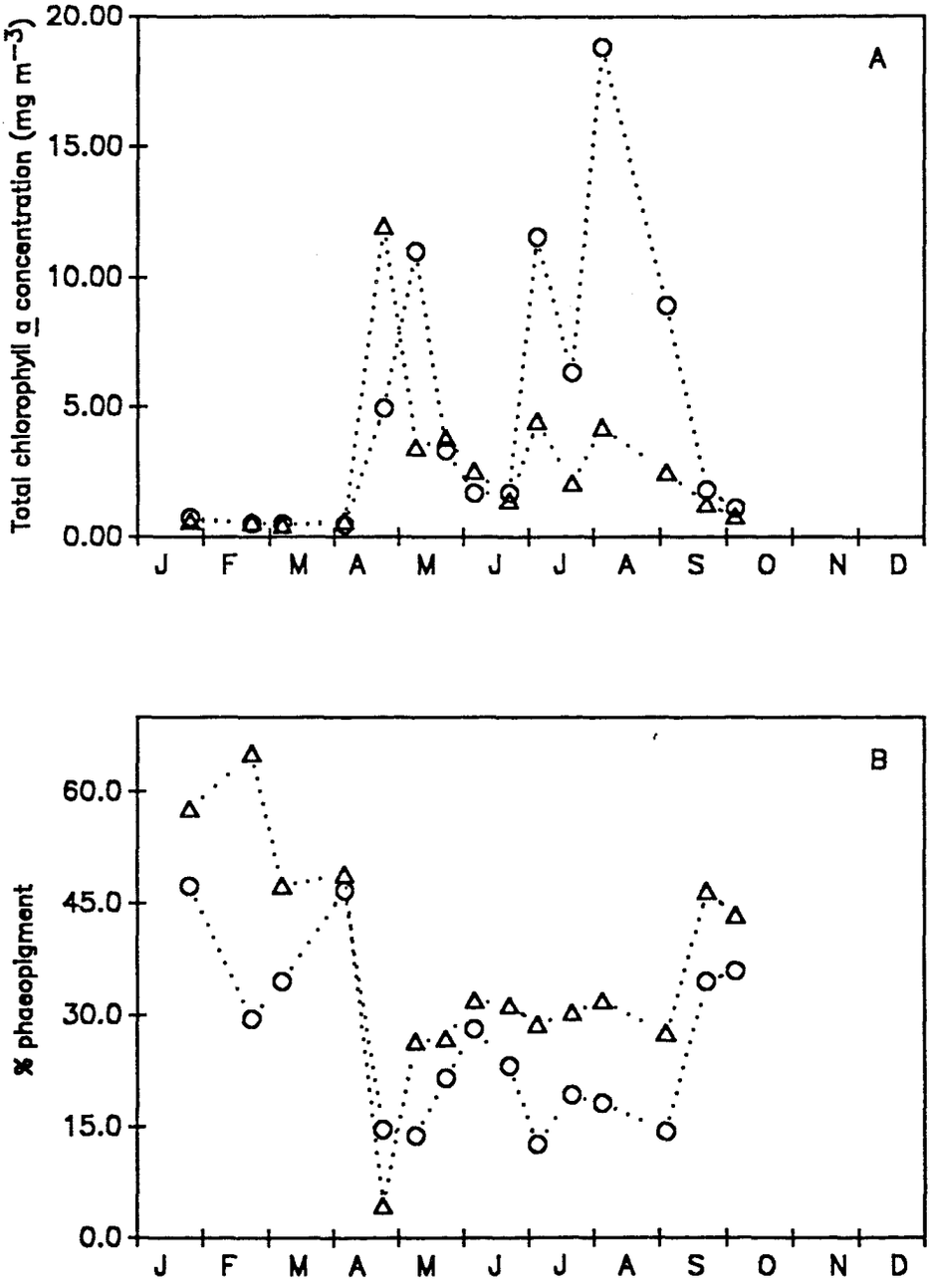

Figure 1.4.1.3. Seasonal distribution of (A) chlorophyll *a* concentration and (B) percentage phaeopigment as measured in the total plankton community (i.e. unfractionated sample) at NW Netley (o) and Calshot Spit (Δ).

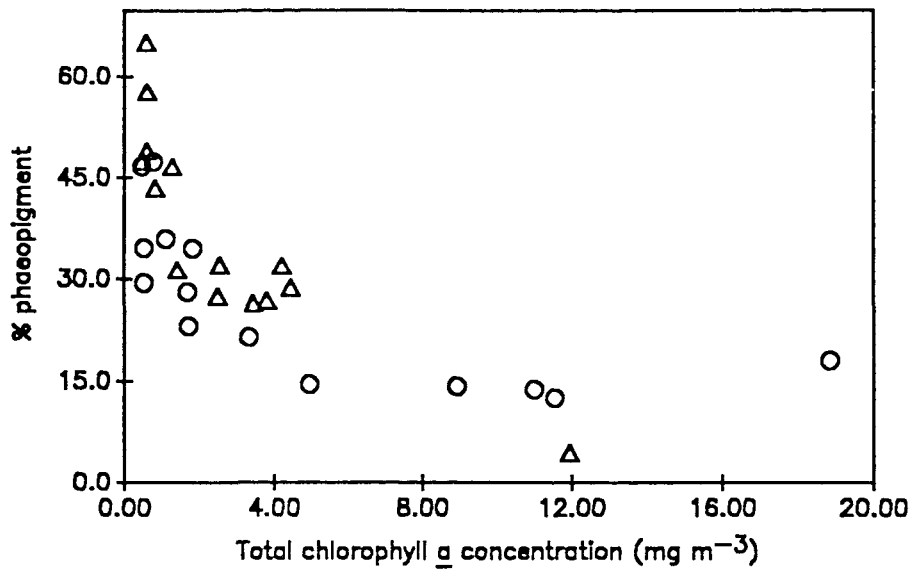

Figure 1.4.1.4. Relationship between the percentage phaeopigment and the chlorophyll *a* concentration in the total plankton community (i.e. unfractionated sample) determined from samples collected during the seasonal survey at NW Netley (o) and Calshot Spit (Δ).

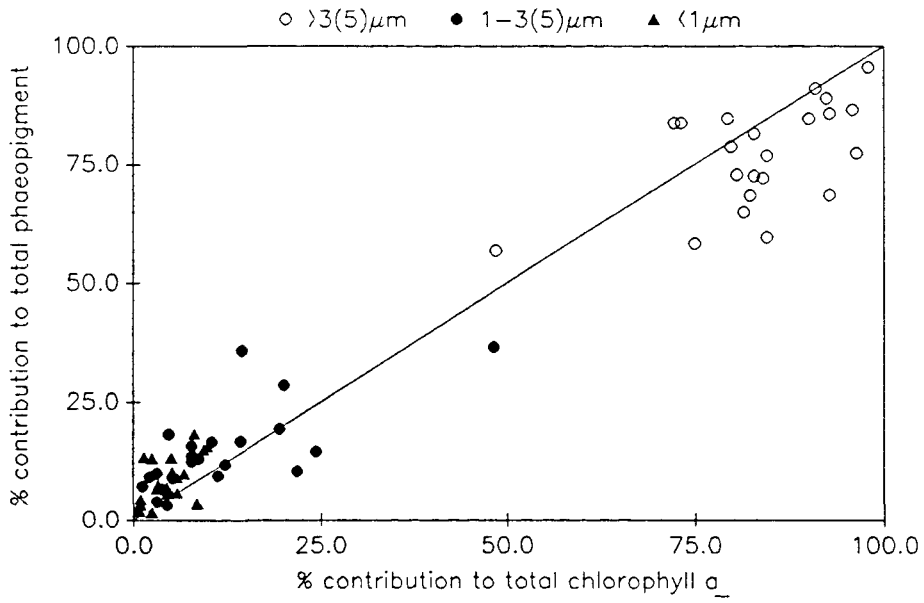

Figure 1.4.1.10. Relationship between the percentage contribution to the total chlorophyll *a* and phaeopigment concentrations (i.e. sum of fractions) for each size fraction, as measured in the seasonal samples from NW Netley and Calshot Spit.

1.4.1.3; Table A.4 in Appendix). Large concentrations of buoyant globular particles were visible in the water samples from NW Netley in this occasion, and microscopic examination revealed the algal bloom to be dominated by the colonial prymnesiophyte *Phaeocystis* sp. and diatoms of the genus *Chaetoceros* sp.. Unlike at Calshot Spit, chlorophyll *a* concentration exhibited high values during the summer at NW Netley, with a maximum of 18mgchl *a* m^{-3} recorded on August the 2nd, when the water column was again visibly discoloured due to the phycoerythrin containing phototrophic ciliate *Mesodinium rubrum*. *Mesodinium rubrum* was observed to aggregate forming dense red patches irregularly distributed along the upper-mid estuary.

The seasonal distribution of total phaeopigment concentration is presented in Table A.5 in Appendix. Expressed as a percentage (i.e. (phaeopigment/(phaeopigment+chlorophyll *a*) x100) the seasonal distribution followed the opposite trend to that of chlorophyll *a* (Figure 1.4.1.3). Highest values were measured during winter and early autumn, with lowest values being recorded during bloom periods. Except during the April peak of chlorophyll *a* the % phaeopigment was always higher at Calshot Spit than at NW Netley, the difference being most significant in winter and at the time of the red water event at NW Netley. This is likely a reflection of the effect of wind driven resuspension of detrital material from the bottom sediments at Calshot Spit in winter. A plot of the % phaeopigment against chlorophyll *a* concentration suggested an inverse exponential relationship between the two variables (Figure 1.4.1.4), with lowest % phaeopigment concentration occurring at high chlorophyll *a* concentration.

1.4.1.5. Size fractionated chlorophyll a concentration

Size-fractionated chlorophyll *a* measurements were undertaken on duplicate samples collected from April to October. The standard difference of duplicate samples expressed as a percentage of the mean was on average higher than for chlorophyll *a* concentrations measured on GF/F filters, 13.1%(SD 16.2). (Note SD stands for standard deviation and SE for standard error throughout the entire thesis). The seasonal distribution is presented in Figure 1.4.1.5 and Figure 1.4.1.6 for Calshot Spit and NW Netley respectively. Data are also

listed in Table A.4, Appendix. For the larger than 3(5)μm fraction the trend followed closely the seasonal pattern described for the total community chlorophyll *a* biomass, at both stations, NW Netley and Calshot Spit. At NW Netley the chlorophyll *a* concentration corresponding to the 1-3(5)μm fraction exhibited two major peaks of 2.1mg m^{-3}, coinciding with the *Phaeocystis* sp. bloom in May and the *Mesodinium* peak in August. The use of 5μm filters instead of 3μm filters on two occasions (22nd of May and 31st of August) may have contributed to the slight increase in chorophyll a concentration observed on these dates. Levels of chlorophyll *a* outside the two major peaks and when 5μm filters were used, were in the range 0.05-0.2mgchl *a* m^{-3}. The chlorophyll *a* contained in particles passing through 1μm filters was low during late winter and spring (0.04-0.08mgchl *a* m^{-3}), increasing during the summer (June to September) with a maximum of 0.56mgchl *a* m^{-3} recorded coinciding with the red water event in August.

At Calshot Spit the trend of variation of the fraction passing through 1μm filters was approximately analogous to that at NW Netley, with high values during the summer months, except that no outstanding peak was recorded in August, values remaining relatively constant (0.13-0.16mgchl *a* m^{-3}) throughout July and August. For the fraction passing through 3(5)μm filters and retained on 1μm filters, highest values (0.79 and 1.18mgchl *a* m^{-3}) were recorded during the two sampling visits in May. In summer, concentrations of about 0.5mgchl *a* m^{-3} were measured during the month of August, coinciding with the use of 5μm filters. For the rest of the sampling period concentrations around 0.1mgchl *a* m^{-3} were determined.

The temporal variation of the contribution by each fraction to the phytoplankton community chlorophyll *a* concentration is depicted in Figures 1.4.1.7 and 1.4.1.8 for Calshot Spit and NW Netley respectively (values are also presented in Table A.6 in Appendix). Throughout the entire period of study the chlorophyll *a* retained in 3(5)μm filters was the dominant fraction, both at NW Netley (72.3-95.9% of the sum of fractions, mean 85.7%(SD 7.7)) and at Calshot Spit (48.6-97.9%, mean 81.7%(SD 13.3)). The second most prominent fraction appeared to be the 1-3(5)μm fraction, for which percentages in the range 3.1-21.9% (mean 9.5%(SD 6.7) at NW Netley and

1.3-48.3% (mean 13.8%(SD 13.4) at Calshot Spit were recorded. The fraction with the lowest percentage contribution to overall chlorophyll *a* was the fraction passing through 1μm filters, values ranging from 0.7 to 9.8% (mean 4.8%(SD 3.6) at NW Netley and from 0.8 to 8.1% (mean 4.5%(SD 4.5) at Calshot Spit.

The seasonal distribution of the percentage contribution to the whole sample chlorophyll *a* concentration showed the fraction passing 1μm filters to be least significant during the spring blooms at both stations with a tendency to become more prominent during winter and autumn and also during periods of lowest overall chlorophyll *a* biomass in summer i.e. June at NW Netley. For Calshot Spit differences between autumn/winter and summer were not so clear.

In the fraction 1-3(5)μm the percentage contribution varied in much the same way as the actual concentration of chlorophyll *a* for both stations. Thus samples from NW Netley displayed two peaks of aroud 20% during the *Phaeocystis* sp. bloom in May and during the *Mesodinium rubrum* peak in August. At Calshot Spit an oustanding maximum of 48.3% was measured on May 22nd when the diatom *Asterionella kariana* was present in significant numbers (D. Kifle personal communication). Values were in the 25% range on May 8th.

A plot of the percentage contribution by the smaller than 1μm fraction against the total (sum of fractions) chlorophyll *a* concentration (Figure 1.4.1.9) suggested an inverse exponential relationship. A linear regression of the semilog plot of the former parameter against the latter yielded a value of $r=0.67$, $n=22$ ($p<0.001$). A similar relationship was not evident for the 1-3(5)μm fraction (Figure 1.4.1.9). The percentage contribution of this fraction was better correlated with the percentage contribution of the larger than 3(5)μm fraction, indicating that the chlorophyll *a* concentration in these two fractions were large enough to influence each others contributions. This was not the case for the smaller than 1μm fraction, reflecting the lower impact of the variations in chlorophyll *a* biomass in this fraction in the relative importance of the larger fractions.

In general, the procedure of fractionation and extraction of chlorophyll *a* from Nuclepore filters resulted in an underestimation of total chlorophyll *a* as measured in unfractionated samples and

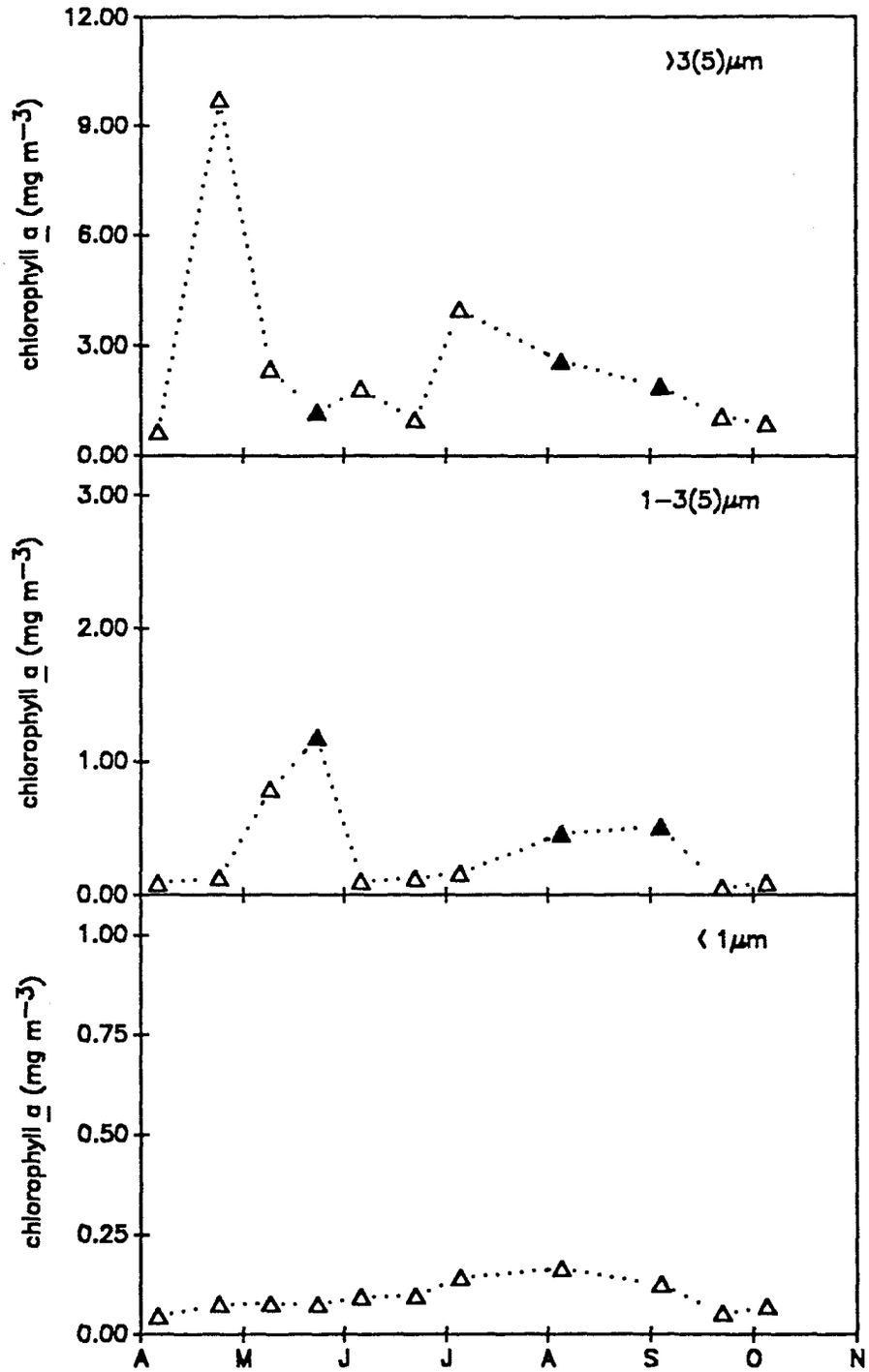

Figure 1.4.1.5. Seasonal distribution of size fractionated chlorophyll *a* concentration at Calshot Spit. Filled symbols denote dates in which 5μm pore-sized filters were used.

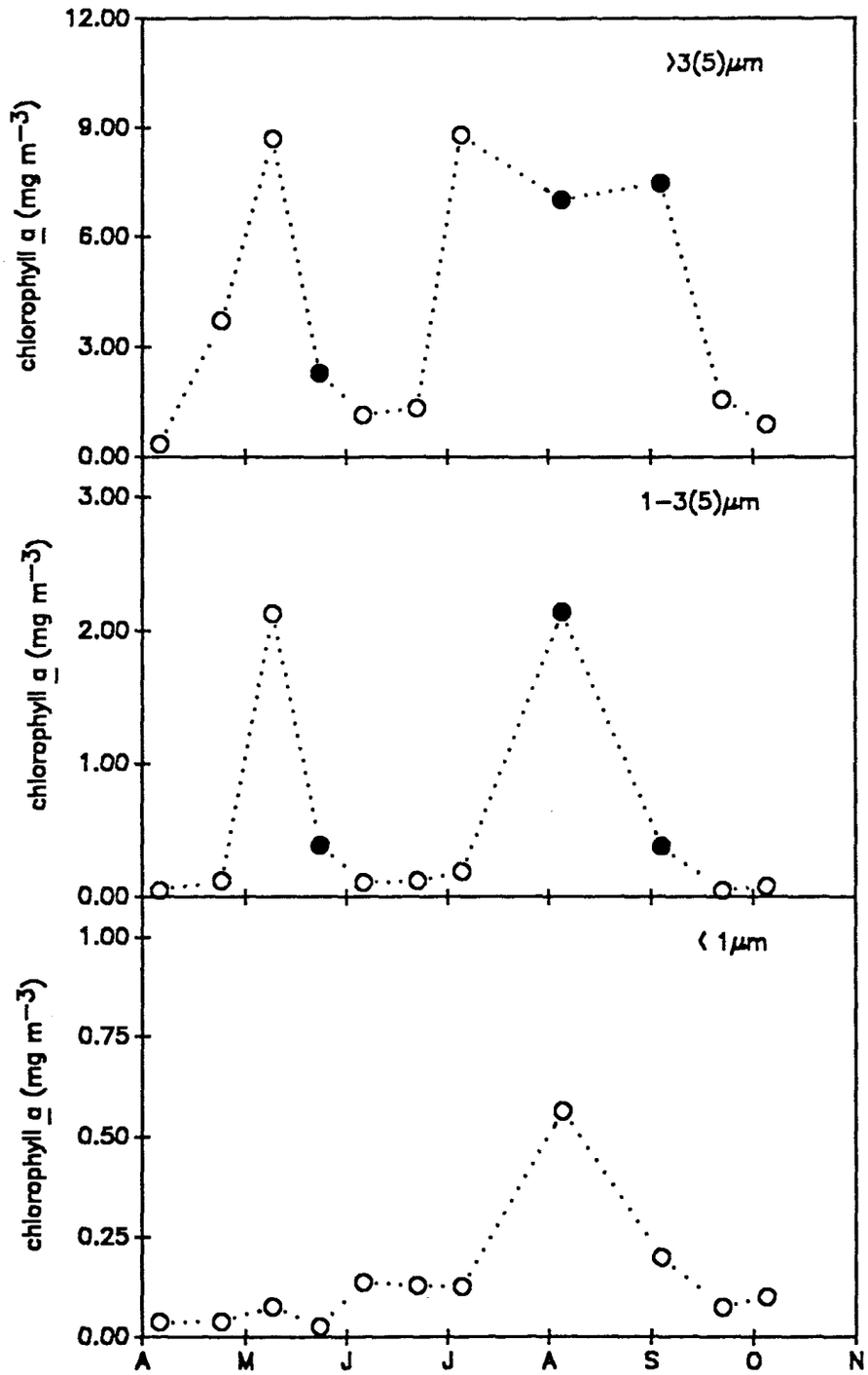

Figure 1.4.1.6. Seasonal distribution of size fractionated chlorophyll *a* concentration at NW Netley. Filled symbols denote dates in which 5μm pore-sized filters were used.

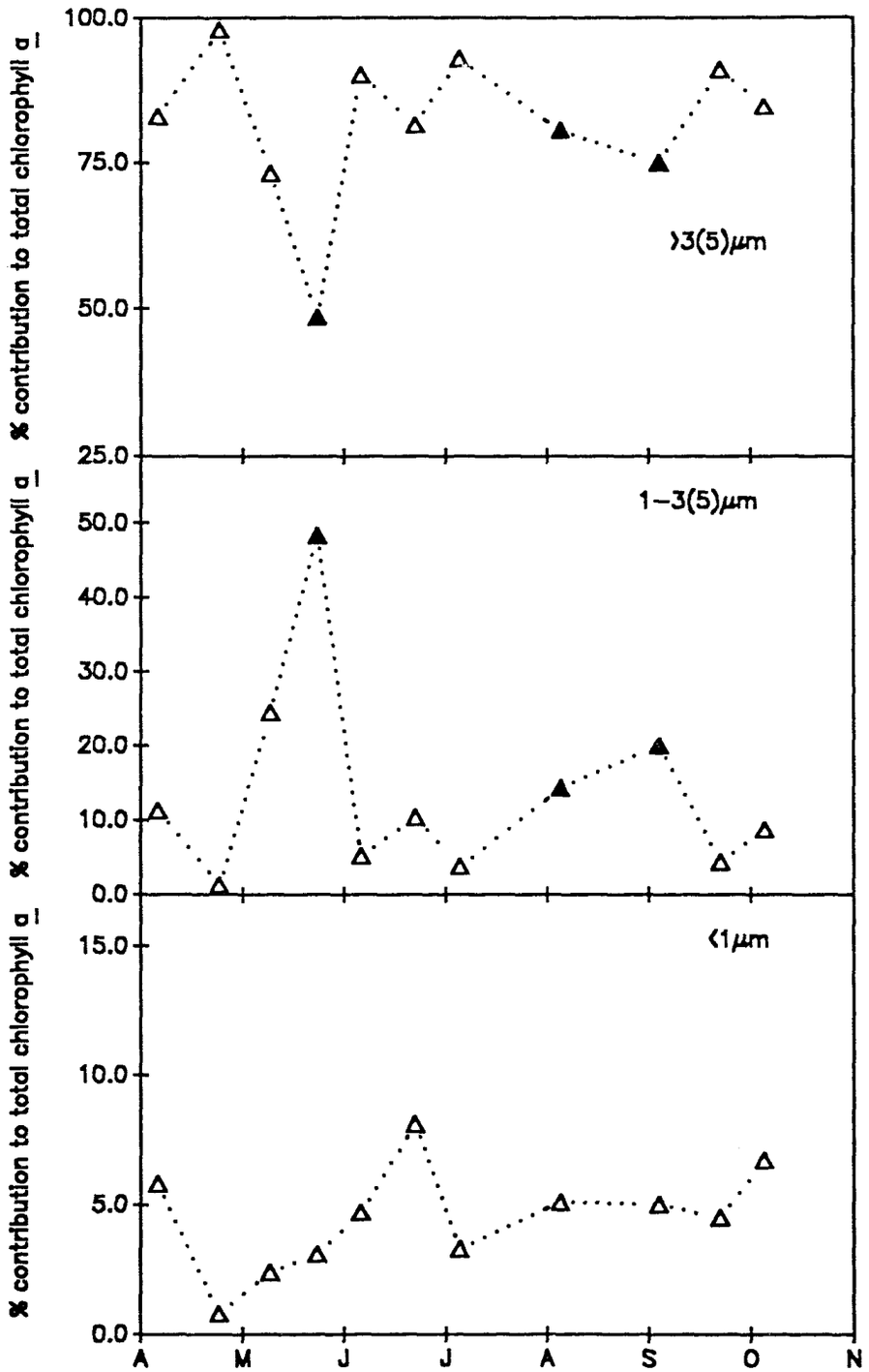

Figure 1.4.1.7. Seasonal distribution of size fractionated chlorophyll *a* concentration expressed as a percentage of the sum of fractions at Calshot Spit. Filled symbols denote dates in which 5μm pore-sized filters were used.

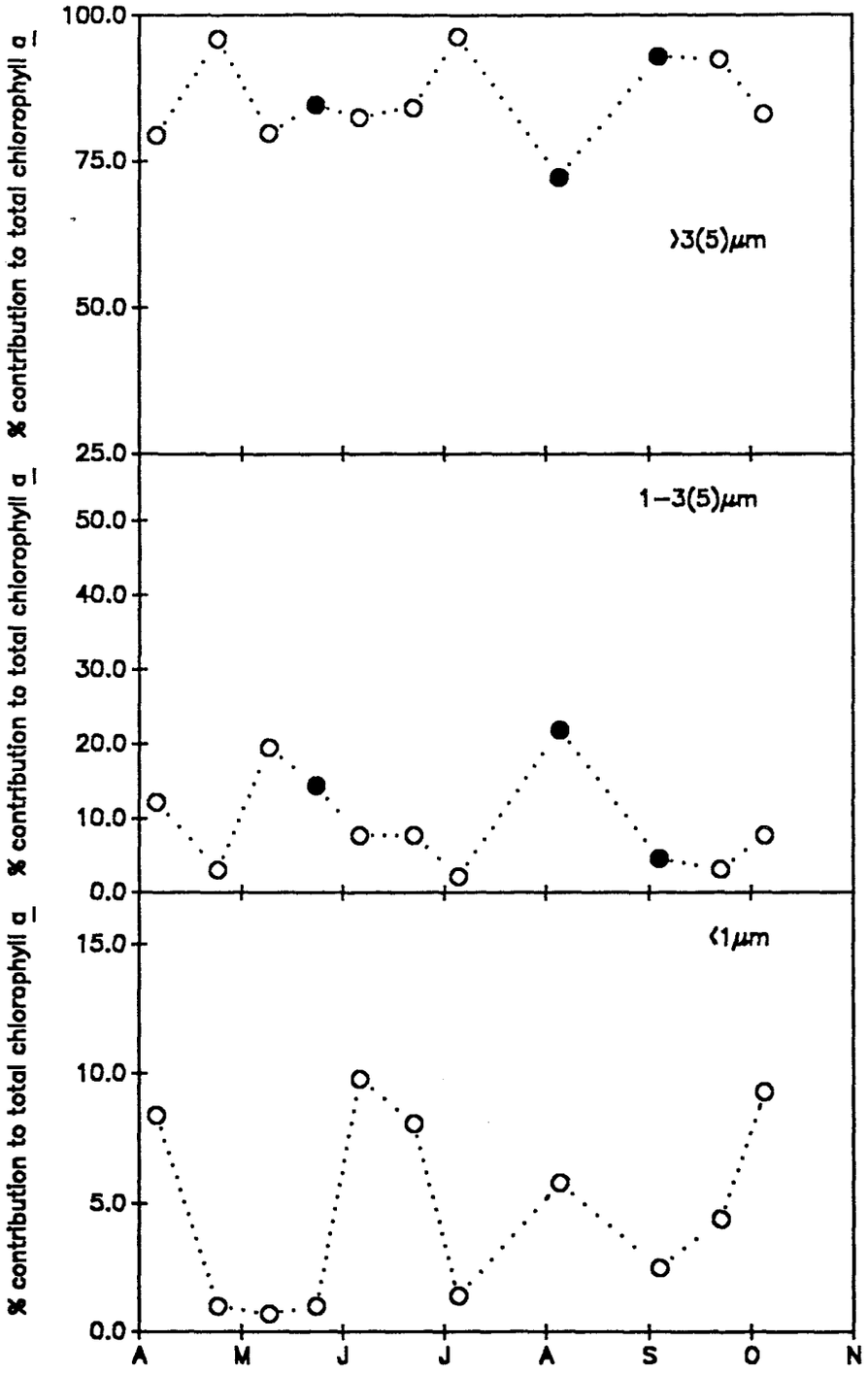

Figure 1.4.1.8. Seasonal distribution of size fractionated chlorophyll *a* concentration expressed as a percentage of the sum of fractions at NW Netley. Filled symbols denote dates in which 5μm pore-sized filters were used.

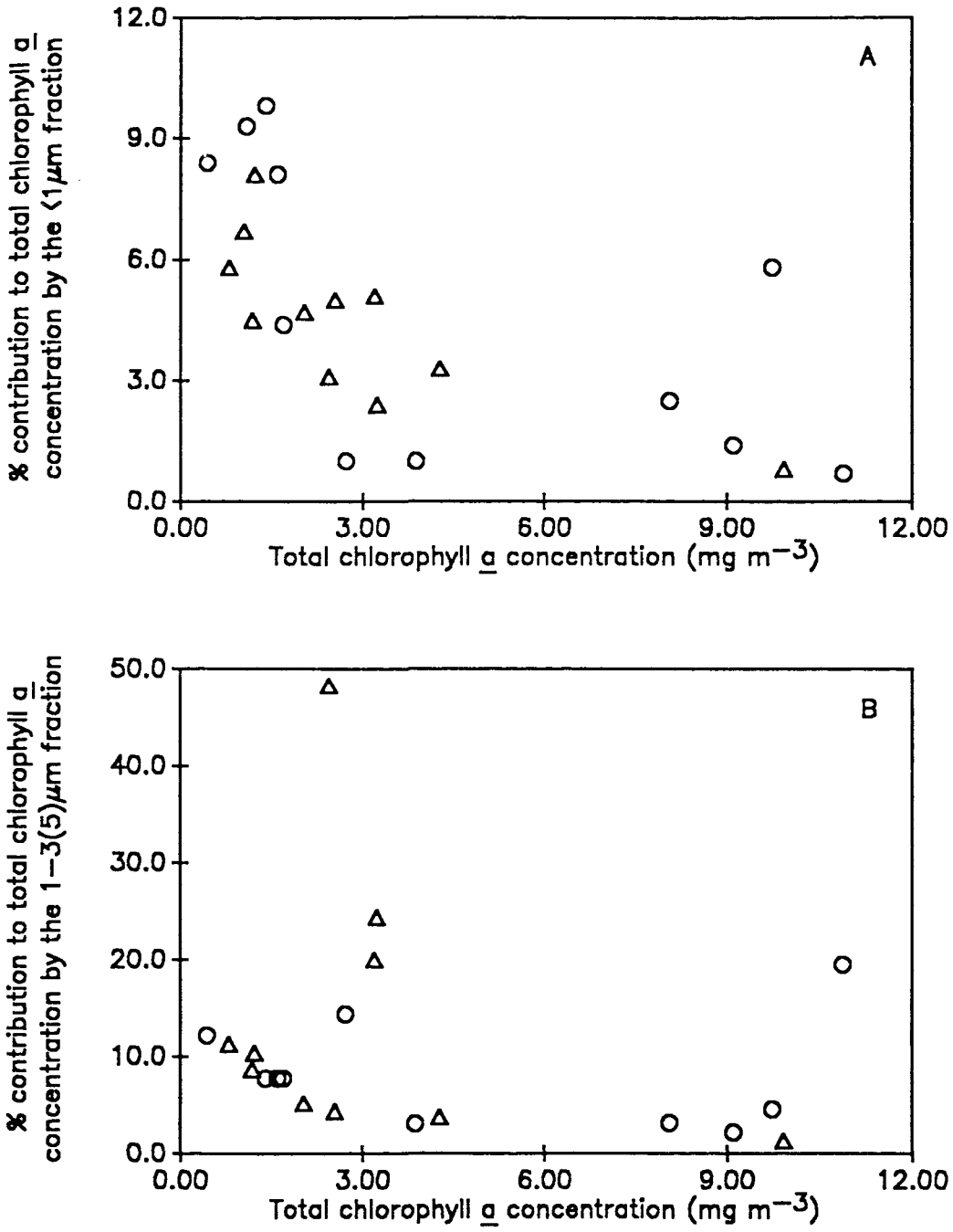

Figure 1.4.1.9. Relationship between the chlorophyll *a* concentration expressed as a percentage of the sum of fractions in the (A) 0.2-1μm and (B) 1-3(5)μm size fractions and the total chlorophyll *a* concentration (sum of fractions) in samples from NW Netley (o) and Calshot Spit (Δ).

extracted from GF/F filters. The comparison of total chlorophyll *a* determined by summing up the various fractions and by "whole sample filtration onto GF/F" yielded an average 16(SD 12)% deviation of the former in relation to the latter (Table 1.4.1.2). This might be attributable either to differences in the structure and hence particle retaining properties of the filters or to the lack of the grinding step in the processing of the Nuclepore filters.

Size-fractionated phaeopigment concentrations at both stations are presented in Table A.5 in Appendix. The distribution of phaeopigment concentration was quite well correlated with active chlorophyll *a* concentration in all fractions. A comparison of the size distribution of the phaeopigments showed that the ratio of phaeopigment to active chlorophyll *a* was higher for the two smaller fractions than for the largest one. Figure 1.4.1.10 is a plot of the percentage contribution to total sample chlorophyll *a* against the percentage contribution to total sample phaeopigment and it shows clearly that the >3(5)μm fraction made a greater impact on the active pool than on the degraded forms (values below the x=y line), whereas the opposite was the case for the 1-3(5)μm and <1μm fractions (values above the x=y line).

1.4.1.6. Picophytoplankton : phycoerythrin containing picocyanobacteria and eukaryotic picophytoplankton

Picophytoplankton cell numbers were counted in the 3(5)μm filtrates from January to October. Phycoerythrin-containing (PE-containing) (yellow-orange fluorescing) chroococcoid cyanobacteria cell numbers varied from $2x10^4$ to $1.3x10^7$cells l^{-1}. The seasonal distribution showed a distinct peak during midsummer at both stations, although NW Netley showed high values over a shorter period of time than Calshot Spit (Figure 1.4.1.11 ; Table A.7 in Appendix). Values during the rest of the months were typically below 10^6cells l^{-1}. PE-containing cyanobacteria and temperature were significantly correlated, but the correlation was not very strong ($r=0.698$ for NW Netley and $r=0.576$ for Calshot Spit ($p\leq0.05$)). Picocyanobacterial numbers were well correlated with chlorophyll *a* concentration in the smaller than 1μm fraction ($r=0.921$ for NW Netley and $r=0.880$ for

Calshot Spit ($p<0.001$), but not with the chlorophyll *a* concentration in the 1-3(5)μm fraction which suggests perhaps the greater influence in the latter fraction of fragments of larger cells and eukaryotic picophytoplankton.

Figure 1.4.1.11 also illustrates the temporal distribution of eukaryotic (red-fluorescing) algal cells passing through 3(5)μm filters. Cell enumeration data are also presented in Table A.7 (Appendix). Concentrations ranged from $8x10^4$ to $2.9x10^8$cells l^{-1}. Low numbers during the sampling visits in January, February and March were followed by a small peak ($2x10^6$cells l^{-1}) during the first week of April, recorded at both stations. Examination of the same samples by light microscopy revealed an outburst of a Cryptomonad like species (D. Kifle, personal communication). However, cells under the epifluorescence microscope fluoresced bright red, and there was no sign of orange fluorescing phycoerythrin. During the second week in May, when the colonial prymnesiophyte *Phaeocystis sp.* was noted in large numbers at NW Netley, cell numbers in the 3μm filtrate increased to $2.9x10^8$cells l^{-1} at NW Netley. Numbers of eukaryotic picophytoplankton from the previous date, when *Phaeocystis* sp. was abundant at Calshot Spit (D. Kifle, personal communication) are unfortunately not available. Another significant peak was recorded in August, $6x10^6$ and $1.2x10^7$cells l^{-1} at Calshot Spit and NW Netley respectively. From June to October the eukaryotic picophytoplankton population was dominated by minute forms, *ca.* 1μm in diameter.

Eukaryotic picophytoplankton numbers appeared to be correlated with PE-containing cyanobacteria at NW Netley ($r=0.783$, $p<0.01$) but not at Calshot Spit ($r=0.238$, $n=12$).

The vertical distribution of the PE-containing picocyanobacteria was investigated on four occasions at NW Netley and two at Calshot Spit during the summer and results are presented in Figures 1.4.1.12 and 1.4.1.13 respectively. On July 19th and August 2nd a pronounced increase in the cell numbers from 1 to 6m was recorded at NW Netley, the difference was largely reduced by August 31st and on September 18th values were slightly higher at 1m. The depth distribution at Calshot Spit was examined on August 31st and September 18th. On neither of the two occassions was there clear evidence of PE-containing cyanobacteria being more abundant deep in the water

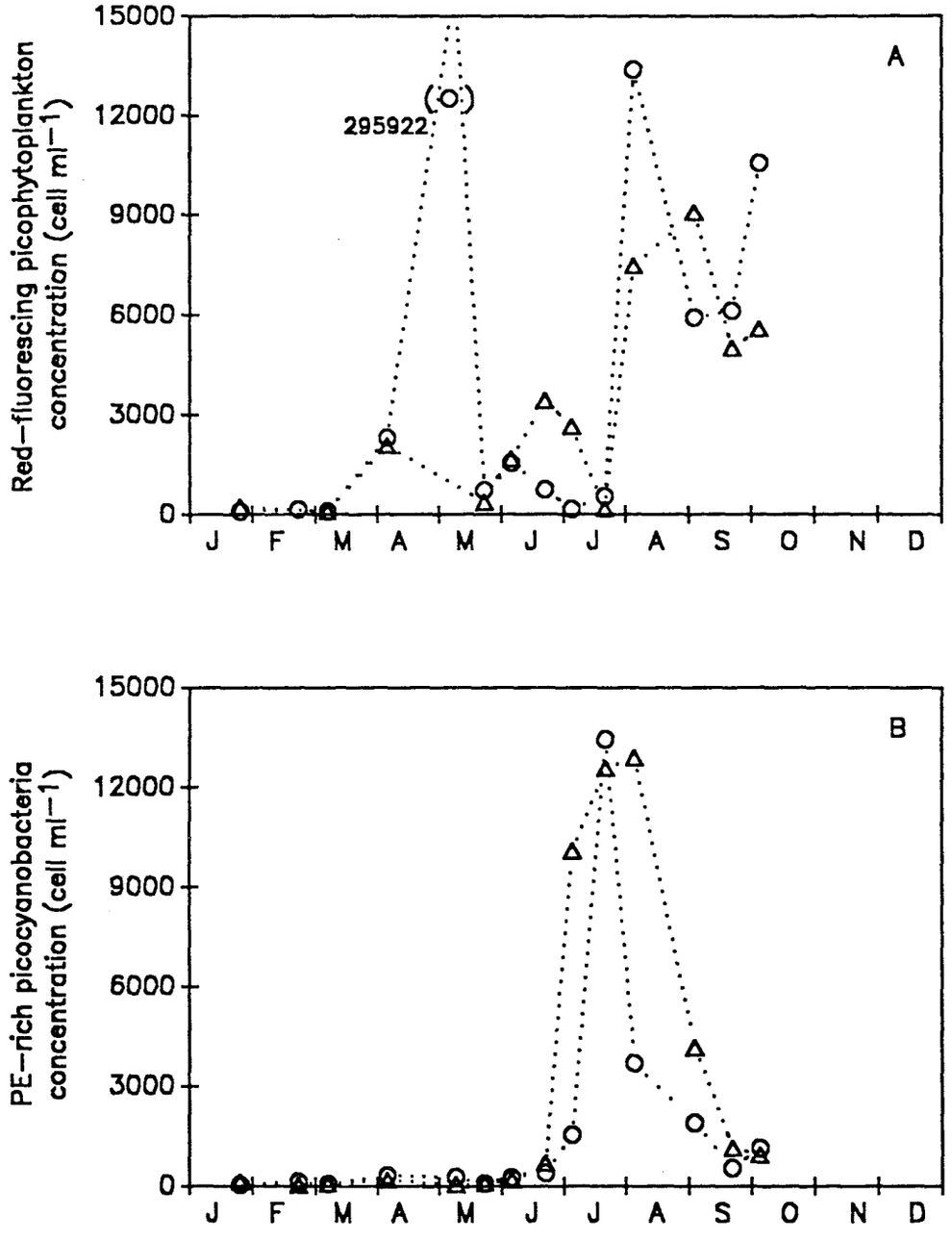

Figure 1.4.1.11. Seasonal distribution of (A) red-fluorescing picophytoplankton and (B) phycoerythrin containing picocyanobacteria cell numbers as measured in the 3(5)μm filter filtrates of the three depth composite samples from NW Netley (o) and Calshot Spit (Δ).

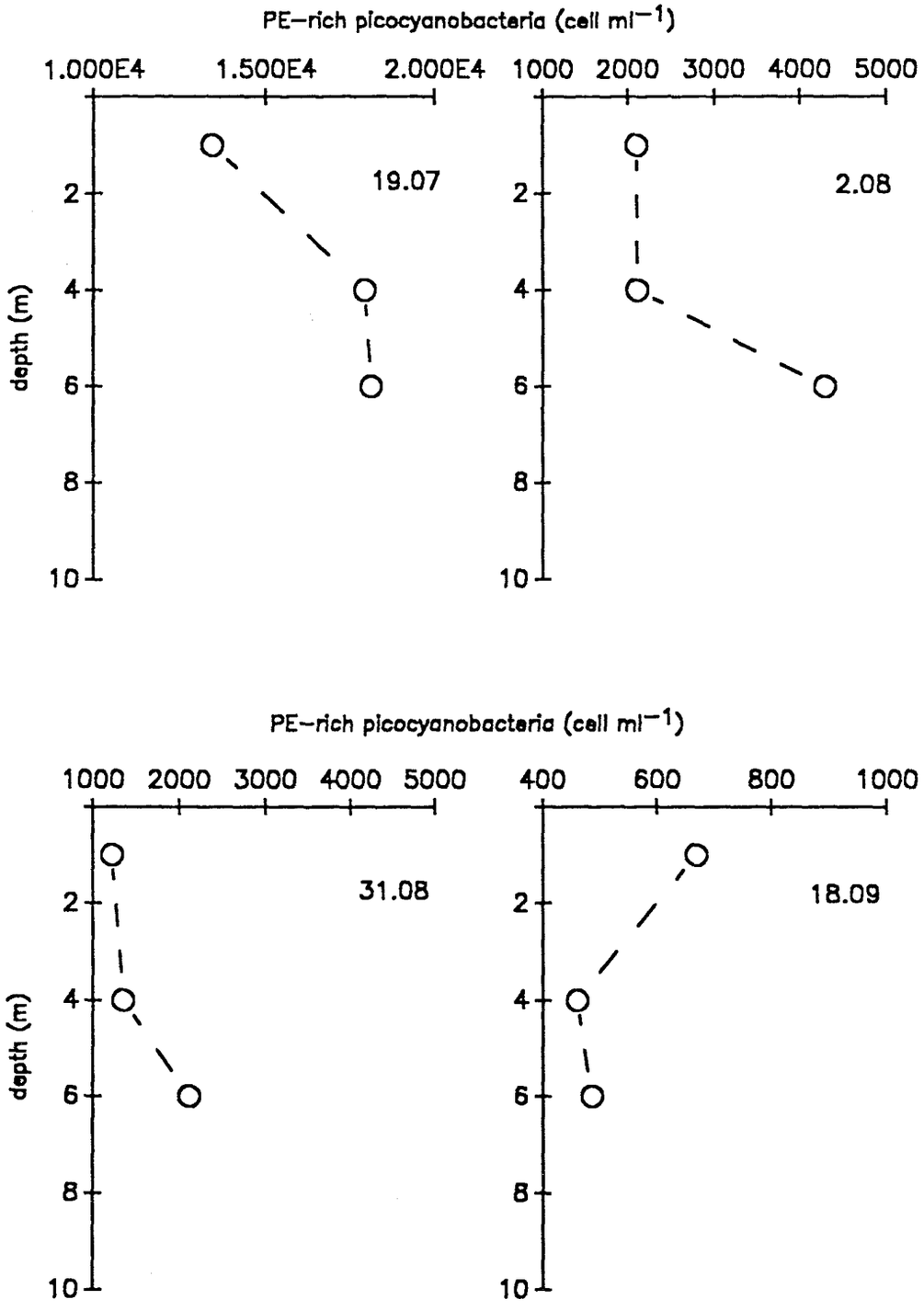

Figure 1.4.1.12. Vertical profiles of PE-containing picocyanobacteria cell concentration at NW Netley on selected dates.

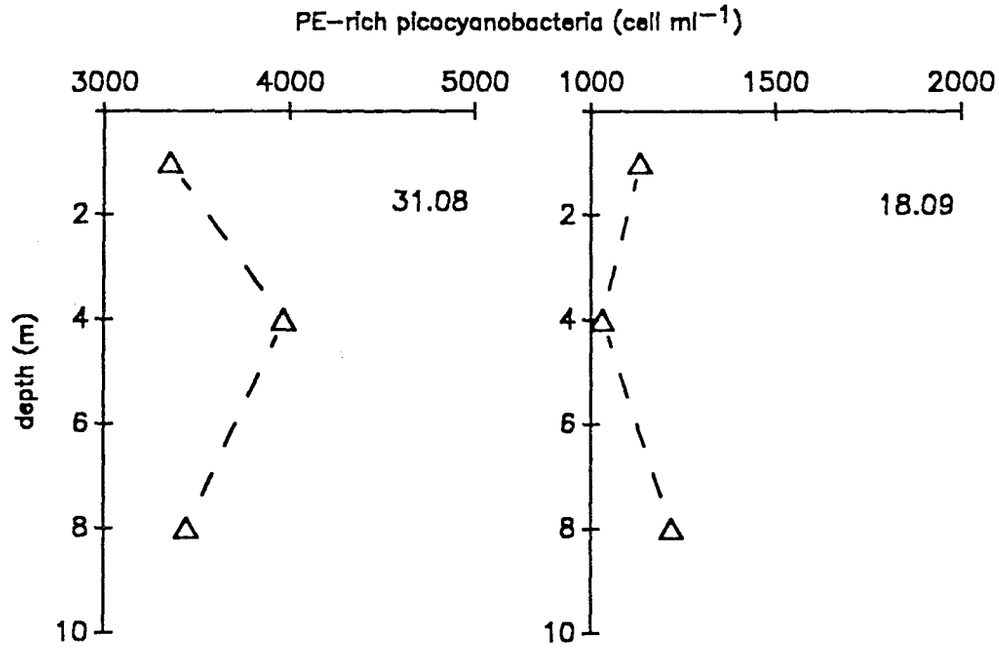

Figure 1.4.1.13. Vertical profiles of PE-containing picocyanobacteria cell concentration at Calshot Spit on selected dates.

column than nearer to the surface.

On August 2nd a slight vertical temperature gradient had developed (Table A.1 in Appendix) and some stratification may have occurred at around 4m. On July 19th there was no salinometer data available but the ambient temperature (21°C) suggests the heat input was also large enough to induce some stratification. On the other two dates vertical profiles of temperature and salinity suggested the water column to be mixed (Table A.1 and Table A.2 in Appendix).

1.4.1.7.Total plankton community rate of carbon fixation into particulate material

Results of seasonal carbon uptake rate (μmolC $l^{-1}h^{-1}$) at the various light levels for the unfractionated and the fractionated samples, as measured in the particulate phase (i.e. retained on filters) are presented in Tables A.8 and A.9 (Appendix). The unfractionated values are based on single measurements and the fractionated values are means of duplicate samples. For the duplicate fractionated values, the standard difference calculated as a percentage of the mean yielded a mean value (calculated for all sampling dates in all fractions) of 14.5(SD 13.9)%. There was no indication that the replication was poorer in any specific size fraction. For each sample at each irradiance level, carbon uptake rate by the various size fractions were added up. A comparison of the sum of fractions with the unfractionated yielded a mean percentage error, calculated as the difference between the two expressed as a percentage of the highest value, of 15.1(SD 9.9)%.

From the carbon fixation rates into particulate material for each size fraction, the depth integrated daily rates of carbon fixation were calculated as detailed in section 1.3.2.6. Data are presented in Table A.10 in Appendix. Values for the various size fractions were then added up for each date to obtain values of carbon fixation rate into particulate material (i.e. excluding DOC) for the total plankton community. Figure 1.4.1.14 is an illustration of the temporal sequence of the daily rates of depth integrated primary production, as estimated from the sum of carbon uptake rates in the various fractions. Estimates ranged between 0.084 and 2.207gC $m^{-2}d^{-1}$

at Calshot Spit and between 0.096 and 3.480gC $m^{-2}d^{-1}$ at NW Netley. At Calshot Spit the temporal variation in depth integrated carbon uptake rate compared well with the chlorophyll *a* distribution pattern. Low winter values were followed by an outstanding peak of 2.207gC $m^{-2}d^{-1}$ during the last week of April, values remaining at around 0.5-1gC $m^{-2}d^{-1}$ during late spring and summer, falling to low pre-spring bloom levels by the end of September. At NW Netley, the temporal sequence of carbon uptake rate showed two main differences with the chlorophyll *a* concentration distribution. The spring maximum of 11µgchl *a* l^{-1} recorded during the *Phaeocystis* sp. bloom in May, was not accompanied by a sizable peak in carbon uptake, values being of less than 1gC $m^{-2}d^{-1}$. In summer, chlorophyll *a* concentrations of similar magnitudes were recorded during samplings in July and August, however, values of carbon uptake rate were substantially higher during the red water event (3.480gC $m^{-2}d^{-1}$) than in the preceding and subsequent samplings.

1.4.1.8. Carbon uptake rate as measured in the dissolved phase (DOC)

From measurements of ^{14}C in acidified and bubbled 0.2µm (occasionally 0.4µm) filtrates of the unfractionated samples, rates of apparent excretion at the various irradiance levels were measured (Table A.8 and Table A.9 in Appendix). Since the activity was counted only in a 5ml subsample, in some cases when the rate of carbon uptake was low, dpm's measured in the filtrates were low and blanks (zero time) were significant in relation to the sample dpm's. Sometimes this resulted in sample dpm's being higher than blank dpm's. These were treated as 0 rates of excretion.

The rates of apparent excretion have also been expressed as a percentage of the total rate of carbon uptake (i.e. as measured in the particulate plus the dissolved phases) at each light level (Table A.8 and A.9 in Appendix). The apparent excretion (mean of light levels) ranged from as low as 1.2% to levels of 39.5% (Figure 1.4.1.15). Highest values were measured at both stations on May 8th, during the mass occurrence of *Phaeocystis* sp. and were also significantly high on another three occassions, April 5th at NW Netley (23.9%) and April 23rd at Calshot Spit, at the time of the first recorded significant occurrence of *Phaeocystis* sp. (D.Kifle, personal communication)

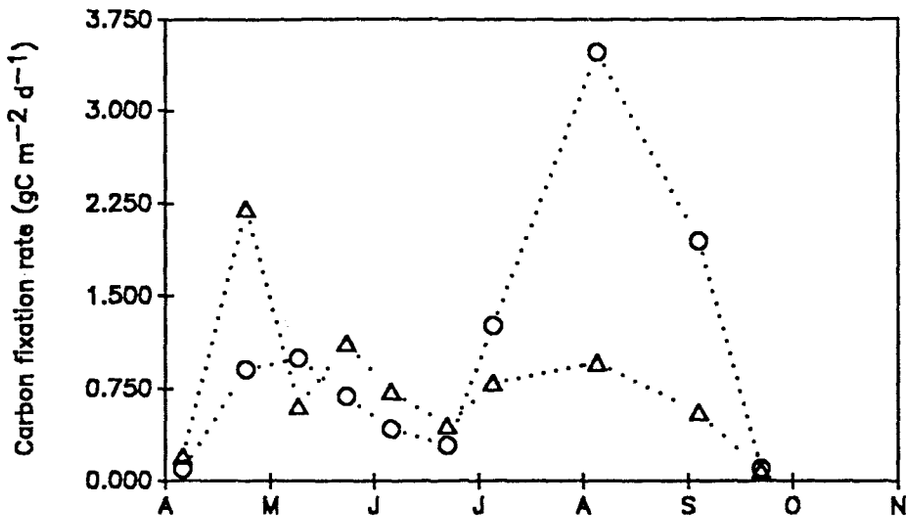

Figure 1.4.1.14. Seasonal distribution of daily water column integrated rates of carbon fixation by the total plankton community (sum of fractions; DOC not included) at NW Netley (o) and Calshot Spit (Δ).

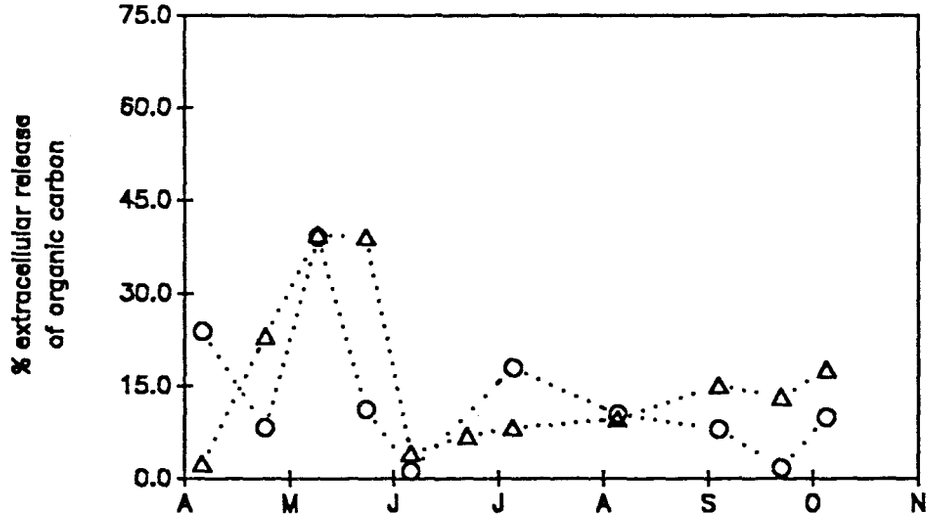

Figure 1.4.1.15. Seasonal distribution of the rate of extracellular release of organic carbon expressed as a percentage of the total carbon fixation (mean of light levels) at NW Netley (o) and Calshot Spit (Δ).

(23.2%) and May the 22nd at Calshot Spit (39.1%), when *Asterionella kariana* was noted in significant numbers (D.Kifle, personal communication).

1.4.1.9.Size fractionated carbon fixation rate

The seasonal variation of water column integrated daily rate of carbon uptake by the various plankton size-fractions is presented in Figures 1.4.1.16 and 1.4.1.17 (Values are listed in Table A.10 in Appendix). At Calshot Spit rates varied from 0.068 to 2.131gC $m^{-2}d^{-1}$ for the larger than 3(5)μm fraction, from 0.010 to 0.307gC $m^{-2}d^{-1}$ for the 1-3(5)μm fraction and from 0.006 to 0.119gC $m^{-2}d^{-1}$ for the smaller than 1μm fraction. At NW Netley, rates span from 0.066 to 2.145gC $m^{-2}d^{-1}$ for the fraction retained in 3(5)μm filters, from 0.010 to 1.062gC $m^{-2}d^{-1}$ for the 1-3(5)μm fraction and from 0.012 to 0.273gC $m^{-2}d^{-1}$ for the fraction passing through 1μm filters.

Some differences were noted in the temporal sequence of carbon uptake in relation to that observed for chlorophyll *a* values for the various size fractions. For the >3(5)μm and the 1-3(5)μm fractions from samples from NW Netley from May 8th (i.e. during *Phaeocystis* sp. massive occurrence), the carbon uptake rates failed to parallel the magnitude of the chlorophyll *a* concentration peaks. This was not apparent in measurements undertaken during the previous sampling visit when *Phaeocystis* sp. was also observed in large numbers at Calshot Spit (D. Kifle, personal communication). Coinciding with the red water event observed at NW Netley in August, however, peaks of carbon uptake were remarkably higher than expected from chlorophyll *a* measurements at NW Netley. For the smaller than 1μm fraction, the overall increase from winter to summer was apparent at both stations but the rates of carbon uptake during the red water event at NW Netley were also higher than indicated from chlorophyll *a* measurements.

The temporal distribution of the percent distribution of each fraction to overall plankton community carbon fixation rates are depicted in Figures 1.4.1.18 and 1.4.1.19 for Calshot Spit and NW Netley respectively (values have been tabulated in Table A.11 in Appendix). The >3(5)μm was the dominant fraction during all sampling visits (61.7-96.5% , mean of 79.9%(SD 9.6)). The 1-3(5)μm fraction

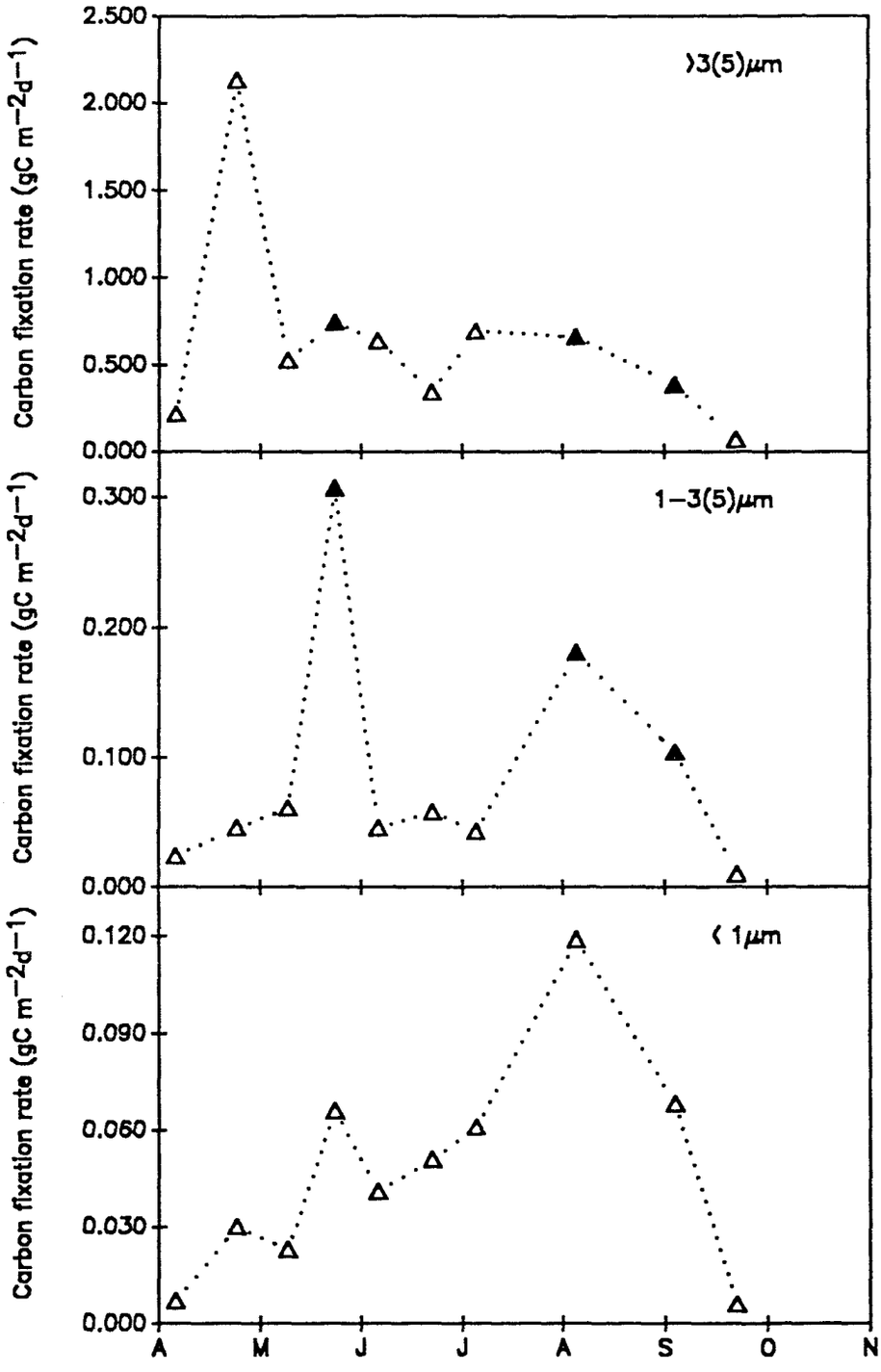

Figure 1.4.1.16. Seasonal distribution of size-fractionated water column integrated daily rates of carbon fixation at Calshot Spit. Filled symbols denote dates in which 5μm pore-sized filters were used.

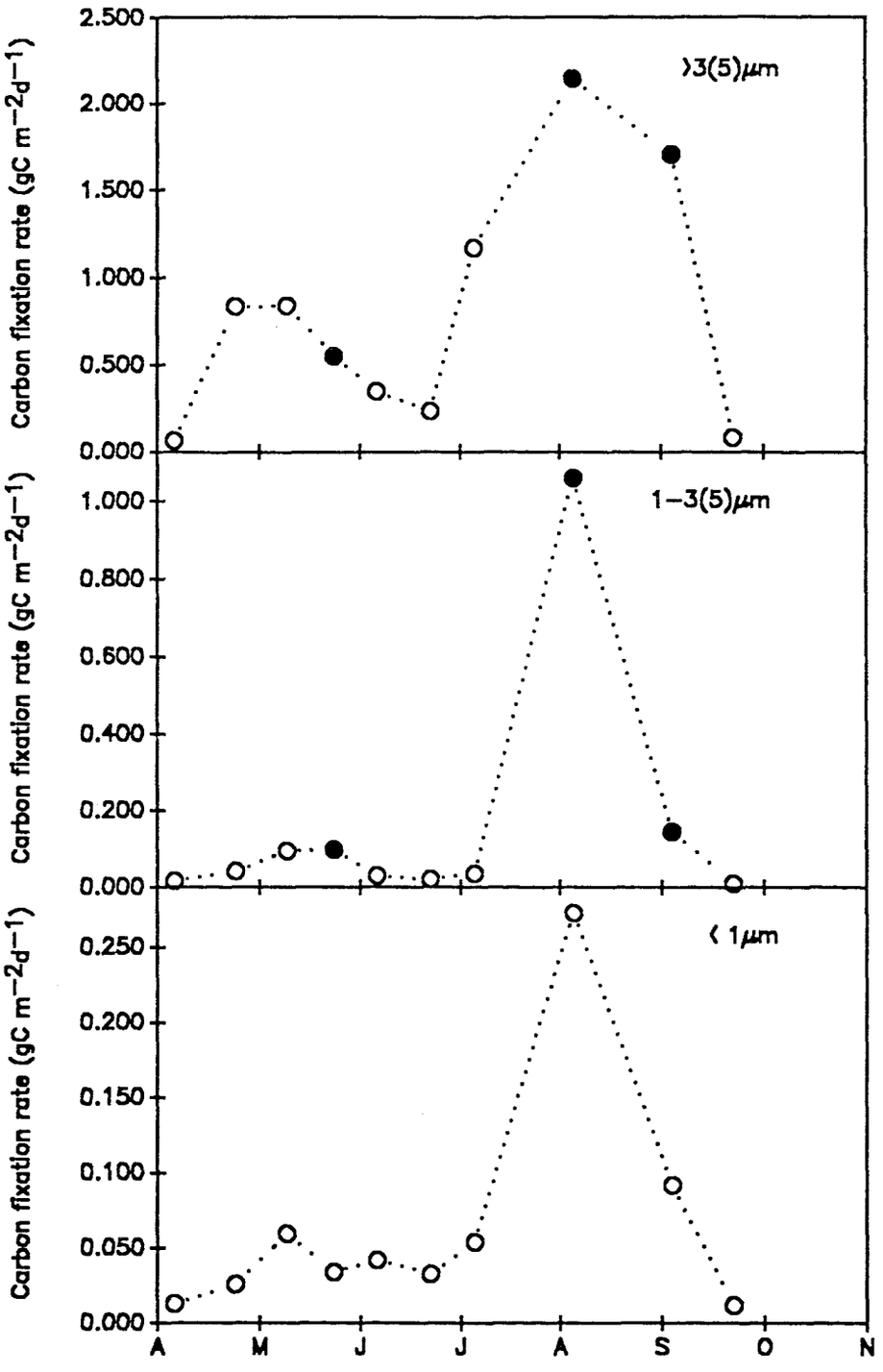

Figure 1.4.1.17. Seasonal distribution of size-fractionated water column integrated daily rates of carbon fixation at NW Netley. Filled symbols denote dates in which 5μm pore-sized filters were used.

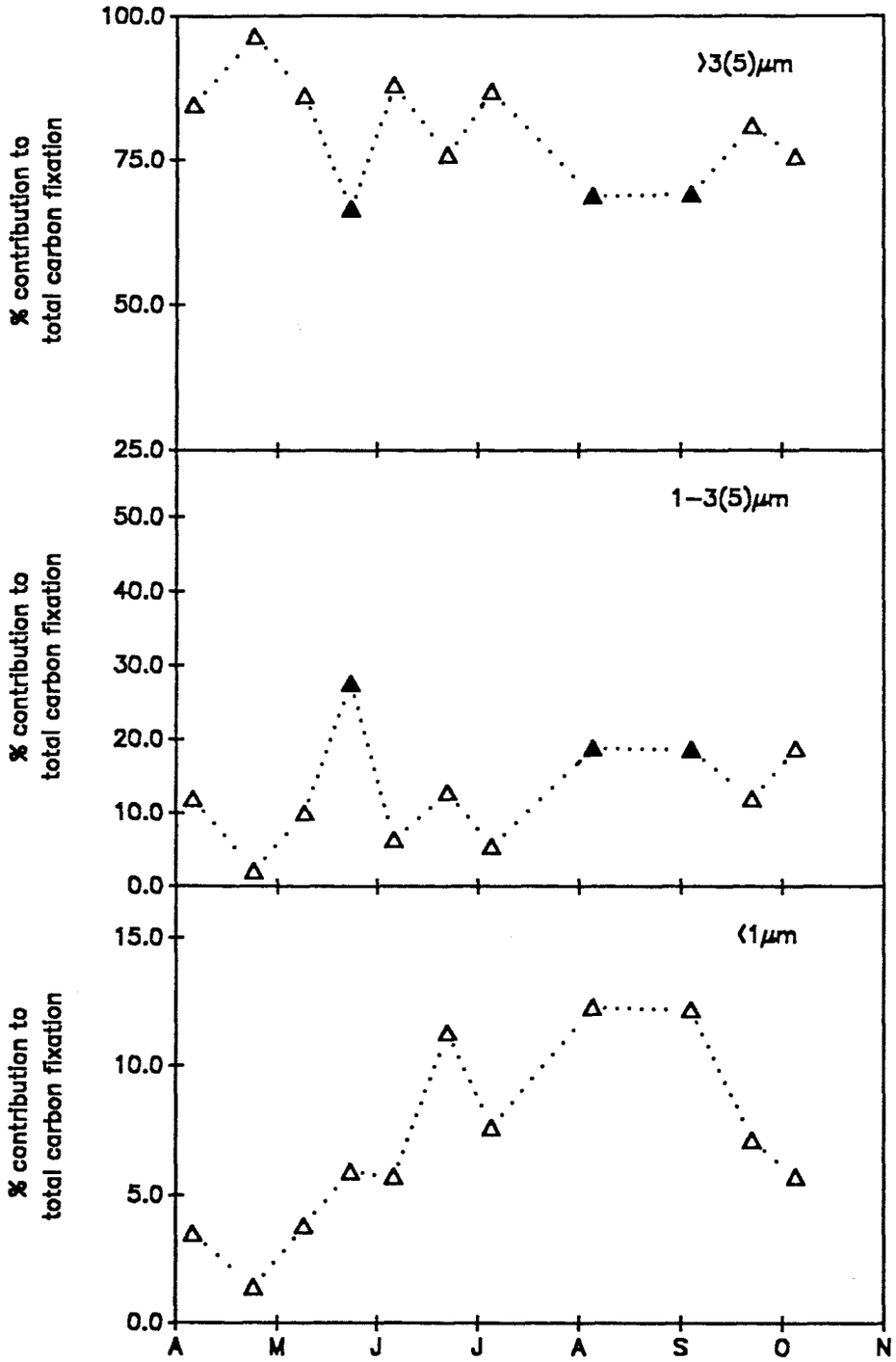

Figure 1.4.1.18. Seasonal distribution of size-fractionated water column integrated daily rates of carbon fixation, expressed as a percentage of the sum of fractions, at Calshot Spit. Filled symbols denote dates in which 5μm pore-sized filters were used.

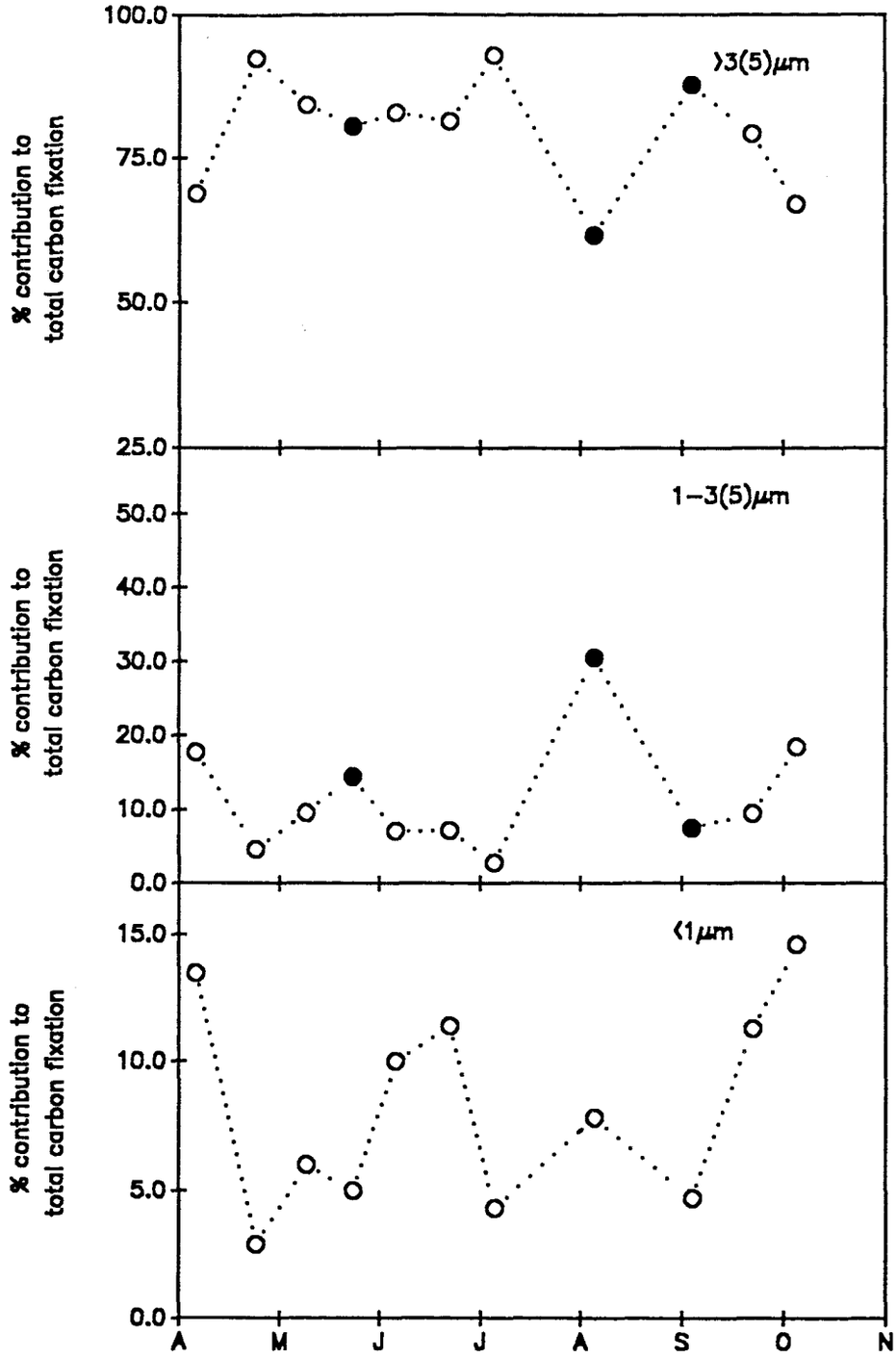

Figure 1.4.1.19. Seasonal distribution of size-fractionated water column integrated daily rates of carbon fixation, expressed as a percentage of the sum of fractions, at NW Netley. Filled symbols denote dates in which 5μm pore-sized filters were used.

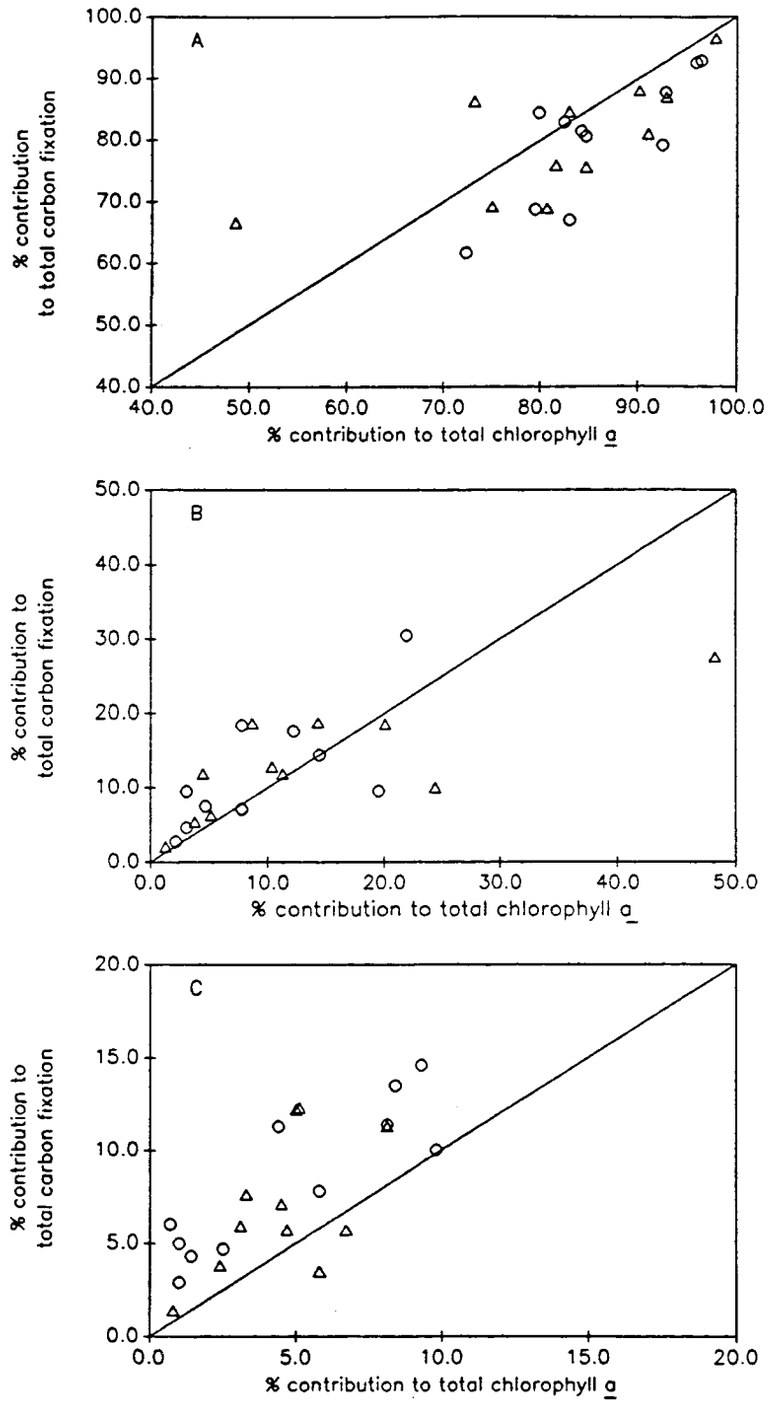

Figure 1.4.1.20. Relationship between the percentage contribution to the total depth integrated daily rate of carbon fixation (i.e.sum of fractions; DOC not included) and to the total chlorophyll a concentration (i.e. sum of fractions) for (A) the >3(5)μm (B) the 1-3(5)μm and (C) the <1μm size fractions, as determined from samples collected during the seasonal survey at NW Netley (o) and Calshot Spit (Δ). Solid line represents x=y.

contributed from 2.1 to 30.5 (12.4%(SD 7.5)) and the contribution of the fraction passing through 1μm filters varied between 1.4 to 14.6% (mean 7.6%(SD 3.8)). The fraction smaller than 3(5)μm contributed a mean of 20% (range between 3.5-38.3%).

A comparison of the percentage contribution to total chlorophyll *a* concentration (sum of fractions) and carbon uptake rate (sum of fractions; DOC not included) by each fraction revealed the fraction passing through 1μm filters to make, in general, a higher contribution to carbon uptake rate than to chlorophyll *a* concentration (i.e. the majority of values are over the x=y line in Figure 1.4.1.20C). For the intermediate fraction, 1-3(5)μm (Figure 1.4.1.20B) and particularly for the fraction >3(5)μm the majority of measurements evidenced a higher contribution to chlorophyll *a* than to primary production (Figure 1.4.1.20A). The >3(5)μm fraction made up for a mean of 83.9% of the total chlorophyll *a* and a mean of 79.9% of the carbon uptake rate. The 1-3(5)μm and <1μm fractions contributed a mean of 11.9% and 4.2% to the total chlorophyll *a*, and a mean of 12.4 and 7.6% to the primary production rate respectively. For the two largest fractions the differences are minor, but for the <1μm fraction the difference of 3.4% means that the contribution to carbon uptake rate was on average 44.7% higher than to chlorophyll *a*.

1.4.1.10. Annual rate of carbon fixation rate

Although fractionation data was only available from April to October, an attempt was made to estimate annual production rates, since it was considered profitable to evaluate the percentage contribution of the various fractions to an annual integrated value of carbon fixation rate. Chlorophyll *a* concentration measurements from unfractionated samples showed levels to be very similar during the sampling visits in January, February, March and first week of April. It was thus assumed that the pre-bloom April value of carbon uptake rate could be representative of winter levels and this value was then adjusted to the monthly mean irradiance level and the number of days in each month to obtain the estimates for the three winter months, January, February and March. No chlorophyll *a* values were obtained for the autumn months of November and December in the present study,

however previous studies have shown chlorophyll *a* values to be fairly similar from October to December in this estuary (Bryan, 1979; Kifle, 1989; Antai, 1990). It was thus assumed the October value as representative of the autumn months and monthly rates of carbon uptake were estimated as for the winter values. It is recognized that there is no information in the literature on variations in the size distribution of chlorophyll *a* during these months in Southampton Water, but Kifle (1989) showed no major differences in the specific composition and size distribution of the phytoplankton in the autumn and winter months. It is thus considered the estimation exercise to be based on reasonable grounds. For the rest of months most of the measurements were taken approximately fortnightly, and each was considered as representative for two weeks. The values of carbon uptake rate were then adjusted for the total irradiation in those two weeks.

The estimates of monthly carbon uptake rates thus obtained (sum of fractions; DOC not included) are shown in Figure 1.4.1.21. August appeared as the most productive month at the inner station; in contrast, at the outer station April was the most prolific month. The total annual rate of carbon uptake (sum of fractions; DOC not included) was estimated to be 130.580 and 176.705gC $m^{-2}yr^{-1}$ for Calshot Spit and NW Netley respectively.

Since DOC production was important during some sampling visits, in order to have more realistic values of annual rate of primary production, it was considered necessary to attempt to estimate also values of annual primary production rate as measured in the dissolved phase. From the monthly rates of primary production, monthly rates of apparent excretion were estimated using the percentage excretion values (mean percentage for the various light levels). For the months in which no carbon uptake measurements were undertaken the same assumptions as for the rates of carbon fixation into particulate material were made. When estimates of carbon uptake measured in the dissolved phase were taken into account, the rates of annual primary production were increased to 161.96 and 207.12gC $m^{-2}yr^{-1}$ at Calshot Spit and NW Netley respectively.

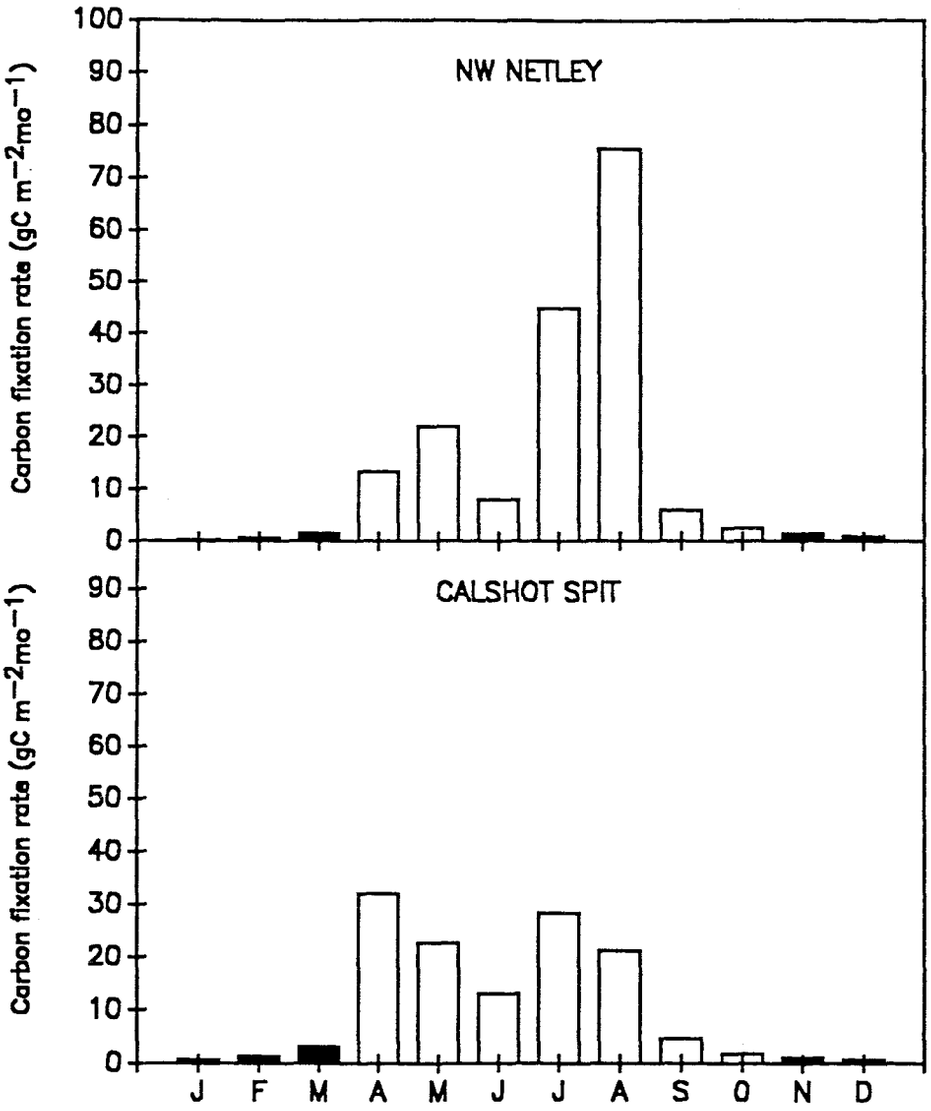

Figure 1.4.1.21. Monthly rates of carbon fixation (excluding DOC), integrated for the water column, by the plankton community, estimated for NW Netley and Calshot Spit. Filled bars denote months in which no measurements of primary production were undertaken and values have been extrapolated as explained in the text.

The distribution by size fractions and by seasons of the annual rate of carbon fixation into particulate material (sum of fractions; DOC not included) is shown in Table 1.4.1.3. A distribution by fractions of the annual rate of carbon uptake was similar at both stations and showed the >3(5)μm to account for 82.8 and 80.7% at Calshot Spit and NW Netley respectively; the 1-3(5)μm fraction to contribute to 10.9 and 13.1% at Calshot Spit and NW Netley, respectively and the <1μm fraction to represent 6.3 and 6.2% at Calshot Spit and NW Netley respectively. A distribution by fractions for each season is presented in Table 1.4.1.4. It is apparent that the >3(5)μm fraction was dominant in all seasons (>65%). The maximum contribution from this fraction was determined in spring (April, May, June) (85.8 and 85.1% at Calshot Spit and NW Netley, respectively). For the 1-3(5)μm fraction the lowest contribution was measured in spring (10.2 and 8.9% at Calshot Spit and NW Netley, respectively) and maximum values were estimated in autumn (October, November, December) and winter (January, February, March) (up to 18.7%). At Calshot Spit, summer (July, August, September) appeared to be the season when the <1μm fraction made the most significant contribution (9.4%). At NW Netley, however, autumn and winter were the periods when the <1μm fraction was most significant (>10%). The distribution by seasons of the annual rate of carbon uptake for each fraction is presented in Table 1.4.1.5. At Calshot Spit, the particulate production showed a seasonal distribution of 4.0, 51.8, 41.5, 2.7% for winter, spring, summer and autumn respectively. At NW Netley, the proportions were 1.4, 24.4, 71.5 and 2.7% respectively. At NW Netley similar trends were observed for all fractions, summer being the most productive season (>68%) and winter being the least productive (<4%). At Calshot Spit, however, for the >3(5)μm fraction the bulk of the production occurred in spring (53.6%); for the 1-3(5)μm fraction spring and summer shared similar proportions and for the <1μm fraction, as at NW Netley, summer was the most prolific season (62.0%).

Table 1.4.1.2. Values of total chlorophyll *a* determined from the sum of fractions, expressed as a percentage of the chlorophyll *a* determined in unfractionated samples, as determined during the seasonal survey at Calshot Spit and NW Netley.

Stat	Date										
	5/4	23/4	8/5	22/5	4/6	20/6	3/7	2/8	31/8	18/9	1/10
Cal	134	63	94	64	79	86	79	76	102	92	127
Net	95	78	99	83	83	93	151	48	90	92	98

Table 1.4.1.3. Carbon fixation rate into particulate material (DOC not included) (gC m^{-2}) by the various size fractions (>3(5)µm, 1-3(5)µm, <1µm) and by the total plankton community (sum of size fractions) integrated for the seasons, at Calshot Spit and NW Netley.

	NW NETLEY				CALSHOT SPIT			
	Total	>3(5)	1-3(5)	<1	Total	>3(5)	1-3(5)	<1
Winter(J+F+M)	2.485	1.709	0.440	0.336	5.227	4.422	0.622	0.183
Spring(A+M+J)	43.174	36.739	3.859	2.576	67.636	57.994	6.913	2.729
Summer(J+A+S)	126.287	100.732	17.990	7.565	54.216	43.078	6.054	5.084
Autumn(O+N+D)	4.759	3.398	0.882	0.479	3.501	2.647	0.655	0.199
Annual(%)		80.7	13.1	6.2		82.8	10.9	6.3

Table 1.4.1.4. Distribution by fractions (%) of the total carbon fixation rate (sum of size fractions; DOC not included) for each season at Calshot Spit and NW Netley.

	NW NETLEY			CALSHOT SPIT		
	>3(5)μm	1-3(5)μm	<1μm	>3(5)μm	1-3(5)μm	<1μm
Winter(J+F+M)	68.8	17.7	13.5	84.6	11.9	3.5
Spring(A+M+J)	85.1	8.9	6.0	85.8	10.2	4.0
Summer(J+A+S)	79.8	14.2	6.0	79.4	11.2	9.4
Autumn(O+N+D)	71.4	18.5	10.1	75.6	18.7	5.7

Table 1.4.1.5. Distribution of the annual carbon fixation rate by seasons (%) for the the various size fractions (>3(5)μm 1-3(5)μm and <1μm) at Calshot Spit and NW Netley.

	NW NETLEY				CALSHOT SPI			
	Total	>3(5)	3(5)-1	<1	Total	>3(5)	3(5)-1	<1
Winter(J+F+M)	1.4	1.2	1.9	3.1	4.0	4.1	4.4	2.3
Spring(A+M+J)	24.4	25.8	16.7	23.5	51.8	53.6	48.5	33.3
Summer(J+A+S)	71.5	70.6	77.6	69.0	41.5	39.8	42.5	62.0
Autumn(O+N+D)	2.7	2.4	3.8	4.4	2.7	2.5	4.6	2.4

1.4.1.11.P *v* I curves: photosynthetic parameters

Carbon uptake rate values were fitted to a P v I type of curve according to the equation by Platt *et al.* (1980) (see section 1.3.1.4). The goodness of fit was sometimes somewhat poor, but on average 86.5(SD 13.5)% of the variance could be accounted for. This procedure allowed the estimation of P_{max} for the dates in which illumination during incubation was not enough to reach saturation of production. In, general, however, since the P_s and β parameters were calculated from 1 or 2 data points, the standard errors were in the majority of cases very large and thus values of P_{max} should be considered with some caution. The estimates for α were more resonable and, on average, standard errors were 19% of the estmated α.

Values of α^B and P_m^B for the various fractions for samples from Calshot Spit and NW Netley are given in Table 1.4.1.6. Values of α expressed as mgC mgchl $a^{-1}h^{-1}(\mu Em^{-2}s^{-1})^{-1}$ (α^B) varied from 0.011 to 0.106 for the fraction retained on 3(5)μm filters, minima being recorded at NW Netley during the *Phaeocystis* sp. bloom on May the 8th and maxima being recorded on June 4th at both stations. For the rest of sampling dates values were reasonably similar and the mean of all measurements from both stations was 0.036(SD 0.023). For the 1-3(5)μm fraction α^B ranged from 0.0071 to 0.124 and a mean value of 0.049(SD 0.029) was estimated. For the fraction passing through 1μm filters α oscillated between 0.043 and 0.155, with a mean value of 0.083(SD 0.029). Paired comparison tests ($p<0.05$) showed α^B for the fraction smaller than 1μm to be consistently larger than α^B for the >3(5)μm and the 1-3(5)μm fractions. However, the test showed α^B for the 1-3(5)μm fraction was not consistently larger than α^B for the >3(5)μm fraction. α and P_{max} expressed on a volume of seawater basis were positively correlated for the three fractions, however when expressed on a chlorophyll *a* basis (α^B and P_m^B) were only correlated for the <1μm fraction ($r=0.711$, $n=19$, $p<0.001$).

At irradiance levels above 800-900$\mu Em^{-2}s^{-1}$ carbon uptake rate was depressed from saturation levels in all fractions.

Table 1.4.1.6. Photosynthetic parameters derived from P v I curves for the various size-fractions and the total plankton community: P^B_m (μgC μgchla^{-1}h^{-1}) α_B (μgC μgchla^{-1}h^{-1}(μEm^{-2}s^{-1})$^{-1}$), determined from samples collected during the seasonal survey at Calshot Spit and NW Netley.

Date	P^B_m				α_B			
	Total	>3(5)	1-3(5)	<1	Total	>3(5)	1-3(5)	<1
				NW NETLEY				
05.04	3.35	2.88	5.16	5.19	0.030	0.025	0.048	0.064
08.05	2.77	3.02	1.11	22.27	0.011	0.011	0.007	0.111
22.05	8.55	(8.45)	(6.63)	28.44	0.034	(0.032)	(0.044)	0.155
04.06	5.52	5.56	6.46	5.43	0.110	0.106	0.056	0.108
20.06	4.59	4.56	4.84	4.18	0.028	0.027	0.027	0.076
03.07	4.91	4.78	5.75	13.00	0.021	0.020	0.030	0.080
02.08	11.90	(9.96)	(17.03)	11.90	0.051	(0.043)	(0.071)	0.102
31.08	8.08	(8.70)	(9.86)	7.36	0.027	(0.025)	(0.044)	0.073
18.09	4.20	8.82	17.49	6.97	0.026	0.022	0.070	0.073
01.10	4.42	3.58	10.59	6.12	0.034	0.028	0.083	0.069
				CALSHOT SPIT				
05.04	7.06	5.45	5.30	2.35	0.057	0.043	0.048	0.059
08.05	4.77	5.66	1.84	6.61	0.020	0.023	0.008	0.037
22.05	11.17	(15.64)	(5.74)	20.68	0.049	(0.065)	(0.036)	0.095
04.06	6.03	5.99	9.03	10.48	0.090	0.085	0.093	0.118
20.06	6.94	6.29	18.05	-	0.035	0.030	0.032	-
03.07	5.25	4.93	7.31	11.50	0.025	0.023	0.038	0.057
02.08	6.94	(5.95)	(9.73)	14.33	0.035	(0.031)	(0.039)	0.100
31.08	4.07	(3.43)	(8.56)	8.28	0.027	(0.025)	(0.021)	0.067
18.09	3.18	2.93	7.08	2.24	0.032	0.027	0.124	0.087
01.10	4.26	3.90	8.93	2.57	0.030	0.027	0.064	0.043

- unreliable value (not included)

1.4.1.12.Correlation analysis

Correlation analysis between a number of environmental parameters (temperature, light availability (i.e. I_o/k), nitrate+nitrite and phosphate concentrations) and picophytoplankton cell number, chlorophyll *a* concentration and carbon fixation rate by the various size fractions was carried out. Results are presented in Table 1.4.1.7 and Table 1.4.1.8 for Calshot Spit and NW Netley respectively.

At Calshot Spit significant positive correlations ($p<0.05$) were found between temperature and a number of variables: chlorophyll *a* concentration and carbon fixation rate by the <1μm size fraction, PE-containing picocyanobacteria and eukaryotic picophytoplankton cell concentrations. Carbon fixation rate by the total plankton community and the >3(5)μm size fraction appeared to be significantly correlated with light availability. There was a weak inverse correlation ($r=0.56$, $p<0.1$) between phosphate concentration and carbon fixation rate by the <1μm size fraction. No other correlations were found between nutrient concentrations and chlorophyll *a* or carbon fixation rate by any size fraction. Chlorophyll *a* concentration and carbon fixation rate were significantly correlated for each size fraction. PE-containing picocyanobacteria numbers were also significantly correlated with chlorophyll *a* concentration and carbon fixation rate by the <1μm size fraction.

At NW Netley, total carbon fixation rate and carbon fixation rate by the >3(5)μm and the <1μm size fractions, as well as PE-containing picocyanobacteria and eukaryotic picophytoplankton cell numbers appeared to be significantly correlated with temperature. No significant correlations were found for the parameters tested with light availability nor nutrient concentration. As for Calshot Spit chlorophyll *a* concentration and carbon fixation rate were significantly correlated for each size fraction. In general there was also significant correlation between fractions. Both PE-containing picocyanobacteria and eukaryotic picophytoplankton cell concentrations were significantly correlated with chlorophyll *a* concentration and carbon fixation rate by the <1μm size fraction and PE-containing picocyanobacteria were correlated with eukaryotic picophytoplankton.

Table 1.4.1.7. Correlation analysis of various physico-chemical parameters, picophytoplankton numbers and chlorophyll *a* concentration and primary production in the various size fractions from data collected during the seasonal survey at Calshot Spit. (ns= not significant for $p<0.05$; ++= significant positive correlation $p<0.05$; +++= significant positive correlation, $p<0.01$).

	T	L	N	P	Ch_T	Ch_3	Ch_1	Ch_{o2}	PP_T	PP_3	PP_1	PP_{o2}	Cy	Euk
T		ns	ns	ns	ns	ns	ns	++	ns	ns	ns	++	++	++
L			ns	ns	ns	ns	ns	ns	++	++	ns	ns	ns	ns
N				+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P					ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ch_T						+++	ns	ns	+++	+++	ns	ns	ns	ns
Ch_3							ns	ns	+++	+++	ns	ns	++	ns
Ch_1								ns	ns	ns	+++	ns	ns	ns
Ch_{o2}									ns	ns	ns	+++	+++	ns
PP_T										+++	ns	ns	ns	ns
PP_3											ns	ns	ns	ns
PP_1												++	ns	ns
PP_{o2}													++	ns
Cy														ns
Euk														

Abbreviations:

T= temperature
L= light availability (I_o/k)
N= nitrate+nitrite concentration
P= phosphate concentration
Ch_T= total chlorophyll *a* concentration
Ch_3= chlorophyll *a* concentration in >3(5)μm size fraction
Ch_1= chlorophyll *a* concentration in 1-3(5)μm size fraction
Ch_{o2}= chlorophyll *a* concentration in <1μm size fraction
PP_T= total daily depth integrated carbon uptake rate
PP_3= daily depth integrated carbon uptake rate by >3(5)μm size fraction
PP_1= daily depth integrated carbon uptake rate by 1-3(5)μm size fraction
PP_{o2}= daily depth integrated carbon uptake rate by <1μm size fraction
Cy= PE-containing picocyanobacteria cell number
Euk= Red-fluorescing picophytoplankton cell number

Table 1.4.1.8. Correlation analysis of various physico-chemical parameters, picophytoplankton numbers and chlorophyll *a* concentration and primary production in the various size fractions from data collected during the seasonal survey at NW Netley. (ns= not significant for $p<0.05$; ++= significant positive correlation, $p<0.05$; +++= significant positive correlation, $p<0.01$).

	T	L	N	P	Ch_T	Ch_3	Ch_1	Cho_2	PP_T	PP_3	PP_1	PPo_2	Cy	Euk
T		ns	ns	ns	ns	ns	ns	++	++	ns	ns	ns	++	++
L			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N				ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P					ns	ns	ns	ns	ns	ns	ns	++	ns	ns
Ch_T						+++	+++	ns	++	+++	ns	ns	ns	ns
Ch_3							++	ns	++	+++	ns	ns	ns	ns
Ch_1								++	++	ns	++	++	ns	ns
Cho_2									+++	+++	+++	+++	+++	++
PP_T										+++	+++	+++	+++	+++
PP_3											++	+++	+++	++
PP_1												+++	+++	+++
PPo_2													+++	+++
Cy														+++
Euk														

Abbreviations:

T= temperature
L= light availability (I_o/k)
N= nitrate+nitrite concentration
P= phosphate concentration
Ch_T= total chlorophyll *a* concentration
Ch_3= chlorophyll *a* concentration in >3(5)μm size fraction
Ch_1= chlorophyll *a* concentration in 1-3(5)μm size fraction
Cho_2= chlorophyll *a* concentration in <1μm size fraction
PP_T= total daily depth integrated carbon uptake rate
PP_3= daily depth integrated carbon uptake rate by >3(5)μm size fraction
PP_1= daily depth integrated carbon uptake rate by 1-3(5)μm size fraction
PPo_2= daily depth integrated carbon uptake rate by <1μm size fraction
Cy= PE-containing picocyanobacteria cell number
Euk= Red-fluorescing picophytoplankton cell number

1.4.2. Transect along the longitudinal axis of the estuary from Redbridge to Calshot Spit

Sampling took place on September 3rd 1990 along the Test sub-estuary down to the vicinity of Calshot Spit Buoy (The Solent). The sampling area is shown in Figure 1.3.3 (section 1.3.2.1). Sampling commenced at the start of the first high water stand at Redbridge.

1.4.2.1. Temperature and salinity distribution

Salinity and temperature data are presented in Table A.12 (Appendix). Samples taken along the transect yielded a range of salinity of 7.5 to 34.5ppt. Surface salinity was very patchy between Redbridge and just before entering Marchwood Channel and a steady seaward increase in salinity was not observed in this sector. The greater increase in salinity was also observed in this area: from 7.5 to 28.3ppt. Salinity along Marchwood Channel ranged from 29.1 to 32.7ppt and from Weston Shelf buoy (at the confluence of the Test and Itchen estuaries) to Calshot Spit buoy (the inner area of the Solent) it span from 33 to 34.5ppt. Surface temperature was less variable along the transect, ranging from 18.7 to 20.3°C.

1.4.2.2. Nitrate+nitrite and phosphate concentration

Values of both nitrate+nitrite and phosphate concentration along the transect can be seen in Figures 1.4.2.1. Data have also been tabulated in Table A.13 of Appendix. Concentrations of both nutrients decreased with salinity. A theoretical dilution line for a consevative behaviour has not been possible to establish because values in one of the end-point members (0ppt) are missing. It is thus difficult to hypothesize on the conservative/non-conservative behaviour of these nutrients. Despite this limitation it is possible to observe an area of phosphate input to the water column in Marchwood Channel (29.1-32.7ppt).

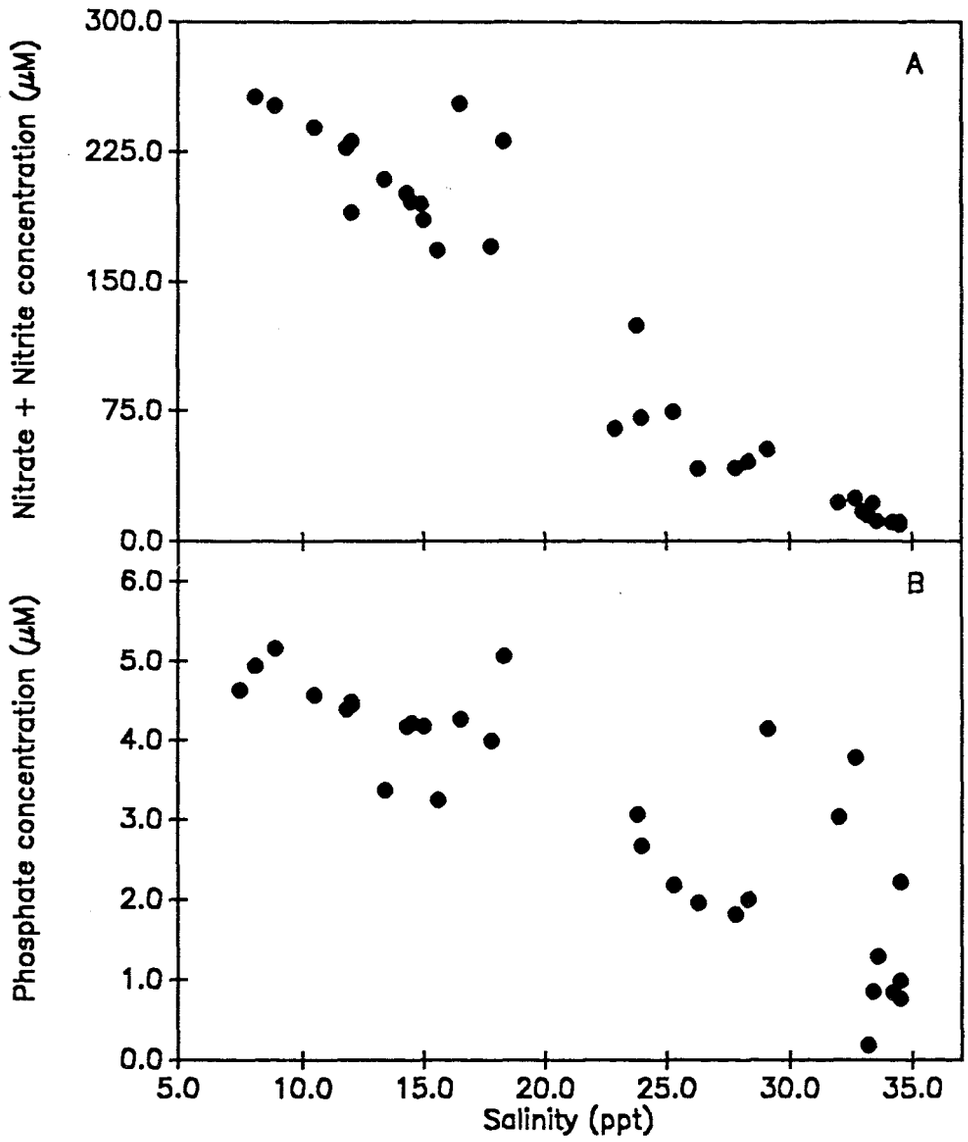

Figure 1.4.2.1. Relationship between salinity and (A) nitrate plus nitrite concentration and (B) phosphate concentration in samples collected along a longitudinal axis of the estuary on September 3rd.

1.4.2.3. Chlorophyll a and phaeopigment concentration

Chlorophyll *a* and phaeopigment concentration was determined by size-fractionation for the >5μm fraction, the fraction between 1 and 5 μm and the 0.2 to 1μm fraction. Measurements were made on single samples. These results are presented in Table A.14 (Appendix). Figure 1.4.2.2 is an illustration of the size cumulative distribution of chlorophyll *a* along the salinity gradient. Clearly, along the entire transect the dominant fraction was the larger than 5μm, with chlorophyll *a* biomass ranging from 0.96 to 11.83mg m^{-3}. Chlorophyll *a* concentration was lower in the 1 to 5μm fraction, spanning from 0.22 to 0.88mg m^{-3}. The fraction with the smallest contribution to the plankton community standing stock of chlorophyll *a* was the <1μm. Chlorophyll *a* levels in this fraction varied from 0.03 to 0.36mg m^{-3}.

In the fraction larger than 5μm, chlorophyll *a* showed an exponential like increase with salinity, up to salinity levels of 34.2ppt (i.e. around Bed 31+33 buoy), and thereafter declined sharply to concentrations of *ca.* 2.5mg m^{-3}, so that levels at Calshot Spit were comparable to levels measured at salinities around 15 to 17ppt (Figure 1.4.2.3). In the 1-5μm fraction chlorophyll *a* concentration decreased slightly with increasing salinity up to a point around 24ppt. Above this level of salinity the value of chlorophyll *a* standing stock showed a marked increase up to salinities around 34.2ppt, beyond which, as for the >5μm fraction, chlorophyll *a* values were reduced significantly (Figure 1.4.2.3). In the <1μm size fraction chlorophyll *a* was also higher at high salinities, but no clear effect of a reduction in concentration towards the seaward end was observed (Figure 1.4.2.3).

The variation in the percentage contribution of each size fraction to the total plankton community chlorophyll *a* biomass with salinity is presented in Table A.15 (Appendix) and illustrated in Figure 1.4.2.4 and Figure 1.4.2.5. The larger than 5μm contributed from 65.5 to 94%. The contribution of the 1-5μm size fraction ranged from 4.4 to 28.68%. The 1-0.2μm fraction accounted only for 1.7 to 8.6%. In the larger than 5μm fraction the pattern of variation of the percentage contribution to the overall plankton community chlorophyll *a* biomass with salinity resembled that of the chlorophyll *a* concentration. An increase with salinity was observed up to 34.2ppt in

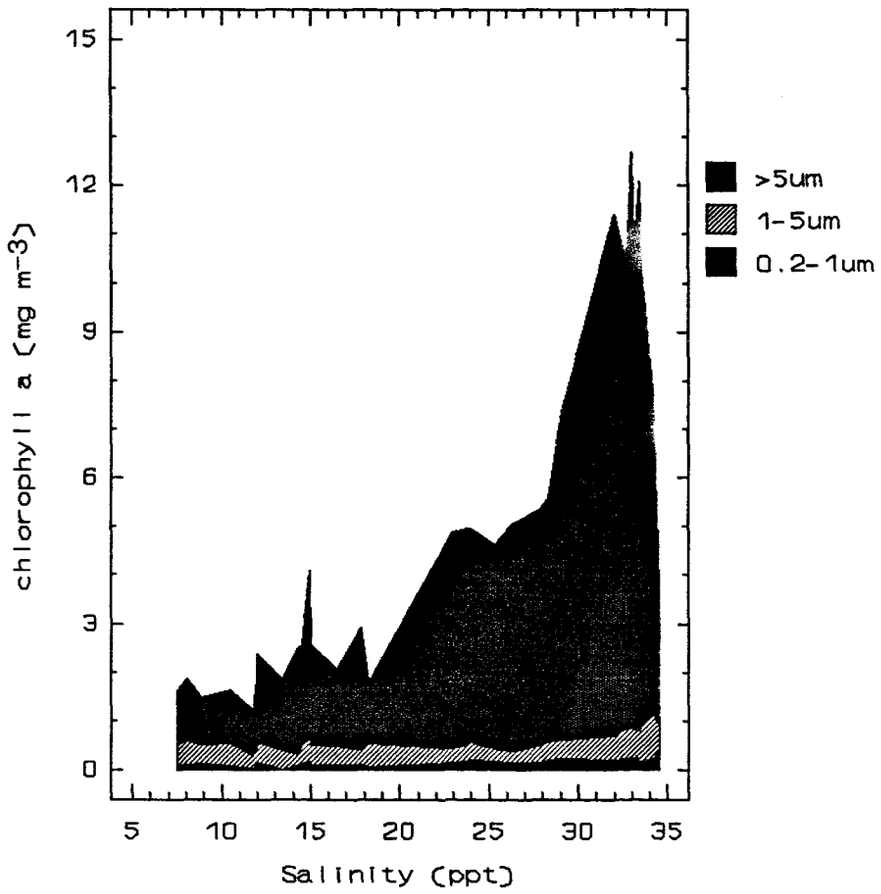

Figure 1.4.2.2. Cumulative line chart of the distribution of chlorophyll *a* concentration in relation to salinity determined from samples collected along a longitudinal axis of the estuary on September 3rd.

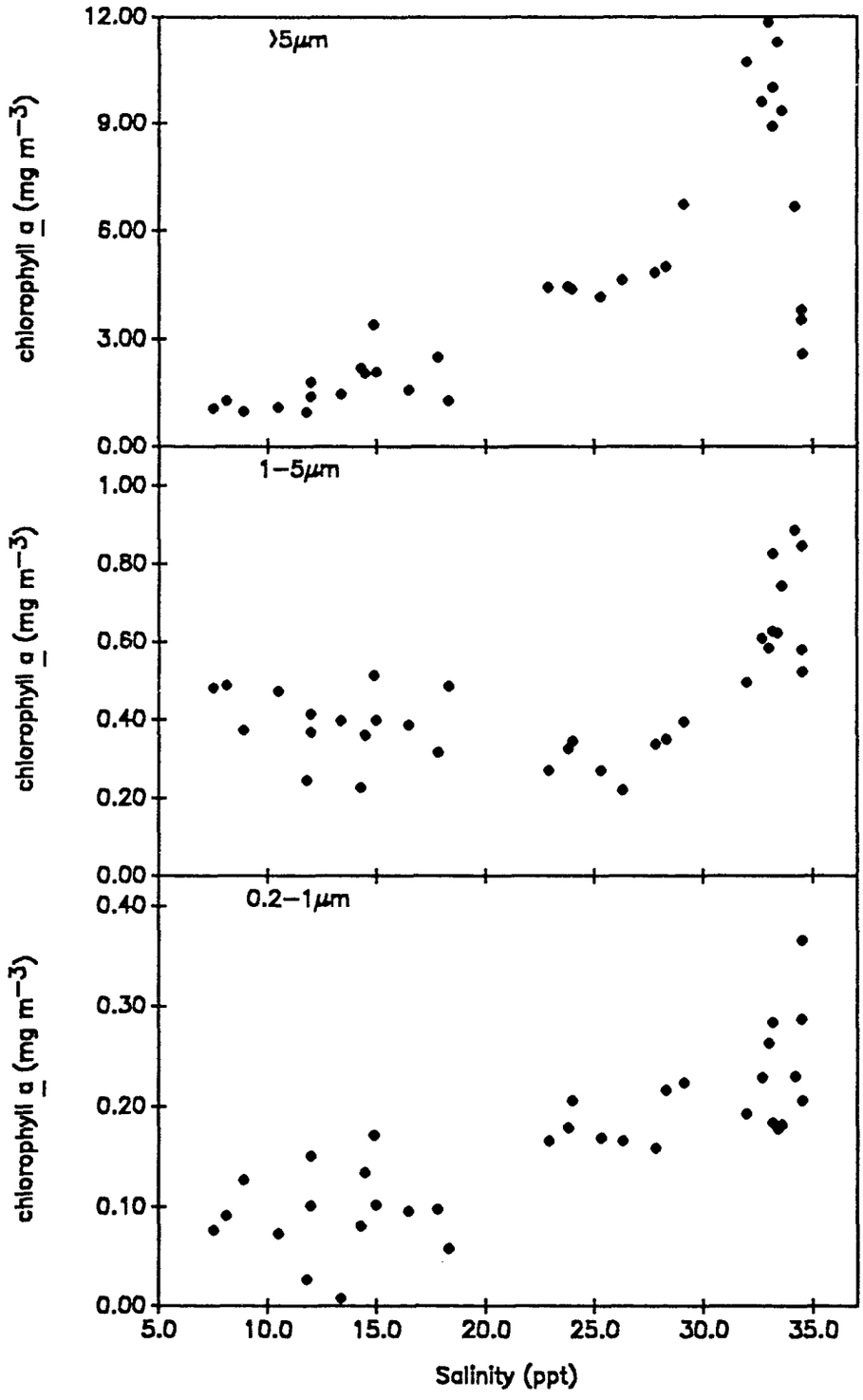

Figure 1.4.2.3. Size fractionated chlorophyll *a* distribution in relation to salinity in samples collected along a longitudinal axis of the estuary on September 3rd.

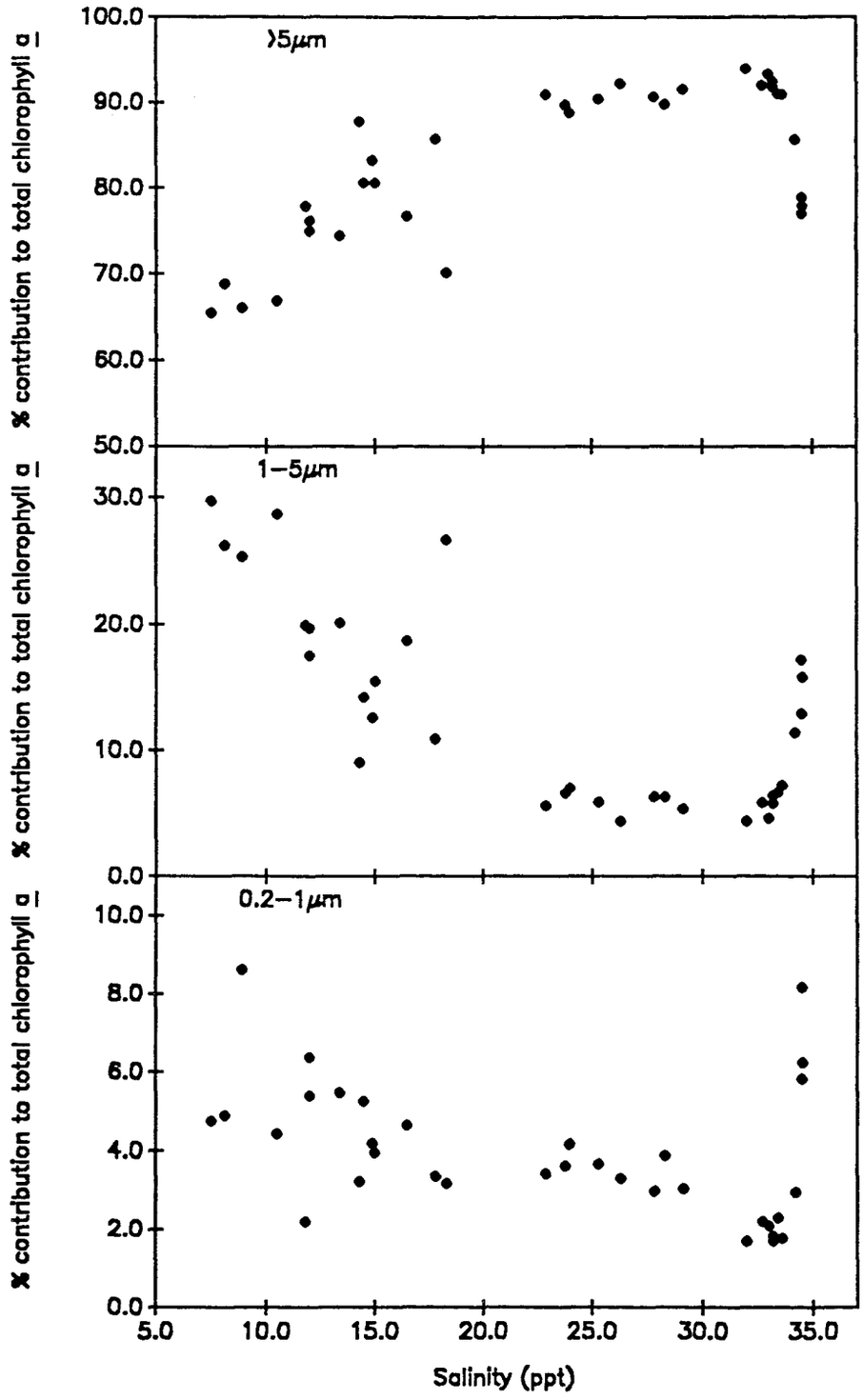

Figure 1.4.2.4. Size distribution of chlorophyll *a* expressed as a percentage of total (sum of fractions) in relation to salinity in samples collected along a longitudinal axis of the estuary on September 3rd.

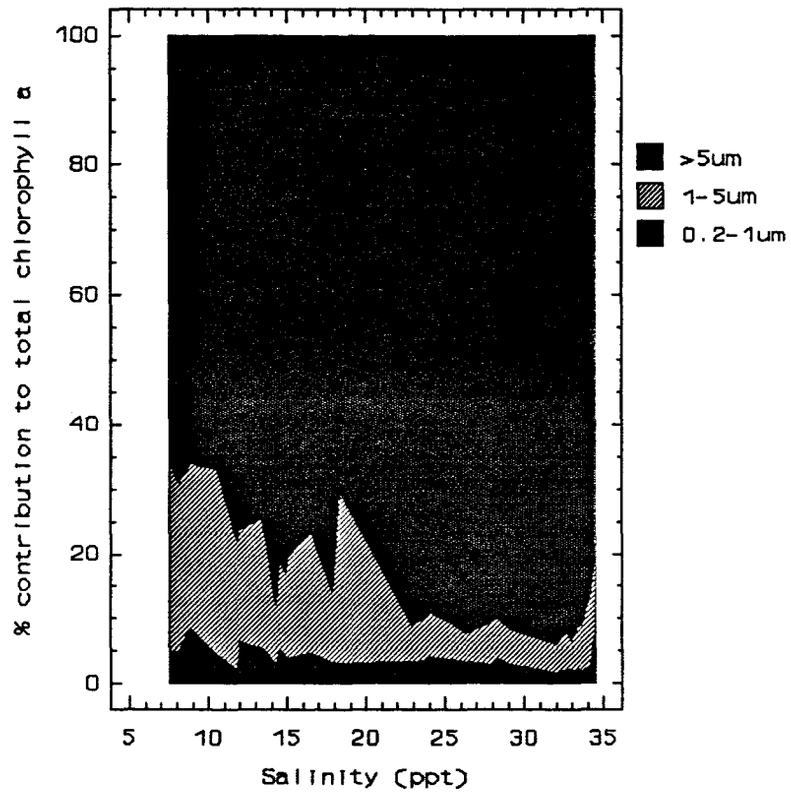

Figure 1.4.2.5. Cumulative line chart of the percentage contribution by each size fraction to the total plankton community chlorophyll *a* concentration (sum of fractions) in relation to salinity, as determined from samples collected along a longitudinal axis of the estuary on September 3rd.

the vicinity of Bed 31+33 buoy, thereafter dropping to levels of 78% around Calshot Spit. In the two smaller fractions (1-5μm and <1μm), however, the trend of variation was the opposite. Both fractions contributed less as salinity increased up to the vicinity of Bed 31+33 buoy. From this point down to Calshot Spit buoy the contribution of both fractions increased to levels of 15.8 and 6.2% respectively. From these considerations it appears that the decrease of chlorophyll *a* at low salinity observed in the three fractions studied was most pronounced in the larger than 5μm. The percentage contribution to total chlorophyll *a* concentration by the 1-5μm and the <1μm fractions showed an inverse relationship with the total chlorophyll *a* concentration (Figure 1.4.2.6).

The percentage phaeopigment (i.e. phaeopigment/(phaeopigment + chlorophyll *a*)x100) decreased with increasing phytoplankton size fraction (Table A. 16 in Appendix; Figure 1.4.2.7). It ranged from 5.4 to 41.6% in the >5μm fraction, from 14.7 to 66.6% in the 1-5μm fraction and from 22.5 to 82.0% in the <1μm fraction. The contribution to the total phaeopigment in each sample by each size fraction (Table A.15 in Appendix) plotted against the contribution to the total chlorophyll *a* concentration (Figure 1.4.2.8) showed clearly that in most cases the two smaller fractions contributed more to the degraded forms of chlorophyll *a* than to the pool of active chlorophyll *a*, whereas the fraction >5μm made a greater input to the active chlorophyll *a* biomass. These results are in agreement with results obtained during the seasonal survey at NW Netley and Calshot Spit. In all three fractions the percentage phaeopigment decreased as salinity increased (Figure 1.4.2.7). Also, the percentage phaeopigment in the total plankton community (i.e sum of fractions) showed an inverse exponential relationship with the total chlorophyll *a* concentration (i.e. sum of fractions) (Figure 1.4.2.9).

The relationship between chlorophyll *a* concentration and nitrate+nitrite concentration was the inverse to that between chlorophyll *a* concentration and salinity (Figure 1.4.2.10). For the >5μm and 1-5μm size fractions at high concentrations of nitrate+nitrite total chlorophyll *a* concentration was minimum and increased exponentially as levels of nitrate+nitrite decreased, up to a point (around 12μM) below which the chlorophyll *a* concentration

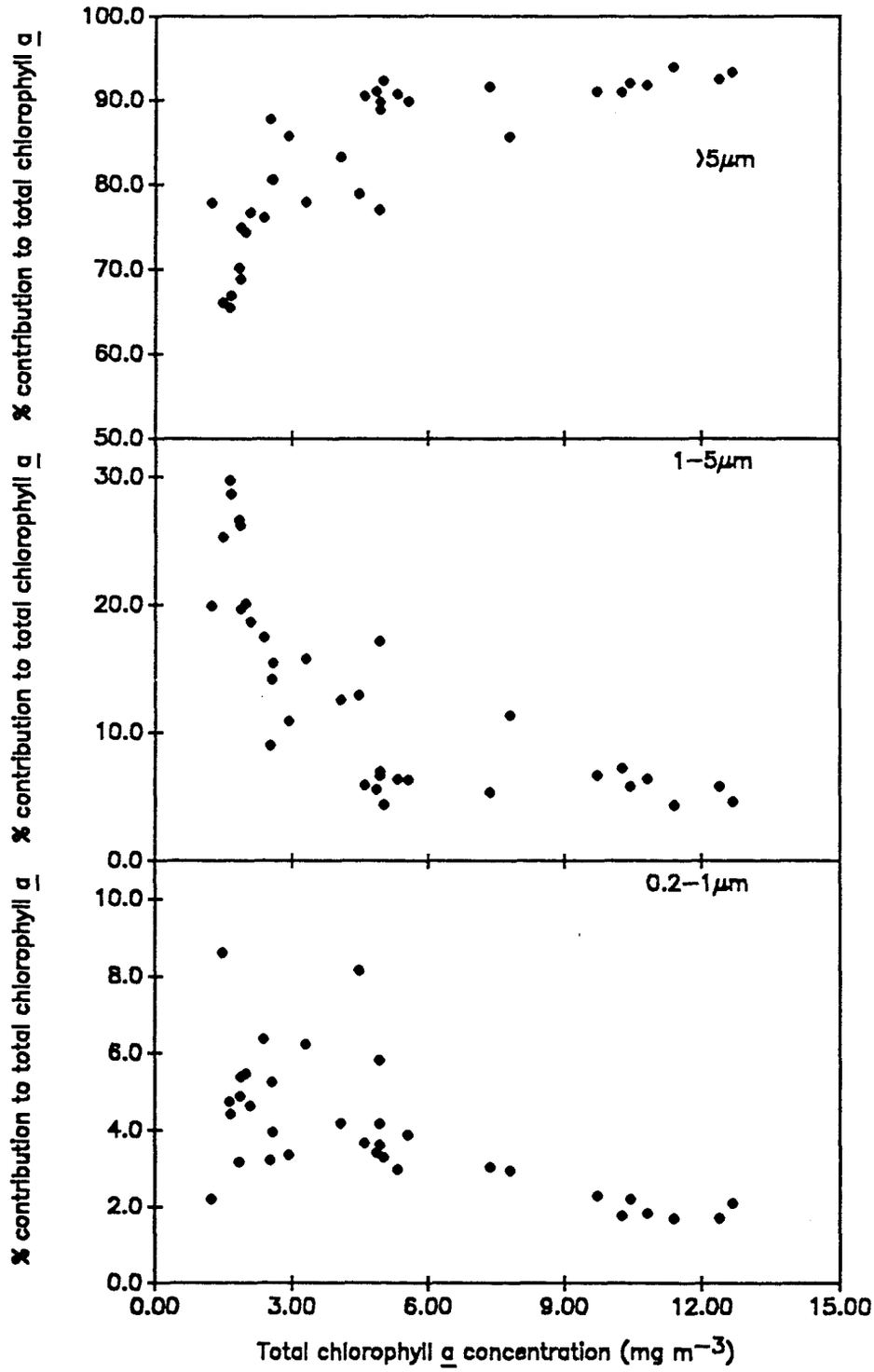

Figure 1.4.2.6. Relationship between total chlorophyll *a* concentration (sum of fractions) and the percentage contribution by each size fraction to total chlorophyll *a* concentration in samples collected along a longitudinal axis of the estuary on September 3rd.

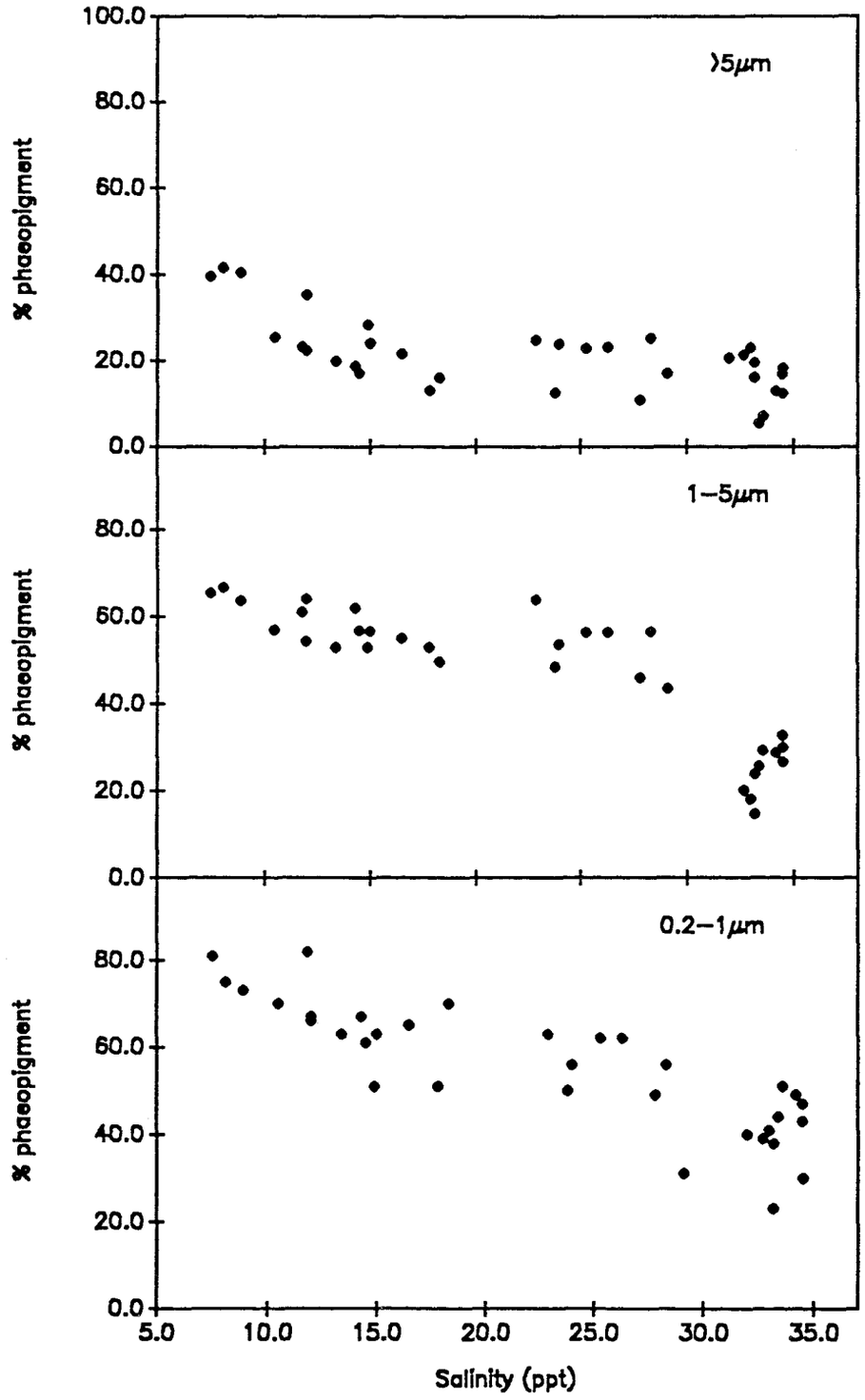

Figure 1.4.2.7. Relationship between salinity and percentage phaeopigment for the various size fractions in samples collected along a longitudinal axis of the estuary on September 3rd.

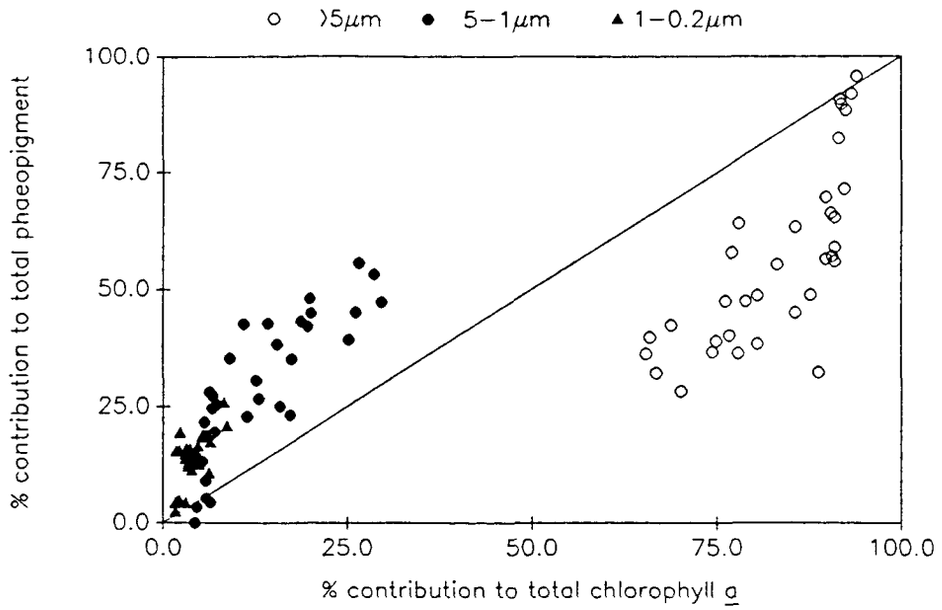

Figure 1.4.2.8. Relationship between the percentage contribution to total chlorophyll *a* and total phaeopigment concentrations (sum of fractions) for each size fraction, as measured in samples collected along a longitudinal axis of the estuary on September 3rd. Solid line represents x=y.

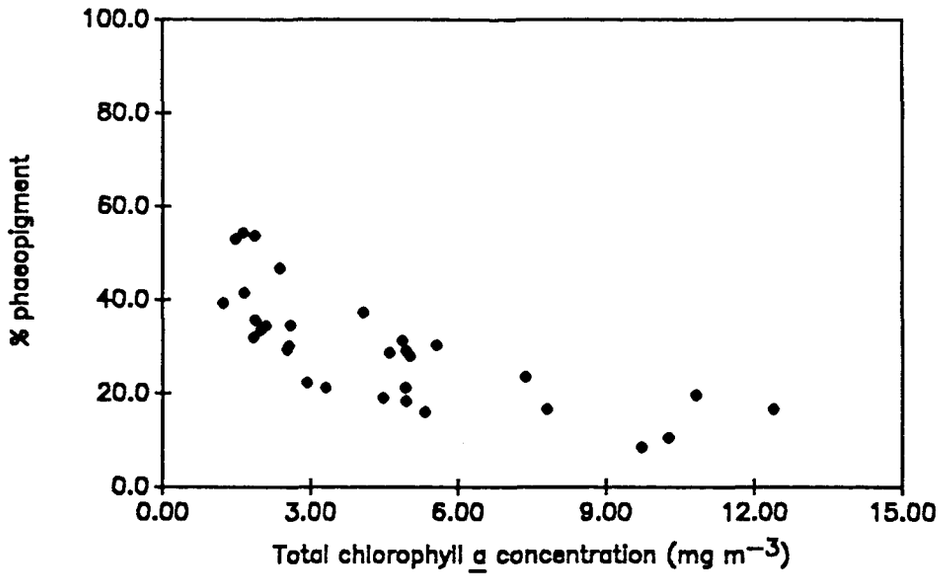

Figure 1.4.2.9. Relationship between the percentage phaeopigment and the chlorophyll *a* concentration for the total plankton community in samples collected along a longitudinal axis of the estuary on September 3rd.

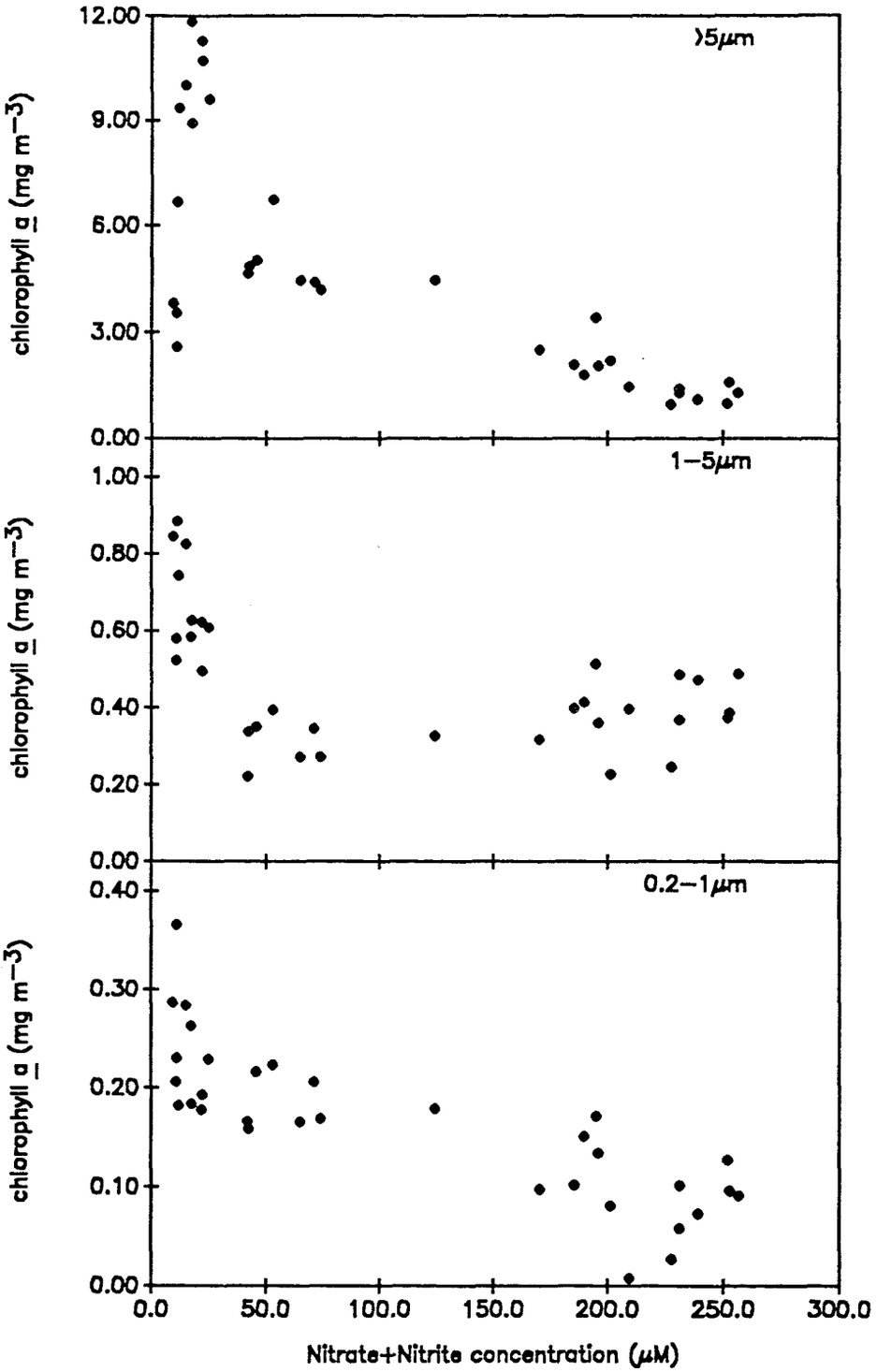

Figure 1.4.2.10. Relationship between nitrate plus nitrite concentration and chlorophyll *a* concentration for the various size fractions in samples collected along a longitudinal axis of the estuary on September 3rd.

decreased sharply. In the <1μm the inverse relationship between nitrate+nitrite and chlorophyll *a* concentration was apparent along the entire nutrient gradient (Figure 1.4.2.10). There was no such clear relationship between phosphate and chlorophyll *a* concentrations.

1.4.2.4. Picophytoplankton: phycoerythrin containing picocyanobacteria and eukaryotic picophytoplankton

Figure 1.4.2.11 is an illustration of the distribution of orange fluorescing phycoerythrin rich picocyanobacteria cell concentration along the salinity gradient. Data can also be found in tabulated form in Table A.12 (Appendix). Numbers varied from 75 to 3609 cells ml^{-1}. Between salinities of 7.5 (Redbridge) and 29ppt (beginning of Marchwood Channel) counts ranged from 75 to 945 cells ml^{-1}. Between salinities of 32 (Marchwood Channel) and 34.5ppt (Calshot Spit) cell numbers showed a marked increase towards the seaward end of the transect.

Figure 1.4.2.11 also depicts the distribution of the less than 5μm eukaryotic algal cells along the salinity gradient (values are also presented in tabulated form in Table A.12 of Appendix). Concentrations also showed a general tendency to increase at high salinities, but the signal was noisier, possibly due to the variations in species composition and the species specific tolerances and preferences of salinity. Algal cells smaller than 5μm clearly outnumbered phycoerythrin containing picocyanobacteria in all samples and showed a variation between 2756 to 13283 cells ml^{-1}.

Although no measurement of individual cell size was made, it was observed that smaller than 5μm red fluorescing algal cells in general tended to be larger and more variable in size in the less saline part of the transect, whereas from Weston Shelf to Calshot Spit minute cells (*ca.* 1μm) were dominant. Thus a linear relationship between cell counts and biomass should not necessarily be found.

High numbers of phycoerythrin containing picocyanobacteria coincided with high levels of chlorophyll *a* in the <1μm fraction plus the 1-5μm fraction (i.e. 0.2-5μm size fraction), but a linear relationship between counts and chlorophyll *a* concentration was not found (Figure 1.4.2.12). Large concentrations of smaller than 5μm

eukaryotic algae were also accompanied by increased levels of chlorophyll *a* in the 0.2-5μm size fraction, but the correlation was also poor (Figure 1.4.2.12). This is a reflection of the noise introduced by the differences in size of the eukaryotic cells, the presence of the picocyanobacteria and possibly the presence of fragments of larger cells.

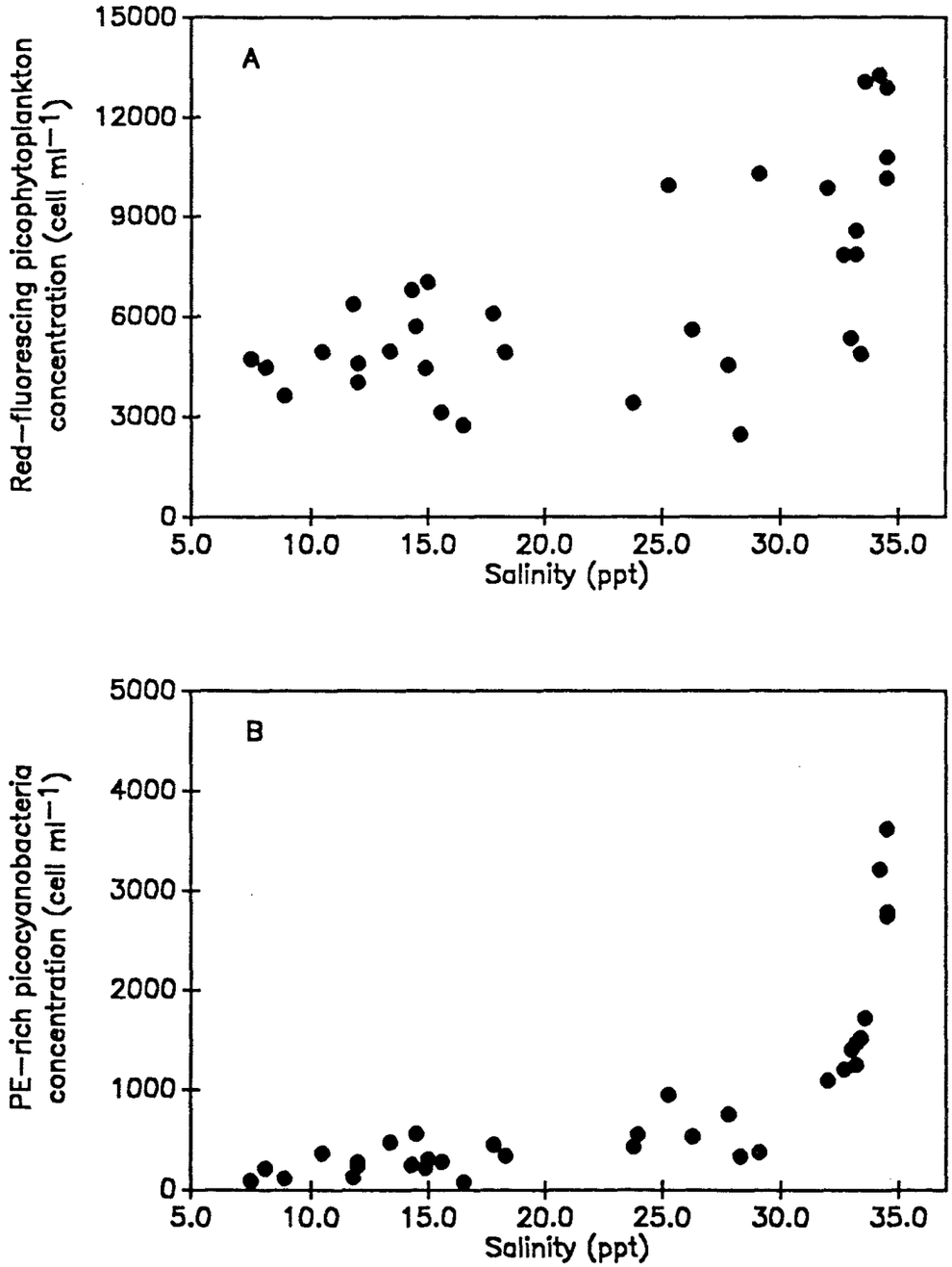

Figure 1.4.2.11. Relationship between salinity and (A) red-fluorescing picophytoplankton and (B) PE-containing picocyanobacteria cell concentration in samples collected along a longitudinal axis of the estuary on September 3rd.

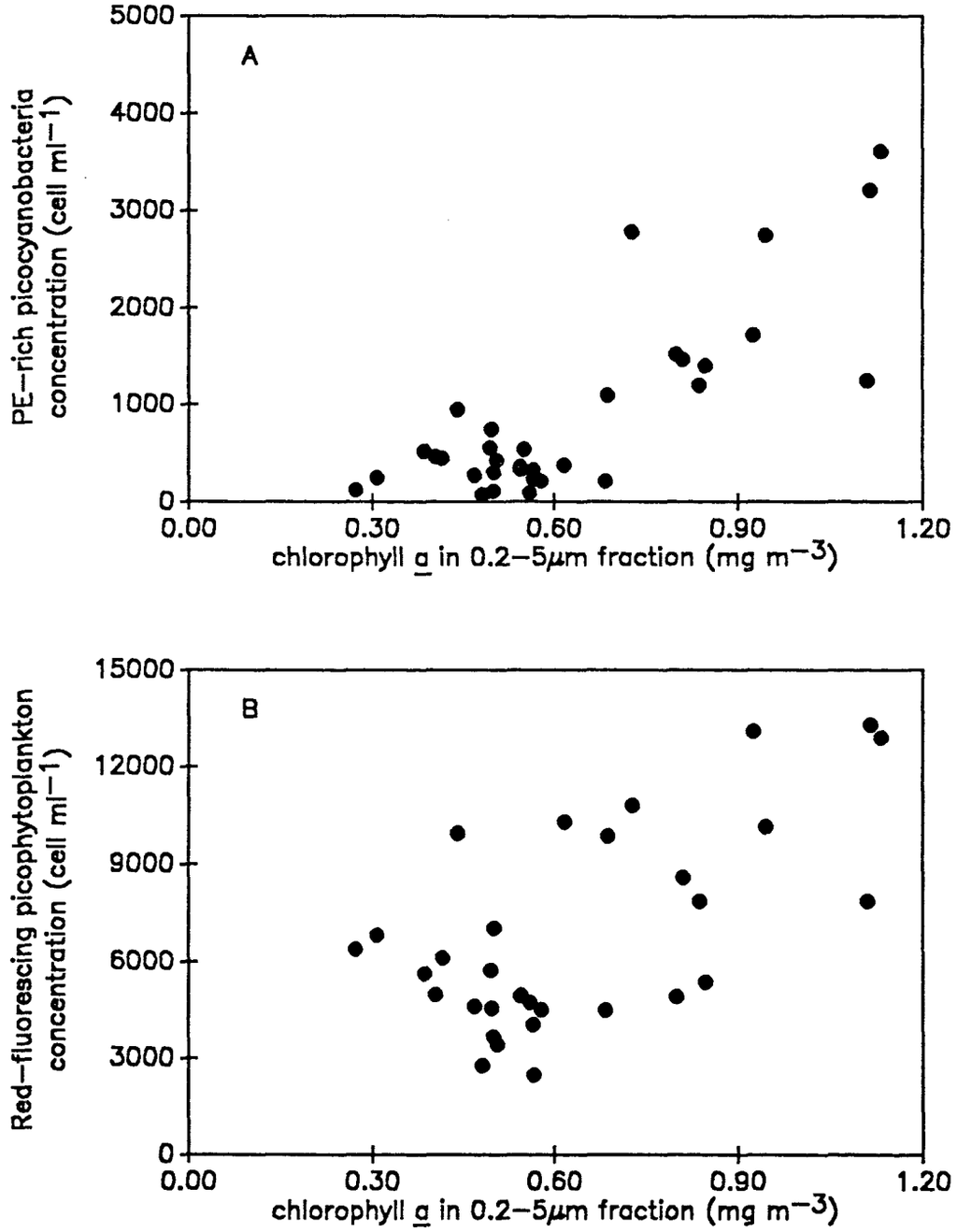

Figure 1.4.2.12. Relationship between chlorophyll a concentration in the 0.2-1μm plus the 1-5μm size fractions and (A) PE-containing picocyanobacteria and (B) red-fluorescing picophytoplankton concentration.

1.4.3. Studies in the North Sea: June/July 1989

1.4.3.1. Physico-chemical characteristics of sampling sites

A summary of the main physical characteristics of the sampling sites are presented in Table 1.4.3.1. Samples were taken between latitude 51.17N and 55.51N and longitude 0.47E and 7.00W, which corresponds to the southern Bight and Central North Sea. The vertical profiles of temperature and salinity indicated that in most locations the water column was mixed (data are not shown). At several sites in the Central North Sea a thermal stratification had developed.

Salinity was fairly uniform (34 to 35ppt) in all samples. Temperature ranged from 10 to 15.8°C, the variation being attributable to differences in latitude and depth of sampling. Extinction coefficients oscillated between 0.12 and 0.69m^{-1}. Highest coefficients were found in two locations in the southern latitudes, close to shore (Experiment 1 and 2). Extinction coefficients were not correlated to chlorophyll *a* concentrations (Figure 1.4.3.1), so suspended material other than the phytoplankton contributed more significantly to the turbidity of the water. Euphotic zone depths calculated as the depth of 1% surface incident irradiance, ranged from 6.6 to 37.4m. The depth of mixing exceeded that of the euphotic zone in sampling locations 1, 2, 3, 7 and 7A. Most of the experimental days were clear and sunny, with mean surface incident irradiances during the illuminated hours of *ca.* 1000$\mu Em^{-2}s^{-1}$ (PAR).

Vertical profiles of the concentration of the major inorganic nutrients obtained from the NERC North Sea Project database, are shown in Table A.17 in Appendix. Concentrations were relatively low, typical of summer periods (Hydes *et al.*, 1989). The depth distribution coincided with the stratification pattern indicated by the vertical temperature distribution. Concentrations were roughly uniform in mixed water columns and tended to increase towards the bottom in stratified locations. In general, the dominant form of inorganic nitrogen was ammonium. At sampling site 5 both ammonium and nitrate levels were much higher than in the rest of samples.

Table 1.4.3.1 Summary of physical characteristics of the sampling sites for the various experiments conducted in June/July 1989 in North Sea waters.

Date	exp no.	Lat	Long	Z_s	T^a	k	I_o	Z_{eu}	Z_m
25.06	1	52.6104N	3.99969E	7.6	15.4	0.55	1098	8.3	26
26.06	2	51.1825N	1.56815E	7.1	15.8	0.69	994	6.6	55
27.06	3	52.9981N	3.43121E	5.3	14.3	0.35	480	12.9	26
28.06	4	54.5820N	4.83077E	15.0	12.0	0.19	862	24.0	16
30.06	5	54.4973N	7.00362E	1.6	14.7	0.30	1011	15.3	16
01.07	6	55.5004N	3.17006E	2.0	13.5	0.13	199	34.1	36
02.07	7	55.3004N	1.11860W	1.5	12.5	0.37	893	12.3	22
02.07	7A	55.2997N	0.31950W	22.0	10.0	0.39	893	11.8	22
03.07	8	54.4103N	0.3297E	17.6	12.8	0.12	-	37.4	10
04.07	9	54.9231N	1.49769E	1.8	14.4	0.13	1199	34.1	33
05.07	10	54.1701N	1.28478E	5.3	13.0	0.16	1131	28.2	16
05.07	10A	54.2005N	0.4610E	3.0	11.9	0.15	1131	31.3	10
05.07	10B	54.2005N	0.4610E	20.0	11.1	0.15	1131	31.3	10

T^a= temperature (^oC)

I_o= daily mean surface incident irradiance (PAR) ($\mu Em^{-2}s^{-1}$)

k= light extinction coefficient (m^{-1})

Z_s= depth at which sample was collected for productivity measurements (m)

Z_{eu}= euphotic zone (m)

Z_m= approximate depth of mixing (m)

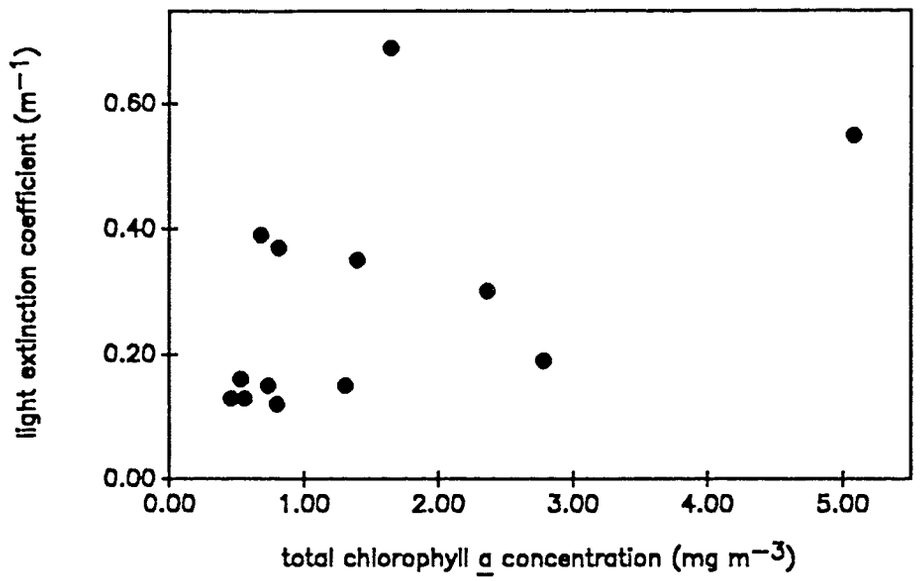

Figure 1.4.3.1. Relationship between the light extinction coefficient and the total chlorophyll *a* concentration in samples from North Sea waters collected during June/July 1989.

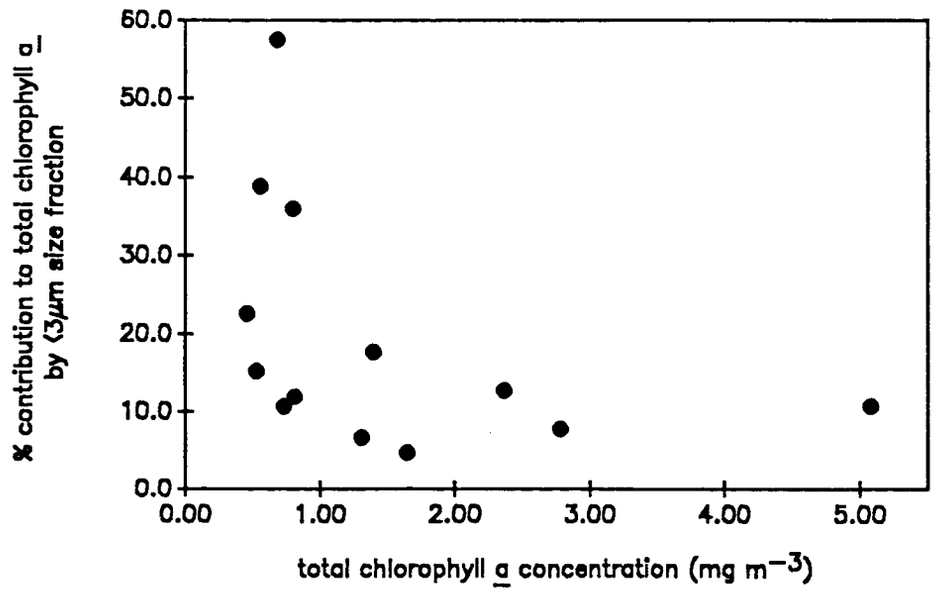

Figure 1.4.3.2. Relationship between the percentage contribution to total chlorophyll *a* concentration by the <3μm size fraction and the total chlorophyll *a* concentration in samples collected in North Sea waters during June/July 1989.

1.4.3.2. Biomass indicators

Chlorophyll *a* concentration was measured in duplicate samples for the total plankton community and the fraction <3μm. The standard difference of the duplicate values expressed as a percentage of the mean, averaged for all measurements was 8.0%(SD 7.1). Chlorophyll *a* concentrations for the total plankton community (unfractionated) varied between 0.46 and 5.08mg m^{-3} and were higher in lower latitudes (Table 1.4.3.2). In the fraction smaller than 3μm chlorophyll *a* ranged from 0.08 to 0.54mg m^{-3}, yielding values between 6.6 and 57.5% (mean of 19.7%) contribution to the total chlorophyll *a* standing stock. The percentage contribution of the fraction passing 3μm filters decreased exponentially with increasing chlorophyll *a* concentration (Figure 1.4.3.2). As for the unfractionated, the smaller than 3μm fraction also showed a marked decrease in chlorophyll *a* biomass at higher latitudes (Central North Sea) in relation to the Southern Bight.

The percentage phaeopigment was similar for both fractions. It varied from 6 to 34.8% (mean 22%) for the unfractionated samples and from 0 to 43.8% (mean 23.4%) for the <3μm fraction (Table 1.4.3.2).

Phycoerythrin-containing picocyanobacteria cell abundance ranged from $6x10^5$ to $4.42x10^7$ cells l^{-1} and were typically between 0.5 and $3x10^7$ cells ml^{-1} (Table 1.4.3.3). Lowest numbers were recorded near the British coast in central North Sea waters. The depth distribution showed no evidence to suggest that phycoerythrin-containing picocyanobacteria were more abundant deeper in the euphotic zone, not even in areas of stratified water column. Only at one station was the number of picocyanobacteria substantially higher at 20m, in comparison to the near surface concentration.

Table 1.4.3.2. Chlorophyll *a* concentration (mg m^{-3}) and percentage phaeopigment in the total plankton community and in the <3μm size fraction. Chlorophyll *a* concentration in the <3μm fraction is also expressed as a percentage of the total chlorophyll *a* concentration.

Exp no	chlorophyll *a*			%phaeopigment	
	TOT	<3μm	<3μm (% TOT)	TOT	<3μm
1	5.08	0.54	10.6	30.3	33.5
2	1.65	0.12	7.6	30.3	35.4
3	1.40	0.24	17.6	26.0	31.8
4	2.78	0.21	7.6	18.1	23.5
5	2.36	0.30	12.7	23.0	24.6
6	0.56	0.22	38.9	24.4	0.0
7	0.81	0.09	11.8	27.7	19.7
7A	0.68	0.39	57.5	34.7	37.2
8	0.80	0.29	36.0	34.8	43.8
9	0.46	0.10	22.6	25.6	22.9
10	0.53	0.08	15.1	10.9	24.3
10A	0.73	0.08	10.6	15.6	8.5
10B	1.31	0.09	6.5	12.1	4.9

Table 1.4.3.3. Depth distribution of phycoerythrin-containing picocyanobacteria (cells $l^{-1} \times 10^{-7}$) at various locations in the North Sea during June/July 1989.

Depth	Experiment no							
(m)	4	5	6	7	8	9	10A	10B
1	nd	2.56	1.79	0.57	nd	nd	nd	0.94
3	nd	1.36	2.37	0.62	0.96	3.78	0.36	0.30
7	3.30	nd	nd	0.39	nd	2.18	0.56	nd
15	1.28	3.32	2.04	nd	nd	2.03	0.29	nd
20	nd	0.79	0.29	0.20	6.18	2.49	0.29	0.06
30	0.64	0.28	2.43	0.15	nd	2.55	0.08	0.12
60	nd	nd	nd	nd	nd	nd	0.05	0.51
72	nd	nd	nd	nd	4.42	nd	nd	nd

nd =not determined

1.4.3.3. Primary production rates

Results of carbon uptake rates at various light levels for the larger and the smaller than 3μm fractions and the total plankton community (sum of both fractions) are presented in Table A.18 in Appendix. The percentage contribution of the fraction passing 3μm filters to the overall community carbon fixation rates is shown in Table 1.4.3.4. Values varied between 1.9 and 32.2% (mean 9.8%(SD 7.4). The contribution showed more variability in between experiments than in between light levels. There was no clear indication that the contribution of the smaller fraction was more significant at lower light intensities.

A comparison between the percentage contribution by the fraction passing through 3μm filters to the plankton community chlorophyll *a* concentration and to the carbon fixation rate, shows that in experiment numbers 3 to 10 the contribution to the carbon fixation rate was substantially lower than to chlorophyll *a* standing stock. These were all experiments in which the 3μm filter used for carbon uptake fractionation was a cellulose nitrate filter (Whatmann). In experiment 1 the contribution was roughly similar to both parameters and in experiment 2 the contribution to carbon fixation rate was considerably higher than to chlorophyll *a* concentration. In these last two cases the 3μm filters for fractionation were nucleation track polycarbonate filters (Nuclepore). For size fractionated chlorophyll *a* measurements Nuclepore filters were used throughout. This observation introduces an uncertainty in terms of the validity of normalizing the carbon fixation rates in the fraction smaller than 3μm to chlorophyll *a* concentration when the filter type for the two fractionations was different.

Results of mixed layer integrated daily carbon uptake rates are presented in Table 1.4.3.5. Values have been expressed in mmolC $m^{-2}d^{-1}$ for an easier comparison with the oxygen production data. Values ranged from 8.019 to 24.415 mmolC $m^{-2}d^{-1}$ in the larger than 3μm fraction and from 0.878 to 3.226 mmolC $m^{-2}d^{-1}$ in the 0.2-3μm. The contribution of the small fraction to the total plankton community water column integrated carbon uptake rate varied from 5.1 to 26.5% (mean 9.9%(SD 7.1)).

Primary production was also measured on deck as changes in dissolved oxygen concentration for the total plankton community (unfractionated). These measurements were based on five replicate samples incubated at each light level, five fixed at zero time and five incubated in the dark. The coefficient of variation of gross primary production rates derived from replicate samples averaged for all measurements was 6.3%(SD 6.3); for net community production rates it was 20.7%(SD 49.6); and for respiration rates it was 5.8%(SD 1.9). Water column integrated gross, net production and respiration rates are given in Table 1.4.3.6. Rates of gross oxygen production rate ranged between 28.32 and 88.21mmolO_2 $m^{-2}d^{-1}$. Estimates of gross production rates with the oxygen technique were generally 2 to 3 times (average 2.5 times) those derived from carbon uptake measurements as indicated from the photosynthetic quotient (Table 1.4.3.7; Figure, 1.4.3.3). Regression analysis indicated values of gross oxygen production rate to be reasonably well correlated with the carbon fixation rate ($r=0.902$, $p<0.001$).

1.4.3.4.Respiration rates

Respiration rates were high, i.e. 0.09 to 0.37 (mean 0.18(SD 0.09) μmolO_2 $l^{-1}h^{-1}$, as compared to other seasons of the year in the North Sea (Purdie *et al.* in prep.). Water column integrated daily rates of respiration were between 43.20 and 184.80mmolO_2 $m^{-2}d^{-1}$ in the surface mixed layer, and in all cases except in sampling site 10 were higher than integrated gross production rates, i.e. net production rates were negative (Table 1.4.3.6).

Size fractionated respiration rate results are presented in Table 1.4.3.8. Respiration rates in the smaller than 3μm fraction were 70 to 100% (mean 80%) of the rates measured in the unfractionated sample. Chlorophyll *a* normalized rates of respiration were about one order of magnitude larger for the <3μm fraction than for the total plankton community, suggesting a greater input of heterotrophic organisms in comparison to autotrophic organisms to the respiration rate in the small fraction than in the total plankton community. The ratio of respiration to light saturated photosynthesis was higher than 70% for the <3μm fraction.

Table 1.4.3.4. Percentage contribution of the fraction smaller than 3μm to the total community primary production at the various percentage surface incident irradiance levels, as measured in North Sea waters during June/July 1989.

Exp no.	% I_o					
	100	58	28	19	10	5.2
1	20.5	7.6	9.9	12.3	12.4	13.4
2	17.7	27.2	23.8	29.1	32.2	25.2
3	11.8	-	8.0	8.7	12.0	17.9
4	4.5	2.7	3.3	8.3	5.3	5.1
5	2.7	3.0	4.1	3.0	2.4	1.9
6	5.7	5.7	4.7	6.8	5.0	6.6
9	7.0	8.3	8.5	7.3	6.7	9.8
10	7.0	7.8	5.3	6.9	7.0	10.8

-: sample lost

Table 1.4.3.5. Surface mixed layer integrated daily rates of primary production (mmolC $m^{-2}d^{-1}$) for the fractions >3μm and <3μm, and the percentage contribution of the <3μm fraction to overall plankton community carbon fixation rate (sum of fractions), as measured in North Sea waters during June/July 1989.

Frac.	Exp. no							
	1	2	3	4	5	6	9	10
>3μm	16.849	8.925	8.019	22.795	14.604	17.793	24.415	15.305
3-0.2μm	2.257	3.226	0.878	1.238	0.846	1.021	2.062	1.202
TOTAL	19.106	12.151	8.897	24.033	15.450	18.814	26.477	16.507
<3μm(%)	11.8	26.5	9.8	5.1	5.5	5.4	7.8	7.3

Table 1.4.3.6. Mixed layer integrated daily rates of gross primary production (GP), net community production (NP) and respiration (R) for the total plankton community (mmolO2 $m^{-2}d^{-1}$) as measured from on deck incubations with the oxygen technique in North Sea waters during June/July 1989.

Exp.no	GP	NP	R
1	58.54	-37.45	96.00
2	32.24	-152.55	184.80
3	28.32	-102.72	131.04
4	55.83	-86.24	142.08
5	88.21	-30.83	57.37
6	43.51	-42.89	86.40
7	37.17	-114.03	151.20
9	47.40	-31.50	79.20
10	48.81	5.61	43.20

Table 1.4.3.8. Rate of respiration of the total plankton community and the fraction <3µm, both in µmolO2 $l^{-1}h^{-1}$ (l) and in µmolO2 µgchl $a^{-1}h^{-1}$ (chl), the percentage contribution of the <3µm fraction to the overall rate of respiration (%) and the percentage ratio of maximum photosynthetic rate to respiration rate (R/P_{max}) for the <3µm fraction and the total plankton community determined in North Sea waters during June/July 1989.

Exp	Respiration rate					R/P_{max} (%)	
no	TOT		<3µm frac			TOT	<3µm
	chl	l	chl	l	%		
2	0.09	0.16	1.03	0.13	83	15.4	89.3
3	0.18	0.25	0.70	0.17	70	-	71.4
4	0.11	0.32	1.20	0.25	80	-	-
5	0.07	0.16	0.55	0.16	100	20.4	83.0

-: no value of Pmax

Table 1.4.3.7. Photosynthetic quotients (moles of O_2 evolved/moles of CO_2 fixed) determined from on deck incubations for the total plankton community at various percentage surface incident irradiance levels during June/July 1989 in North Sea waters.

Exp no	100%	54%	31%	15%	11%	5%
1	6.75	2.69	2.51	2.98	3.03	2.35
2	2.19	2.54	2.82	2.88	2.77	2.50
3	2.36	-	2.35	2.40	3.44	6.51
4	2.13	2.36	2.21	1.94	2.10	3.27
5	1.99	1.82	1.56	1.69	1.74	2.30
6	2.07	2.00	2.16	2.52	3.20	1.70
9	2.25	1.57	1.71	1.83	1.73	2.09
10	3.15	2.59	2.42	2.65	3.08	4.13

Range: 1.5-6.75 $\bar{x}$= 2.57(SD 1.02)
Regression line y= 0.074 + 1.879x (r^2= 0.813, n=47)
where y= gross oxygen production ($\mu molO_2\ l^{-1}h^{-1}$)
x= carbon uptake rate ($\mu molC\ l^{-1}h^{-1}$)

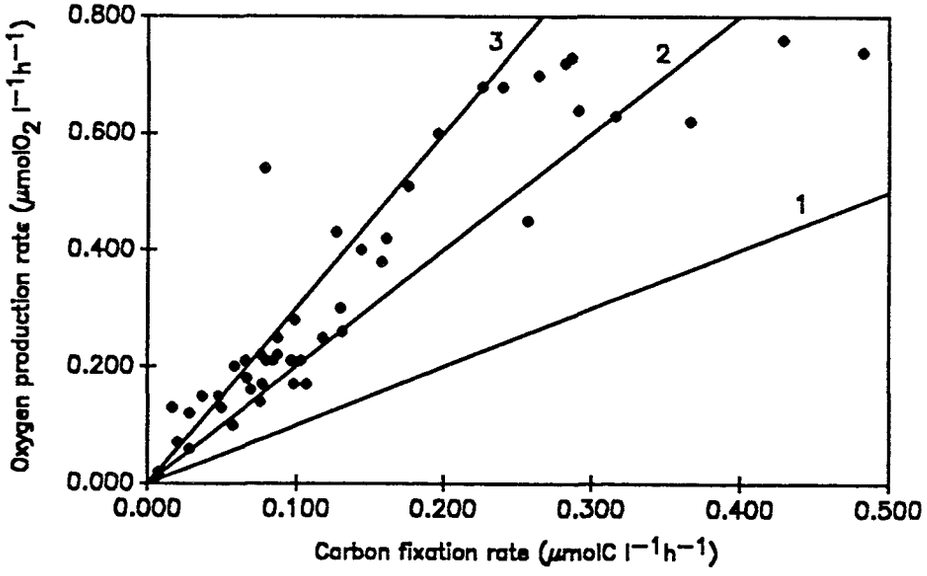

Figure 1.4.3.3. Relationship between the rate of gross oxygen production and the rate of carbon fixation in the total plankton community from parallel on deck incubation measurements during June/July 1989 in the North Sea. Solid lines represent the lines of PQ=1, PQ=2 and PQ=3.

1.4.3.5. Excretion rates

Rates of extracellular release of photoassimilated carbon as measured with the ^{14}C technique were relatively low. Results of percentage extracellular relase at each light level are presented in Table 1.4.3.9. The proportion of carbon fixed excreted as dissolved organic carbon ranged from 0.5 to 32% (mean 9.5%).

1.4..4. Studies in the North Sea: March 1989

During the cruise in March samples were collected from the southern Bight between latitudes 52.11N and 52.60N and longitudes 3.30W and 4.24W. Some hydrographic characteristics of sampling locations are summarized in Table 1.4.3.10. In this cruise few samples were collected mainly owing to bad weather conditions. Phycoerythrin-containing picocyanobacteria cell numbers varied from 0.16 to 1.68×10^{7} cells l^{-1} (Table 1.4.3.11) and were in the range of values estimated during the June/July cruise in areas of the central North Sea.

Size fractionated chlorophyll *a* concentration was measured in two occasions. The contribution by the smaller than 3μm fraction to total plankton community chlorophyll *a* concentration was in the range of percentage contributions determined during June/July (13.7-25.6%). Similarly to results obtained during June/July, the proportion of chlorophyll *a* and phaeopigment was similar for both size fractions.

Only two size fractionated ^{14}C on deck experiments were carried out and, given the variability found in the June/July samples, it is difficult to make comparisons between seasons. In one of these experiments the fraction smaller than 3μm contributed a mean (between light levels) of 60% and in the other 24% (Table 1.4.3.12). Nuclepore filters were used for both 0.2μm and 3μm filters.

Table 1.4.3.9. Percentage extracellular release of organic carbon at various surface incident light levels (%I_o) as measured in North Sea waters during June/July 1989.

Exp no	% I_o					
	100	58	28	19	10	5.2
1	11.3	12.6	ud	ud	ud	ud
2	0.5	6.5	3.2	1.8	21.1	18.3
3	ud	ud	ud	ud	ud	ud
4	-	8.9	2.8	0.6	3.1	14.0
5	16.4	1.9	1.3	ud	0.7	ud
6	4.8	14.8	13.6	7.7	19.1	32.0
9	3.8	ud	3.4	12.3	ud	ud
10	14.6	4.3	9.9	7.9	3.5	11.8
$\bar{x}$	8.6	8.1	5.7	6.1	9.5	19.0
s.d.	6.4	4.9	4.9	4.8	9.8	9.0

ud= undetected
-: lost sample

Table 1.4.3.12. Percentage contribution by the <3μm size fraction to total plankton community primary production at various percentage surface incident light levels (%I_o) as measured with the ^{14}C technique from on deck incubations in North Sea waters in March.

Exp no	% I_o						
	100	58	28	19	11	5.2	$\bar{x}$±SD
2	17	62	82	84	77	32	59±28
4	19	28	21	21	40	13	24±9

Table 1.4.3.10. Physical characteristics of the sampling locations in North Sea waters during March 1989.

Date	Exp	Lat(N)	Long(W)	Z_s	T^a	k	I_o	Z_{eu}
16.03	11B	52.5667	3.66717	5.8	7.5	-	-	-
18.03	12	52.3328	3.37255	6.6	7.6	0.54	622	8.5
20.03	13	52.4532	3.47103	6.1	7.6	-	-	-
21.03	14	52.2749	4.24555	5.8	7.6	0.79	639	5.8

T^a= temperature (°C)

Z_s= depth of sampling (m)

k= light extinction coefficient (m^{-1})

Z_{eu}= euphotic zone (m)

-= not available

Table 1.4.3.11. Cell concentration (cells $l^{-1}x10^{-7}$) of phycoerythrin -containing picocyanobacteria (PEP), chlorophyll *a* concentration (mg m^{-3}) and percentage phaeopigment in the total plankton community and the <3μm size fraction and the percentage contribution of the <3μm size fraction to the overall plankton community chlorophyll *a* concentration in samples from North Sea waters during March.

Exp no	PEP	chlorophyll *a*			% phaeopigment	
		TOT	<3μm	<3μm %	TOT	<3μm
11	1.68	1.22	0.17	13.7	31.3	41.6
12	0.65	nd	nd	nd	nd	nd
13	0.16	0.57	0.14	25.6	48.4	49.1
14	0.17	nd	nd	nd	nd	nd

nd= not determined

1.5. DISCUSSION

1.5.1. Studies in Southampton Water

1.5.1.1. Temporal variation in plankton community chlorophyll a and primary production

A prominent feature of the seasonal cycle of phytoplankton in Southampton Water is the recurrent occurrence of red water events during the summer (Williams, 1980; Rees and Williams, 1982; Soulsby *et al.*, 1985; Kifle, 1989; Crawford, 1991). These are caused by blooms of the phototrophic ciliate *Mesodinium rubrum*. The chlorophyll concentrations attained during these events (up to 100mg m^{-3}) have caused Southampton Water to be described as an essentially summer blooming estuary (Williams, 1980). More recent detailed annual phytoplankton surveys have shown, however, the development of a series of transient blooms (mainly diatoms) occurring in spring, which, although generally overshadowed by the magnitude of the summer maximum, nevertheless can reach levels of around 25mg m^{-3} (Kifle, 1989; Antai, 1990). At the mouth of the estuary (i.e. vicinity of Calshot Spit buoy), however, chlorophyll *a* maxima have been shown to occur in spring (Kifle, 1989; Antai, 1990), a pattern closer to typical coastal offshore conditions in temperate regions (Legendre, 1990).

The seasonal distribution of chlorophyll *a* observed in the present study undertaken during 1990 also showed the spring and summer peaks at the inner estuarine station (NW Netley). A first outburst of phytoplankton biomass accumulation was observed during spring (May). This was identified as a bloom of the colonial prymnesiophyte algae *Phaeocystis* spp.. *Phaeocystis* spp. is an important algae in Antarctic and Arctic ecosystems, as well as in temperate coastal waters. Blooms of this algae are a common feature during spring or early summer in European coastal waters: in the English Channel (Holligan and Harbour, 1977; Boalch, 1987), the Irish Sea (Jones and Haq, 1963) and the southeastern North Sea (Lancelot *et al.*, 1987). *Phaeocystis* spp. have also been reported to bloom in some years in adjacent estuarine areas, e.g. Bristol Channel (Joint and Pomroy, 1981), the Ems estuary (Colijn, 1983), but 1990 was the first year in which a bloom of

Phaeocystis spp. has been reported in Southampton Water. The spring maximum was followed by a brief period of low chlorophyll *a* biomass. Midsummer was characterized by a build up of chlorophyll *a* that reached its maximum during the red water event. The present study, however, did not show the pre-eminent magnitude of the summer peak as reported in previous studies, 50-100mg m^{-3} (Bryan, 1979; Soulsby *et al.*, 1985; Kifle, 1989; Antai, 1990) since a maximum of only 18mgchl *a* m^{-3} was measured. Peak chlorophyll *a* values, though, compare well with concentrations reported by Rees and Williams (1982) during a survey in 1981.

In accordance with previous works (Bryan, 1979; Kifle, 1989; Antai, 1990), the outer area of the estuary (Calshot Spit) was characterized by a spring maximum and during the summer chlorophyll *a* levels were less significant than in the inner estuary in the present study.

Mesodinium rubrum is characterized by an extremely patchy spatial distribution in the estuary. As noted in this work it generally occurs as dense aggregates with a tendency to accumulate at well defined depths (Rees and Williams, 1982; Kifle, 1989; Crawford 1991), as a result of rheotactic and phototactic responses (Smith and Barber, 1979; Lindholm, 1985) and possibly also in response to water turbulence (Crawford and Purdie in press). This highly uneven spatial distribution poses serious difficulties for representative sampling and may partly be responsible for the interannual variability as determined from relatively low spatio-temporal resolution sampling programmes. Also, year to year variations in climatological conditions can have a major influence on the timing, duration and magnitude of algal blooms.

Algal blooms have been defined as rapid increases in biomass, caused by locally enhanced primary production that results in abnormally high cell concentrations (Legendre, 1990). This definition excludes passive accumulation of cells as a result of mere physical aggregation, that has been included in other definitions (Holligan, 1987). Blooms can thus be considered to be the consequence of transient imbalances between the rate of primary production and the rate of loss of photosynthetically fixed carbon (i.e. respiration, grazing and advection) (Legendre, 1990). In the marine environment the

major limiting factors to primary production are considered to be light and nutrients (Raymont, 1980; Parsons *et al.*, 1984b); thus enhanced rates of production require a covariance of light and nutrients (Margalef, 1985). If the imbalance between production and loss processes that is necessary for a bloom to occur, is a result of a rapid increase in primary production rate, a rapid increase in covariance between water column light availability and nutrient concentration must occur (Legendre, 1990) and this is generally attributed to rapid changes in one of the two variables. But, although high rates of primary production are a necessary condition for a bloom to occur, blooms are not necessarily only triggered by changes in light and/or nutrient availability, since the imbalance can be the result of a rapid decline in the rate of loss of phytoplankton biomass (e.g. sudden reduction of grazing pressure, a large increase in the residence time of the water in the estuary).

In temperate and subpolar regions the spring phytoplankton outburst is generally explained in terms of increased rate of primary production following a marked increase in the mean light level in the surface mixed layer (i.e. rise in surface incident irradiance levels and in many instances a decrease in the depth of the surface mixed layer resulting from a thermal stratification (e.g. Celtic Sea, Pingree *et al.*, 1976).

For a pronounced increase in phytoplankton biomass the mean irradiance in the surface mixed layer must reach a minimum critical value estimated to be around 40gcal $m^{-2}d^{-1}$ (Riley, 1967). As a consequence of the increased concentration of suspended matter, resulting from increased river runoff and stronger wind driven resuspension of bottom sediments, extinction coefficients were high in Southampton Water during the winter, and Riley's "critical irradiance" value was not attained until April in the outer estuary, i.e. at Calshot Spit. However, the mean surface mixed layer irradiance value was already double this value at NW Netley by March, mainly because the surface mixed layer was shallower in the inner estuary, as a consequence of haline stratification. The vertical salinity profiles suggest that the freshwater input was considerable until April-May, which is also indicative of accordingly high flushing rates. It seems likely that in the inner estuary the residence time of the water was

too short to allow a substantial accumulation of phytoplankton until later in the year, whereas irradiance was non-limiting. The observation that during the April maxima of chlorophyll *a* (15.7μg l^{-1}) at Calshot Spit, the level at NW Netley only reached 4.9μg l^{-1}, suggests that this algal population (mainly *Phaeocystis* spp., D.Kifle, personal communication) had bloomed offshore and was brought into the estuary with the tidal incursion. Light and temperature were probably adequate by April to trigger a bloom offshore, but the low residence time of the water in the estuary hindered the growth of an autochthonous population of *Phaeocystis* spp. until later in the season (first week of May).

A previous study by Bryan (1979) suggested light is non-limiting to phytoplankton production throughout the year in Southampton Water. The development of the bloom in Southampton Water has been claimed to be predominantly determined by the relationship between growth rate and flushing time (Rees and Williams, 1982; Souza Lima and Williams, 1978) and temperature has been suggested as influential (Bryan, 1979). However, this interpretation should be treated with caution, since the phytoplankton growing period was identified as a single summer bloom and environmental parameters controlling the onset of the spring and summer blooms seem to be different.

An imbalance in the timing of the spring phytoplankton bloom between the estuary and adjacent offshore waters is a common feature. Sinclair *et al.* (1981) argued that the stratification induced by the vertical salinity gradient during periods of high river runoff caused the onset of the spring bloom to occur earlier in an estuary than offshore. This seems to be the case in many estuaries, e.g. Narragansett Bay (Hitchcock and Smayda, 1977), Bedford Basin (Platt and Irwin, 1971), How Sound (Stockner *et al.*, 1977), Loch Creran (Tett and Wallis, 1978). However, other estuaries are late bloomers. In these waters, blooms typically appear to be retarded until the freshwater input decreases considerably. Increased levels of turbidity and subsequent light limitation and/or short residence time of the water in the surface layer within the estuary, both associated with high freshwater input, seem to be the reasons behind the delays. Southampton Water is not a particularly turbid estuary (minimum depth

of the euphotic zone (1% surface light level) reached in winter was 2.3m), however, the flushing rate may be high. Some of the late blooming estuaries are fjords and Sinclair *et al.* (1981) argued that the narrowness of these estuaries prevents the establishment of complex lateral circulation patterns that would increase the residence time of the mixed layer. Other late blooming estuaries are not fjords, e.g. lower Saint-Lawrence (Sinclair, 1978), Forleague Bay (Randall and Day, 1987). The seasonal cycle of freshwater input appears thus as a major controlling factor in the timing of the winter-spring transition phytoplankton blooms in estuaries (Sinclair *et al.*, 1981) and this seems to be the case for the spring bloom in Southampton Water. The occurrrence of early or late blooms with respect to offshore waters will thus depend largely on interannual climatological changes.

At the time of the spring maxima of chlorophyll *a*, nutrient levels decreased considerably at both stations, particularly at NW Netley (*ca.* 1.8μM of nitrate+nitrite and 0.13μM of phosphate). The precise determination of the concentration at which nutrients become limiting for phytoplankton growth requires detailed measurements of growth rates in nutrient enrichment experiments (D'Elia *et al.*, 1986; Granéli, 1987) and/or measurements of algal physiological parameters indicative of nutrient limitation, e.g. alkaline phosphatase activity in the case of phosphate limitation (Chiaudani and Vighi, 1982). A simple more limited approach has been employed by Fisher *et al.* (1988). These authors argued that levels of nutrients below the half saturation constant for nutrient uptake reduce considerably the uptake rates, whereas levels above it would only increase the uptake rates 1-2 times. On reviewing data in the literature for half saturation constants for nutrient uptake rate, they found values between 1 and 2μM for nitrate+nitrite+ammonia, between 0.1 and 0.5μM for phosphate and between 1 and 5μM for silicate. They then considered the upper limit of the range as the critical value below which the specific nutrient would become limiting for phytoplankton growth, i.e. 2μM for dissolved inorganic nitrogen, 0.5μM for phosphate and 5μM for silicate.

Ammonia concentration was not measured in the present study, however, the concentration of nitrate+nitrite was above 2μM during the entire survey, except at the time of the May chlorophyll maximum at NW

Netley (1.8μM). It is likely that if the concentration of ammonia was included, this would be above the critical value. Levels of phosphate, however, decreased to concentrations below 0.5μM during the May chlorophyll *a* maximum at NW Netley and it is interesting to hypothesize that phosphate limitation might have at least partly been responsible for the bloom termination. However, since the bloom was not followed throughout its development, it is not easy to establish if this was the case. The possibility of nutrient limitation causing the collapse of the spring bloom in Southampton Water has previously been pointed out (Kifle, 1989). However, the spring bloom of 1988 was dominated by diatoms (*Skeletonema costatum* and *Rhizosolenia delicatula)* and silicate limitation was suggested as a primary factor determining the spring bloom termination (Kifle, 1989). The silicate concentration, however, would not explain the collapse of the *Phaeocystis* spp. population during the spring bloom of 1990 (the present study), though it must have been of importance to the diatom populations of *Chaetoceros* spp. also present abundantly at this time (D.Kifle, personal communication). It is not uncommon to find significant numbers of diatoms of the genus *Chaetoceros* during blooms of *Phaeocystis* spp. It has been suggested that the setae of these diatoms act as a surface to which the motile forms of the prymnesiophyte algae attach and become palmelloid. These non-motile forms then pass into a colonial stage in which they can accumulate in vast numbers giving rise to massive blooms (Boalch, 1987). Boalch (1987) has further suggested that *Chaetoceros* spp. might not provide solely a physical support, but may produce a chemical that triggers the change from the motile to the non-motile form of the cells of the prymnesiophyte. The decline of blooms of *Phaeocystis* spp. in coastal waters of the southern bight of the North Sea has frequently been related to phosphate limitation (Weisse *et al.*, 1986; Veldhuis *et al.*, 1986), although in some cases nitrate has been identified as the limiting nutrient (Lancelot and Billen, 1984).

Another possible explanation for the decline of the spring bloom is herbivorous grazing. The possible impact of grazing by herbivorous zooplankton in Southampton Water is not well documented, mainly as a result of the spatio-temporal asynchrony between the sampling programmes of zooplankton orientated and phytoplankton orientated

surveys carried out in recent years. The dominant herbivorous zooplankton are calanoid copepods and cirripede larvae (Raymont and Carrie, 1964; Zinger, 1989; Lucas, 1991). A typical seasonal cycle of herbivorous zooplankton biomass in Southampton Water shows low winter values, increasing thereafter to peak in spring. This spring peak is followed by a dramatic reduction in late spring and the concentration of herbivorous zooplankton remains low during the summer, finally showing some recovery during the autumn (Zinger, 1989; Lucas, 1991). The late spring decline is likely to be primarily associated with the appearance of gelatinous predators such as the coelenterate *Aurelia aurita* and the ctenophore *Pleurobrachia pileus* (Williams *et al.*, in prep. cited in Lucas, 1991). The spring peak in copepod biomass was also recorded in 1990, during the *Phaeocystis* spp. outburst (Lucas, 1991), however, from the available data, it is not clear whether the copepods were grazing on the prymnesiophyte algae. There is some controversy so as to the effect of *Phaeocystis* spp. on other planktonic organisms and of its edibility by herbivorous zooplankton. Savage (1930) reported that herring populations avoided *Phaeocystis* spp. blooms in their migration. Jebram (1980) observed toxic effects of the prymnesiophyte on some bryozoans and Martens (1981) noted a breakdown of the copepod population during a *Phaeocystis* spp. bloom development. Weisse (1983), however, found a positive correlation between the *Phaeocystis* spp. concentration and the copepod concentration, suggesting the microalgae as an important food source and Jones and Haq (1963) showed copepods can feed on *Phaeocystis* spp.. Similarly, Admiraal and Venekamp (1986) reported growth of tintinids on the prymnesiophyte algae. Estep *et al.* (1990) have suggested that predation upon *P. pouchetii* colonies depends on the physiological state of the algae, healthy colonies not being consumed and susceptible colonies being grazed at rates higher than diatom populations. On the other hand, scenescent blooms of *Phaeocystis* spp. have been observed largely to sediment to the bottom (see Peinert *et al.*, 1989).

After being largely reduced during the spring bloom, some recovery in nitrate+nitrite and phosphate concentrations was observed to occur by June/July at the inner station. The possible sources of inorganic nutrients in Southampton Water during summer months are,

a)external sources: river flow and sewage and industrial effluents, and b)internal sources: remineralization of organic matter in the water column and/or in the sediment, and dissolution of diatom silicate frustules. Collins (1978) estimated a nutrient budget for Southampton Water from a survey carried out in 1974. He concluded that in summer the major sources of inorganic nitrogen to the estuary were sewage and industrial effluents, and this occurred largely in the form of ammonia. The heaviest load was estimated to be from industrial discharges, however, most of these are released in the lower half of the estuary, whereas most of the sewage outfalls are located nearer to the head of the estuary (see Savari *et al.*, 1991), and Collins argued that sewage has a greater impact on the steady levels of ammonia in the estuary. Respiratory processes in the water column were also estimated to supply a substantial proportion of ammonia at this time of the year. Sewage effluents were identified as the main source of phosphate.

In many estuaries the sediment has been identified as an efficient remineralization site of organic matter, supplying a large proportion of the nutrients used by primary producers in the water column during the summer months (Boynton *et al.*, 1980; Callender and Hammond, 1982). Estuarine features such as the shallow depths (Nixon, 1981) and the relatively frequent sequence of destabilization-restabilization of the water column (i.e. semidiurnal and fortnightly tidal mixing variations) are considered can ease a rapid return of the regenerated nutrients to the water column. In Southampton Water, however, the flux of inorganic nutrients from the sediments was suggested to be minor (Collins, 1978). If these findings by Collins are applicable to 1990, it can be hypothesized that little of the organic matter derived from the spring phytoplankton bloom sediments to the bottom within the estuary; hence, it is largely either consumed by herbivorous zooplankton and/or microheterotrophs or exported to offshore waters by tidal flushing, where it possibly sediments to the bottom. It is now believed that in coastal areas sedimentation maybe of the order of half of the primary production (Conover and Mayzaud, 1984; Skjoldal and Wassmann, 1986; Laws *et al.*, 1988).

Increased nutrient levels in summer were followed by an increase in the phytoplankton biomass, which reached a maximum during

the red water event. *Mesodinium rubrum* appears to be present in the plankton of Southampton Water all year around (Kifle, 1989), but outbursts of growth are typically restricted to summer months (Kifle, 1989; Crawford, 1991). Stability of the water column seems to be a critical parameter triggering the red water bloom (Crawford, 1991). As in the present study, the bloom period has been observed to coincide with calm, sunny weather conditions (Crawford, 1991) and this may be a generalized pattern for this phototrophic ciliate (Lindholm, 1985). When examining factors controlling blooms of *M. rubrum*, emphasis has frequently been placed on light availability (Smith and Barber, 1979). Crawford (1991), however, has hypothesized that energy costs involved in swimming to keep itself in a well lit depth in a turbulent environment may result in a considerable reduction of growth rate for the ciliate. A stable water column would thus be a prerequisite for *M. rubrum* to bloom. In this context, the variation in water column stability associated with neap/spring tidal variations are anticipated may be important in determining the timing of the *M. rubrum* bloom and possibly other increases in phytoplankton biomass too, as has been suggested in other estuaries (Sinclair *et al.*, 1981; Litaker *et al.*, 1987; Cloern, 1991). However, the frequency of sampling was too low to observe a pattern in relation to spring/neap tidal variations.

During the summer, chlorophyll *a* concentrations in the outer estuary were much lower than in the inner estuary. At the mouth of the estuary, although levels of nitrate increased in summer, phosphate concentration fell below the critical value of 0.5μM (*sensu* Fisher *et al.*, 1988) after the spring chlorophyll *a* maximum in April, and did not increase to concentrations above it until late August, reflecting the stronger dilution effect by the offshore, nutrient poor seawater coming in with the tidal incursion (Collins, 1978). Thus, if the assumptions made following Fisher *et al.*, (1988) are valid, phytoplankton were probably phosphate limited at Calshot Spit. The lower phytoplankton biomass concentration, however, could be the result not only of reduced rates of primmary production due to nutrient limitation, but also of restricted accumulation of algal biomass due to tidal flushing. The bloom of *Mesodinium rubrum* has commonly been observed to be restricted to inner areas of the estuary and the ciliate always appears in low concentration in the vicinity of

Calshot Spit buoy. There is evidence to suggest that *M. rubrum* avoids being flushed to the outer estuary by positioning itself below the seaward flow during the ebb period of the tidal cycle, thus maintaining itself in waters of higher nutrient concentration and water column stability (Crawford and Purdie, in press).

Daily rates of primary production for Southampton Water are in good agreement with values reported by Bryan (1979). In the present study low autumn-winter rates of 0.08-0.09gC $m^{-2}d^{-1}$ raised to rates as high as 2.2-3.4gC $m^{-2}d^{-1}$ during the spring and summer blooms. Bryan's estimates for primary production ranged from lower than 0.1gC $m^{-2}d^{-1}$ in winter to 2-3gC $m^{-2}d^{-1}$during the summer bloom. The range is in fair agreement with estimates for a variety of estuaries and coastal waters (see compilation by Pennock and Sharp, 1986). Estimates of annual rate of primary production suggest Southampton Water as a reasonably productive estuary (for a comparison see Table 1.5.1), though much less productive than the most productive ones e.g. Great South Bay, Puget Sound. Moderate production in estuaries is frequently associated with light limitation by turbidity e.g. Wassau Sound (Turner *et al.*, 1979), Bristol Channel (Joint and Pomroy, 1981) lower San Francisco Bay (Cole and Cloern, 1984), Delaware Estuary (Pennock and Sharp, 1986). Southampton Water can not be considered as a very turbid estuary (i.e. phytoplankton production was not light limited, except in winter), but the combination of a high flushing rate (freshwater flow and tidal flow) and a relatively moderate input of riverine nutrients may be the reasons, although the importance of this latter factor may be questionable in an environment where regenerative processes and effluent discharges can possibly supply a large proportion of the nutrients required by the phytoplankton community (except the diatoms) during the summer months. Estimates of annual primary production rate made during 1990 appear to be higher than those obtained in a previous study carried out from February 1974 to March 1975 (Bryan, 1979), particularly in the outer estuary. The major difference seems to derive from the low productivity found by the latter author during the spring months. Year to year climatological variations and the low temporal resolution of the sampling in both studies (i.e. maximum twice per month) may be responsible for these differences. To test any possible trend of progressive eutrophication

in the estuary, productivity measurements would need to be undertaken with higher resolution sampling programmes and over a number of years.

Table 1.5.1. Annual rate of primary production in selected estuarine ecosystems (gC $m^{-2}y^{-1}$).

Location		Primary production	Reference
Beaufort Channel, N.Carolina		225	Williams & Murdoch (1966)
Wassau Sound Estuary		90	Turner *et al.* (1979)
Peconic Bay, New York		162-213	Bruno *et al.* (1980)
Lower San Francisco Bay		130	Cole & Cloern (1984)
Bristol Channel	inner estuary	6.8	Joint & Pomroy (1981)
	outer estuary	165	
Lower Hudson Estuary		200	Malone (1977a)
Delaware Estuary		307	Pennock and Sharp (1986)
Great South Bay, New York		450	Lively *et al.* (1983)
Puget Sound, Washington		465*	Winter *et al.* (1975)
Southampton Water	mid estuary	*ca.* 150*	Bryan (1979)
	outer estuary	*ca.* 80*	
	mid estuary	207	Present study
	outer estuary	162	

*derived from graph for regions in the estuary similar to those from the present study.

1.5.1.2. Temporal variations in picophytoplankton abundance

The discovery of a group of minute chroococcoid cyanobacteria, previously overlooked in the marine planktonic microflora (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979), as major primary producers in tropical and subtropical regions (Li *et al.*, 1983; Platt *et al.*, 1983) has prompted the necessity to quantify the impact of the small size-class of the phytoplankton in other marine habitats.

Chroococcoid cyanobacteria seem to be ubiquitous and have been found in almost all seas where they have been searched for. In the marine environment PE-containing chroococcoid cyanobacteria are typically reported in concentrations of the order of 10^7cells l^{-1} (see Table 1.2.2 in section 1.2.1). In coastal and inshore areas seasonal peaks generally attain 10^8cells l^{-1} (see Table 1.2.2, section 1.2.1).

In the present study in Southampton Water, levels of PE-containing picocyanobacteria were within the range generally

reported in the marine environment, but peaks of only $1.5x10^7$cells l^{-1} were reached in the summer. Low summer maxima of $4x10^7$cells l^{-1} and around $2x10^7$cells l^{-1} have also been measured in Funka Bay (Japan) and Chesapeake bay sub-estuary respectively (Odate, 1989; Ray *et al.*, 1989). In a previous study in Southampton Water maximum levels of PE-containing cyanobacteria of $1.5x10^6$cells l^{-1} were reported (Leakey, 1989), but fading of fluorescence by delayed counting was realized to cause some underestimation, and although a correction factor was applied, this may not have fully accounted for the losses.

Data on eukaryotic picophytoplankton is very scant in the literature. In the marine environment, a typical concentration value is considered to be around 10^6cells l^{-1}, one order of magnitude lower than for PE-containing picocyanobacteria (Joint, 1986; see Table 1.2.2 in section 1.2.1). However, in estuarine and coastal waters sporadic blooms of exceptional magnitudes, such as the "brown tide" of *Aureococcus anophagefferens* (smaller than 2μm) that reached levels higher than 10^9cells l^{-1} during 1983 in Narragansett Bay (Hargraves *et al.*, 1989), may be a common feature in coastal and estuarine waters in temperate regions (Ryther, 1954; Malone, 1977a; Wilhelm *et al.*, 1982; Hargraves *et al.*, 1989). In Southampton Water largest numbers of eukaryotic phytoplankton in the smaller than 3(5)μm size fraction ($2.9x10^8$cells l^{-1}) occurred during the bloom of the colonial prymnesiophyte *Phaeocystis* spp.. Most of these cells are likely to have derived from the disruption of colonies, rather than representing free-living single cells. Although the size of a single cell of *Phaeocystis* spp. is in the range 3-8μm in diameter, when aggregated they can form colonies that may exceed 10mm diameter (Kayser, 1970; Gieskes and Kraay, 1975). These colonies function as biological entities, rather than aggregations of cells (Lancelot and Mathot, 1987; Verity *et al.*, 1988) and, from a physiological and trophodynamical point of view, colonial *Phaeocystis* spp. cannot be regarded as behaving as a collection of small cells. The consideration of these cells as part of the picoplankton is thus at least arguable. Except for during the bloom of *Phaeocystis* spp., peaks of red-fluorescing picophytoplankters of similar size range as the PE-containing picocyanobacteria (*ca.* 1μm) were found in late summer (August to September). In agreement with these results in Southampton

Water, picophytoplankton other than the PE-containing picocyanobacteria were found to reach an annual maximum of abundance in late summer (August) in Funka Bay (Odate, 1989). Lively *et al.* (1983) reported that in Great South Bay chlorophytes of 2-4μm were numerically dominant throughout the year and contributed to about half of the total phytoplankton standing stock.

Data suggest that the biomass of eukaryotic picophytoplankton gains importance in relation to that of the picocyanobacteria from offshore to coastal areas (Johnson and Sieburth, 1982; Furnas, 1983; Ray *et al.*, 1989). This was apparent in the present study in Southampton Water, where at times eukaryotic picophytoplankters numerically dominated the picophytoplankton, in contrast to oceanic regions where PE-containing picocyanobacteria are typically dominant (Murphy and Haugen, 1985).

In agreement with a previous survey in Southampton Water (Leakey, 1989), PE-containing cyanobacterial numbers were low in winter and spring and experienced a sharp increase, reaching a peak, in summer; concentrations declined considerably again by autumn. This seems to be a generalized pattern in temperate regions, since virtually all studies of seasonal variations in picophytoplankton in coastal and estuarine waters show maxima of abundance during summer months (Krempin and Sullivan, 1981; Hag and Fogg, 1986; Waterbury *et al.*, 1986; Shapiro and Haugen, 1988; Jochem, 1988; Leakey, 1989; Odate, 1989; Vaulot and Xiuren, 1989). Occasionally peaks of abundance of PE-containing picocyanobacteria have also been recorded during winter (Shapiro and Haugen, 1988; Vaulot and Xiuren, 1988).

In some studies the increase in picocyanobacteria appears to be very rapid (Hag and Fogg, 1986; the present study); in others the numbers of picocyanobacteria show a progressive rise, starting during the spring months (Krempin and Sullivan, 1981; Waterbury *et al.*, 1986). Waterbury *et al.* (1986) noted that the onset of the increase in *Synechococcus* numbers in Woodshole Harbor occurs consistently at temperatures of around 6°C. In agreement with the present study in Southampton Water, on a seasonal basis, maxima of abundance of picocyanobacteria have been found to occur at temperatures ≥17.5°C (Krempin and Sullivan, 1981; Hag and Fogg, 1986; Waterbury *et al.*, 1986; Jochem, 1988).

Few explanations have been put forward for the seasonal distribution pattern of picophytoplankton in temperate regions. Based, both on seasonal and latitudinal variations, many workers have been able to establish a positive relationship between abundance of picocyanobacteria and temperature (Murphy and Haugen, 1985; Caron *et al.* 1985; Hag and Fogg, 1986; Joint, 1986; Waterbury *et al.*, 1986; Jochem, 1988; Odate, 1989). It is well documented that cyanobacteria are, in general, mesophiles (Carr and Wyman, 1986). Some of them are truly thermophilic, showing optima for growth at temperatures as high as 50-60°C (Brock, 1978). The other prominent genus in the marine environment (*Trichodesmium* = *Oscillatoria*) is an organism typical of tropical regions and requires a minimum temperature of 20°C for growth (Carpenter, 1983). *Synechococcus* spp. in culture appears to grow optimally at temperatures between 20-25°C and fails to grow at temperatures in excess of 30°C (Waterbury *et al.*, 1986). The effect of low temperature on *Synechococcus* spp., however, is less well documented. In the present study in Southampton Water a positive correlation between PE-containing picocyanobacteria cell concentration and temperature was also found, however, this correlation was not very strong and suggests that, although temperature may exert an influence, it is unlikely that a direct effect of temperature on the physiology of *Synechococcus* spp. will be the only factor determining the variations in its distribution and abundance. Smith *et al.* (1985) found levels of *Synechococcus* spp. higher than 10^6cells l^{-1} in the Antarctic at temperatures below 0°C and Shapiro and Haugen (1988) reported a peak of *Synechococcus* spp. in winter in Boothbay Harbor (USA). A possible explanation for this winter peak was either the existence of a cold northern race, that could correspond with the one found in the Antarctic by Smith *et al.* (1985), or the suppression of some grazers that can be affected by the low temperature.

Hag and Fogg (1986) and Joint (1986) have suggested light availability, a parameter that correlates with temperature, as a possible controlling factor. However, picocyanobacteria have been shown to be able to grow at fast rates at low irradiance (Morris and Glover, 1981; Kana and Glibert, 1987a,b) and it is difficult to envisage them being light limited for example in spring in temperate waters, when larger phytoplankton are not.

In Southampton Water the number of microflagellates that survive the winter (Kifle, 1989) suggests picophytoplankton are likely to be controlled effectively by grazing in spring when conditions are favourable for rapid growth rates. Trophodynamic modelling studies show this can be a generalized pattern (Murray, 1991). Phytoplankters subject to reduced grazing pressure and with a substantial storage capacity i.e. large diatoms or colonial forms, are probably better suited to succeed during the spring bloom (Malone, 1980). In summer, a combination of factors, such as an increased rate of production (enhanced by increased temperature) and the increased residence time of the water in the estuary (i.e. low freshwater inflow), together with an increased bacterial concentration (Antai, 1990), possibly enable picocyanobacteria biomass accumulation rate to rise above the grazing rate by protozoans. Bacterial concentrations and growth rates have been shown to increase markedly in summer and the seasonal distribution of microflagellates has been shown to follow closely the bacterial distribution pattern in this estuary (Antai, 1990). Bacterial growth rate appeared to be roughly matched by the grazing rate up to a level of growth rate, above which grazing rate could not keep pace with the increasing growth rate, thus resulting in a substantial net population growth of bacteria (Antai, 1990). Microflagellates are recognized to be one of the most important grazers of picophytoplankton (Sherr and Sherr, 1984; Sherr *et al.*, 1991). As for bacteria, the increased temperature may enhance specific growth rates of picophytoplankters, although from the present data there was no clear indication that P_m^B increased with temperature in the small size fractions. Also, the increased bacterial concentration in summer is likely to result in a release in grazing pressure by microflagellates and ciliates upon the picophytoplankton, since the former have been shown to reach a saturation point of ingestion rate with increasing prey concentration (Fenchel, 1982; Antai, 1990). Recently, Ferrier and Rassouzaldegan (1991) have shown that increased protozoan concentration increases picoplankton specific growth rates and have suggested the nutrient recycling potential of protozoans as responsible.

1.5.1.3. Vertical distribution of PE-containing picocyanobacteria

Although a greater number of observations would have been desirable, the vertical distribution profiles of PE-containing cyanobacteria suggested a possible preferential accumulation at depth under stratified conditions, but no clear pattern was found in mixed water columns. Maxima of abundance of picoplankton have been recorded deep in the euphotic zone in stratified oceanic waters (Li *et al.*, 1983; Takahashi and Hori, 1984; Murphy and Haugen, 1985; Glover *et al.*, 1986; Furnas and Mitchell, 1988; Legendre *et al.*, 1988). This distributiion pattern may be a response to the availability of nutrients, it being more advantageous to live deeper in the water column, close to the nutricline in stratified waters. Indeed, in the open ocean the vertical distribution of picophytoplankton has been suggested to be more related to the nitracline than to the thermocline (Herbland *et al.*, 1985). The peak of abundance deep in the euphotic zone has often been associated with low light photoadaptational characteristics of picophytoplankton. However, the observation in many marine environments that picophytoplankton can be equally abundant, with fast growth rates at shallower depths suggests that these minute phytoplankters are not exclusively adapted to low light (Landry *et al.*, 1984; Iturriaga and Mitchell, 1986; Furnas and Mitchell, 1988) and thus it can be hypothesized that either there are various strains with different photoadaptational characteristics or else this minute phytoplankters are not genotypically shade adapted, but show a great plasticity to adapt to various illumination conditions.

1.5.1.4. Filtration as a technique for size discrimination: an assesment

For the discrimination of cell size in planktonic populations visual (e.g. microscopy, microautoradiography) and electronic (e.g. Coulter counter, flow cytometry) techniques can be employed. For the discrimination of size specific rates of activity (e.g. inorganic carbon uptake, respiration) and other biomass indicators (e.g. chlorophyll, particulate organic carbon (POC), ATP) the physical fractionation by differential filtration is the most widely used method.

Despite its simplicity and widespread use, a precise control on the filtration technique is difficult and this can result in inaccuracies. For a 100% effectiveness of the size separation, filters should only retain particles with diameters equal to or larger than their stated pore sizes. In practice, however, there are various factors affecting the passage and retention of particles through filters and the nominal pore size of a filter does not always have a correspondance with the lower limit of the diameter of the particles retained and the upper limit of the diameter of the particles passing through (Li, 1986).

The filter type can be influential. Nylon and wire screens are quite effective sieves (Sheldon, 1972), but the pore-diameter required for the separation of small nanoplankton and picoplankton can only be provided by membrane and glass fiber filters. The matrix nature of the glass fiber filters gives an imprecise boundary of retention and passage and these filters have not been used to define the upper limit of a size-class. GF/F's (nominal pore size 0.7μm diameter), however, have been employed as lower boundaries for picoplankton (Li *et al.*, 1983), but there are questions as to their suitability (Munawar *et al.*, 1982; Phinney and Yentsch, 1985). Cellulose ester filters (e.g. Millipore filter), for reasons of filter structure, capillarity and absorption, are claimed to retain particles much smaller than their stated pore size (Sheldon, 1972). Nucleation track perforated polycarbonate filters (e.g. Nuclepore filters) have cylindrical pores of precisely defined size and can act more effectively as screens (Sheldon, 1972).

The vacuum applied during filtration can also be critical; too high vacuum can cause cell rupture (Sharp, 1977); on the other hand, particles smaller than the filter pore size may adhere to the filter and a slight vacuum may be required to overcome electrostatic and Van der Waals forces (Li, 1986).

According to the classification of Sieburth *et al.* (1978) the picoplankton is composed of planktonic organisms in the 0.2-2μm size range. However, discrepancies over the functional boundaries of the size class to be adopted for these minute organisms have arisen in the literature throughout the past decade and thus 5, 3, 2 and 1μm have been used as upper limits. The majority of workers, although in

agreement with Sieburth's classification, have assumed that most of the organisms in this size range can pass through 1μm Nuclepore filters (Li *et al.*, 1983; Platt *et al.*, 1983; Joint and Pomroy, 1983, 1986; Joint *et al.*, 1986; Iturriaga and Mitchell, 1986; Smith *et al.*, 1985; Raimbault *et al.*, 1988; Chavez, 1989; Magazzú *et al.*, 1987) and the picoplankton has operationally been defined as plankters passing through 1μm Nuclepore filters (Li *et al.*, 1983). It is evident, however, that a large proportion of coccoid cyanobacteria can be retained in 1μm Nuclepore filters (up to 90% in studies by Li (1986) and Craig (1986)) and some can also be retained in 3μm Nuclepore filters (about 30% in the studies by Li (1986) and Ray *et al.* (1989)). Picoplankton numbers from 5μm filters seem to be undiminished in relation to unfractionated samples (Li, 1986), but organisms larger than picoplankton can also get through these filters. A 3μm filter has thus been recommended as a reasonable upper boundary (Craig, 1986; Li, 1986). For the lower limit, 0.2μm Nuclepores are the most commonly used type of filters, although some authors have used GF/F's as well. 0.2μm Nuclepore filters have been reported to allow the passage of 10-15% of the coccoid cyanobacteria, or even higher proportions, depending on clonal differences (Li, 1986). 0.1μm Nuclepore filters are claimed to have a potential for 99% retention capacity (Li, 1986), but these filters have rarely been reported to have been used in the literature for primary production studies. All these percentages of passage, of course, only serve as an orientation, since they will vary depending on the specific conditions of the sample and mainly on the population density.

The use of large vacuum should be avoided, but a small pressure of 20-50mm Hg is more effective than gravity alone for the 3 and 1μm filters (Li, 1986). For 0.2μm and smaller pore sized filters a vacuum of at least 100mm Hg is required to avoid prolonged filtration times, but vacuums higher than 200mm Hg should not be applied.

Other effects are related to the characteristics of the particles in the samples, such as cellular shape, fragility and flexibility, as well as population density. Samples with high concentrations of particles, either detrital or living material, can cause clogging of the filter, thus avoiding passage of particles smaller than the filter pores. However, the choice of volume of sample

filtered must also take into account the minimum concentration required to detect the least concentrated or active fraction according to the sensitivity of the techniques used. On the other hand, a number of phytoplankton species are well known for their extreme fragility and fragments of larger cells can be included in the small size fractions. Similarly, the small size fractions will be overestimated when cells larger than a given filter pore size are flexible enough or have such a shape so as to squeeze themselves through the filter pores. Although these factors can pose serious difficulties for the interpretation of results, they are largely overlooked in size-fractionation studies, primarily because they are hardly quantifiable and it is almost impossible to asses their repercussion in the measurements (Jochem, 1989).

In the present study in Southampton Water, during the colonial *Phaeocystis* spp. bloom in May, colony disruption is likely to have occurred during filtration since passage of some cells through 3μm pore-sized filters was apparent. Thus the cell number and chlorophyll *a* concentration measurements included some of these cells as part of the 1-3μm fraction. The size-fractionated carbon uptake measurements are also confusing, since the phytoplankter accumulates an appreciable amount of its organic carbon in the colony matrix (Lancelot *et al.*, 1986; Veldhuis *et al.*, 1986), and if the colonies are disrupted during the filtration, the mucilaginous substances of the matrix solubilize, passing through the filters, giving spuriously large values of excretion rate (Lancelot, 1983).

During the summer the presence of the phototrophic ciliate *Mesodinium rubrum* also caused some difficulties. *M. rubrum* (40μm in diameter, D.Kifle, personal communication) is well known for its extreme fragility (Lindholm, 1985) and there is evidence of cell rupture during incubation in bottles and during filtration (Throndsen, 1978; Smith and Barber, 1979). It is likely that cell fragments of this organism may have caused some overestimation of the two smaller fractions. However, chlorophyll *a* concentration was also large (around 10mgchl *a* m^{-3}) and some clogging of the filter may have partly neutralized the effect. It is difficult to evaluate the magnitude of this effect on the picoplankton fractions and some caution should be exercised in the interpretation of the results.

1.5.1.5.Carbon uptake measurements : blank and pre- or post-incubation fractionation options

The ^{14}C technique for measuring aquatic primary production, although extensively used since its introduction in 1952 by Steeman Nielsen, has long been recognized for its multiple analytical as well as interpretative difficulties (Carpenter and Lively, 1980; Peterson, 1980; Williams and Robertson, 1991). Some of these interpretative difficulties appear to be more acute when the technique is used in size fractionated experiments in the picoplankton size range (Li, 1986). A fundamental problem is that in a mixed plankton population not all the ^{14}C uptake can be attributed to photosynthesis. A proportion of it will be ascribable to the generically termed "dark CO_2 uptake", which includes inorganic carbon uptake through both chemosynthetic and anaplerotic reactions. The former are restricted to a small group of bacteria, the latter, although mainly put down to bacteria, are carried out by all organisms that include the Krebs cycle in their metabolism. Another proportion of ^{14}C fixation may be due to uptake of labelled organic algal exudates by the microheterotrophs. Finally, some of it may be due to passive incorporation.

Substraction of a dark incubated value from the light incubated value was proposed in the original method (Steemann Nielsen, 1952) to exclude non-photosynthetic fixation. It was then argued that, although non-photosynthetic, anaplerotic reactions could result in net fixation of CO_2, and using a dark blank would underestimate the global CO_2 fixation (Morris *et al.*, 1971). Furthermore, there are indications that the Krebs respiratory cycle and the bacterial activity in general might not be invariant over the light-dark cycle (Burris, 1980; Sieburth, 1984; Li, 1986). As a result, there is great uncertainty as to how to deal with dark ^{14}C uptake values (Peterson, 1980; Li, 1986). This problem becomes even more acute when dealing with the picoplankton size fraction, since in this size range dark uptake at times represents a large proportion of the light uptake (Li *et al.*, 1983; Herbland *et al.*, 1985).

The use of metabolic inhibitiors, such as dichlorophenol dimethyl urea (DCMU), which acts on the cytochrome Q and plastoquinone , inhibiting the non-cyclic photophosphorylation, has been suggested

as an alternative (Legendre *et al.*, 1983). However, this practice does not seem to have been widely adopted, particularly because of concern of specificity of action, since there are indications that the DCMU can also affect heterotrophic activity (Li and Dickie, 1985).

Li (1986) in a review of the subject in relation to picoplankton studies has recommended to ignore the dark ^{14}C values when trying to evaluate the rate of carbon fixation in terms of photosynthesis. It is on these grounds that the zero time blank protocol was adopted in the present study. Therefore, values of carbon fixation rate in the <1μm fraction cannot be assumed to strictly represent photosynthetic rate.

Bacterial uptake of algal exudates cannot be accounted for neither by using dark blanks or DCMU treated blanks. This can be largely eliminated by physically separating the bacteria from the large phytoplankton through size-fractionation by filtration, when the fractionation is performed prior to the initiation of the experimental incubation. This procedure, though, does not exclude bacterial uptake of exudates released by picophytoplankton, but excretion in this fraction would be negligible in relation to the excretion by larger algae (Ward, 1984). McCarthy *et al.* (1974) found post-incubation screening consistently yielded lower estimates than pre-incubation screening and advocated the pre-incubation filtration practice. Some workers have followed this recommendation (Herbland and Le Bouteiller, 1981; Waterbury *et al.*, 1986), however, the vast majority of studies on size-fractionated primary production are based on post-incubation fractionations (Malone, 1977a; Platt *et al.*, 1983; Li *et al.*, 1983; Joint and Pomroy, 1983,1986; Cole *et al.*, 1986; Jochem, 1989; Furnas and Mitchell 1988; Legendre *et al.*, 1988; Prézelin *et al.*, 1986; Magazzú and Hull, 1985; Glover *et al.*, 1986; etc.). Authors in favour of post-incubation screening claim the pre-incubation fractionation can introduce other biases such as a release in grazing pressure exerted by the larger organisms over the smaller ones, separation of sources of nutrient regeneration, promotion of photosynthetic activity when the phytoplankton community is segregated into less dense aggregates (e.g. reduced shading), and damage to small cells with passage through filters (Furnas, 1987). It thus seems that there is no "optimum" method for size fractionated studies of carbon uptake rate

(Li, 1986). For consistency with the majority of studies carried out on picoplankton, the post-incubation screening protocol was adopted in the present study.

1.5.1.6. Temporal variation in size fractionated chlorophyll a biomass and carbon uptake rate

The seasonal sequence of chlorophyll *a* in the 1-3(5)μm and <1μm fractions generally followed the succesion observed in picophytoplankton cell numbers. The seasonal distribution of size-fractionated rate of carbon uptake, in turn, followed reasonably closely the variations in size-fractionated chlorophyll *a* concentration ($r= 0.944$ ($p<0.01$) for the >3(5)μm fraction, $r=0.883$ ($p<0.01$) for the 3(5)-1μm fraction, $r=0.883$ ($p<0.01$) for the <1μm at Calshot Spit and $r= 0.790$ ($p<0.01$) for the >3(5)μm fraction, $r=0.705$ ($p<0.05$) for the 3(5)-1μm fraction, $r=0.972$ ($p<0.01$) for the <1μm fraction at NW Netley). Exception was during the sampling visit in which *Phaeocystis* spp. was in bloom at NW Netley (May 8th), when the rates of carbon uptake in the >3(5) and 3(5)-1 μm failed to reach the magnitude expected from the chlorophyll *a* peaks. This is likely to be the result of colony disruption and subsequent solubilization of the colonial organic matrix (Lancelot *et al.*, 1986; Veldhuis *et al.*, 1986). However, this mismatch between the magnitudes of the chlorophyll *a* concentration and primary production rate peaks was not observed to occur in samples collected on April 23rd at Calshot Spit, when a chlorophyll *a* concentration of 15.68μg l^{-1} was recorded and *Phaeocystis* spp. was identified as numerically dominant (D.Kifle personal communication). It can be hypothesized that on May 8th the *Phaeocystis* sp. bloom was in a more advanced state of development at NW Netley than it was on April 23rd at Calshot Spit and that the algal colonies were probably in a more scenescent state and hence more susceptible to rupture during filtration. This was substantiated by the difference in apparent excretion rate between these two dates: 23% percentage extracellular relaease (PER) on April 23rd at Calshot Spit and 39% at both stations on May 8th. Both chlorophyll *a* and carbon uptake values in the 1-3(5)μm and smaller than 1μm fractions, may have been overestimated during the *Mesodinium rubrum* peak of abundance at

NW Netley, since, as discussed above, the ciliate is well known for its fragility (Lindholm, 1985). Throndsen (1978) carried out size-fractionated primary production measurement during a *Mesodinium* bloom and observed that when rinsing the filters with a slightly hypotonic solution *Mesodinium* cells were disrupted and the liberated endosymbionts, passed through 5μm screens.

The seasonal distribution of the rate of carbon uptake into particulate material (excluding DOC) integrated for the year showed pronounced differences between the inner and the outer stations of the estuary. At the more marine station (Calshot Spit) the largest proportion of the annual particulate production occurred during spring (51.8%), most of it taking place during a short period of time of high chlorophyll *a* concentration. During summer, production was substantial (41.5%), but this was more evenly distributed during the season than in spring. In contrast, at the more estuarine station (NW Netley) the bulk of the primary production took place during the summer (71.5%). This variation in primary production rate between the mid and outer estuary is in reasonable agreement with the previous study of Southampton Water by Bryan (1979).

The distribution of the annual carbon uptake rate in each fraction showed, however, that in the smaller than 1μm fraction the major portion of the annual rate of carbon fixation (excluding DOC) occurred during the summer months at both stations. Summer carbon fixation appeared to be about twice (60-70% of annual) that fixed during the spring months (20-30% of annual) for this size fraction. For the 1-3(5)μm fraction, primary production was similar in spring (April, May, June; total 6 dates) (48.5%) and summer (July, August, September; total 4 dates) (42.5%) at Calshot Spit. The high production for spring was mainly the result of the substantial amount of chlorophyll *a* concentration and carbon uptake rate determined in this fraction on May 22nd, however, an accordingly high concentration of picophytoplankton cells was not detected on this date. The reasons for this are at present unclear. At NW Netley, however, in the 1-3(5)μm fraction 77.6% of the primary production occurred during the summer. There are no previous data of size fractionated chlorophyll a biomass and primary production for the 3-1μm and the 1-0.2μm phytoplankton size-classes for Southampton Water with which these results can be

compared.

The greater proportion of the annual production for the 1-5μm and the <1μm fractions in the Celtic Sea (Joint *et al.*, 1986) and for the smaller than 3μm fraction in the Kiel Bight (Jochem, 1989) and in Stockholm archipelago (Larsson and Hagstrom, 1982) was also found to take place during the summer.

The significantly high correlation between the carbon uptake rate and chlorophyll *a* concentration for the smaller than 1μm fraction would suggest the carbon uptake in this size-fraction was essentially photosynthetic, and given the overall significant correlation with picophytoplankton cell numbers, a substantial part of it can probably be attributed to picophytoplankton.

Bacterial abundance and rate of activity is typically well correlated with temperature (Albright, 1977; Ducklow and Kirchman, 1983; Iriberri *et al.*, 1985) and chlorophyll concentration (Bird and Kalff, 1984). Bacterial numbers and production have also been shown to increase with temperature and chlorophyll *a* biomass in Southampton Water, reaching maxima during summer at both stations, Calshot Spit and NW Netley), showing particularly high numbers during the *Mesodinium rubrum* peak of abundance at NW Netley (Antai, 1990). Increased DOC production during the ciliate bloom was assumed to be partly responsible for this trend (Antai, 1990), however, rates of extracellular release of DOC by phytoplankton were not measured. It is difficult from the data in the present study to determine the level of excretion rate during the *Mesodinium* maximum, because an unknown proportion of it may be attributable to material leaked from the ciliate cell upon breakage during filtration. However, some overestimation of the carbon uptake by phytoplankton in the <1μm fraction due to bacterial uptake of carbon derived from algal exudates cannot be ruled out.

In temperate coastal areas the contribution of picoplankton to the annual primary production in the water column is estimated to be about 20-30% (Larsson and Hagstrom, 1982; Joint *et al.*, 1986; Maita and Odate, 1988). The contribution of picoplankton to overall phytoplankton community chlorophyll and primary production has been reported to increase during the summer months to values up to 40-60% (Larsson and Hagstrom, 1982; Maita and Odate, 1988; Raimbault *et al.*,

1988). There are few reports from estuarine areas, but the input seems to be somewhat lower than in coastal areas and values around 10% have been reported for the < 3μm fraction (Jochem, 1989; Madariaga and Orive, 1989; Ray *et al.*, 1989), although these studies include measurements for different periods of the year and thus estimates are not strictly intercomparable. The contribution to the annual total plankton community carbon fixation rate (excluding DOC) by the fraction passing through the 3(5)μm filter was estimated to be 19.3% at NW Netley and 17.2% at Calshot Spit and 6% in both stations for the less than 1μm fraction. Carbon fixation by the <1μm fraction thus represented 32.1 and 36.5% of the carbon fixation by the <3μm fraction. There are virtually no data in the literature for the contribution by the smaller than 1μm fraction to the plankton community chlorophyll a biomass and carbon fixation rate in estuaries. The value of 6% obtained in the present study in Southampton Water shows the relatively minor importance of the picocyanobacteria in temperate estuarine waters in comparison to their impact on the primary production in oceanic waters. In absolute terms this 6% accounted for 10.956 and 8.195gC m^{-2} yr^{-1} at NW Netley and Calshot Spit respectively. This is substantially lower than the 23.06gC $m^{-2}yr^{-1}$ estimated for the same fraction in the Celtic Sea (Joint *et al.*, 1986).

Carbon fixation rate by the <1μm size fraction appeared to be inversely correlated with phosphate concentration, although the correlation was not very strong ($p<0.1$). This suggests that this size fraction was not nutrient limited at either of the two stations. The inverse relationship between primary production rate by the picophytoplankton and the nutrient concentration has also been noted in other areas (e.g. Funka Bay, Maita and Odate, 1988).

In the outer estuary the contribution of the smaller than 1μm fraction to the total community carbon fixation rate when analyzed by seasons, was found to be maximum during the summer (9.4%), coinciding with the maximum of abundance of picophytoplankton. This appears to be a generalized pattern in temparate coastal and estuarine waters where the major annual bloom occurs during the spring (Larsson and Hagstrom, 1982; Maita and Odate, 1988; Jochem, 1989). In these areas the microphytoplankton are generally dominant only for a brief period,

during the spring outburst, when they can easily account for >90% of the primary production (McCarthy *et al.*, 1974; Durbin *et al.*, 1975; Malone, 1977a; Throndsen, 1978; Joint *et al.*, 1986; Jochem, 1989). During the rest of the year the nanophytoplankton generally constitutes the major component (Joint *et al.*, 1986). The picophytoplankton are most abundant in summer (Joint *et al.*, 1986; Jochem, 1989), but only occasionally can overcome the nanophytoplankton level of significance. The bulk of the annual primary production can be attributed to the nanoplankton size-fraction (Joint *et al.*, 1986).

Results obtained in the present study show that in estuarine areas where the major productive season is the summer this temporal sequence of the size-structure of the phytoplankton community does not apply. In Southampton Water the bulk of the annual chlorophyll *a* and primary production was associated with particles >10μm (D.Kifle, personal communication) and although divisions at the microplankton-nanoplankton level i.e. larger than and smaller than 20μm, have not been carried out, the nature of the major blooming organisms, large diatoms and colonial *Phaeocystis* spp. (up to 10mm diameter) in spring (D. Kifle, personal communication) and the ciliate *Mesodinium rubrum* (40μm diameter) in summer, suggests microplankton as possibly dominating the annual rate of primary production. Previous reports of size fractionated biomass in Southampton Water include a study by Savage (1965) who found 70% of chlorophyll *a* related to particles <20μm, and a study by Leakey (1989) who reported a mean of 61% of the chlorophyll was associated with <10μm fraction in Southampton Water. But these estimates were means of all sampling visits during the study period and thus are not representative of an integral value for the whole year as the estimates of carbon fixation rate from the present study are. At the inner station, when analyzed by seasons, the picoplankton attained its highest contribution to the total plankton community carbon fixation rate in winter and autumn.

1.5.1.7. Photosynthetic parameters

The range (2.8-11.9) and mean (5.9) assimilation number value (P^B_m), i.e. light saturated rates of chlorophyll *a* specific

photosynthesis (μgC μgchl a^{-1} h^{-1}) (Harrison and Platt, 1980), determined for the total phytoplankton community in Southampton Water during the period of study, compared well with the general range of 1-15 and the annual mean of around 5 estimated for temperate coastal waters (Harrison and Platt, 1980; Falkowski, 1981). The range (0.011-0.110) and mean (0.039) chlorophyll *a* normalized initial slope of the *P v I* curve expressed as μgC μgchl a^{-1} h^{-1} ($\mu Em^{-2}s^{-1}$)$^{-1}$ (α^B) measured in the present study are also in good agreement with results obatined in other coastal and estuarine areas (see compilation by Pennock and Sharp, 1986).

The spatio-temporal variability in these two parameters can be large and this variability is observable over time scales of a few hours (Harrison and Platt, 1980; Harding *et al.*, 1986). Diel variations have been shown to approach seasonal variations (Harrison and Platt, 1980). This constrains somewhat the comparability in the present study, since the commencement of the experimental incubations varied 1 or 2 hours (12.00-14.00 hours).

Identified variables influencing the values of P^B_m and α^B in phytoplankton include light history, nutrient status, temperature, species composition and cell size (Harrison and Platt, 1980; Falkowski, 1981). In Southampton Water the only environmental parameter that significantly correlated with P^B_m for the total plankton community was temperature at NW Netley ($r=0.709$, $p<0.05$) but not at Calshot Spit. When analyzed by fractions, the temperature-P^B_m relationship was only apparent for the >3(5)μm fraction, but not for the two smaller fractions. The temperature-P^B_m relationship in the outer estuary may be masked by the effect of nutrient limitation during the summer (Falkowski, 1981), but this is not clear from the data. P^B_m *versus* temperature plots can commonly be fitted to exponential curves (Li, 1980), although, Harrison and Platt (1980) argue that the fit is generally not statistically different from linear. Pennock and Sharp (1986) observed that temperature sets an upper limit to the maximum P^B_m, a pattern similarly to that found by Eppley (1972) for the maximum growth rate. The physiological basis for this positive correlation with temperature lies in the temperature enhancement of the activity of rate-limiting enzymes (Falkowski, 1981; Harrison and Platt, 1980), since P^B_m is mainly limited by the rate of

activity of the enzymes of the dark reactions of photosynthesis (Rabinowitch, 1951).

A deficient supply of amino acids precursors of photopigment-protein complexes, following nitrogen limitation, can result in reduced concentrations of chlorophylls and accesory pigments (see references in Perry *et al.*, 1981). Both photosynthetic unit (PSU) size and numbers have been shown to decrease in starved phytoplankton cells (Perry *et al.*, 1981). The effect these changes in PSU can have on α^B and P^B_m, however, is not so clear. Howard and Joint (1989) consider P^B_m would be increased under nitrogen starvation, whereas Falkowski (1981) and Harrison and Platt (1980) consider that chlorophyll specific photosynthesis would be depressed. Reduction in PSU numbers and size results in higher rates of photosynthesis per unit of chlorophyll *a* value i.e. reduced package effect, however, since nitrogen will also limit synthesis of amino acids for enzymes of dark reactions, and the rate of activity of these is probably the major limitation to light saturated rates of photosynthesis, P^B_m need not necessarily be enhanced under nitrogen limitation. In the present study in Southampton Water it was not possible to establish a relationship between nutrient availability and photosynthetic parameters at any of the two stations sampled. At NW Netley nutrients did not seem to be limiting at any time during the period sampled. At Calshot Spit, the nutrient limited period coincided with increased temperatures, and since these two factors have opposite effects on P^B_m, they may have cancelled out each other. Harrison and Platt (1980) did not observe a relationship between P^B_m and nutrient status in the natural environment.

Variations in α^B and P^B_m can also result from photoadaptational responses. Adaptation to low light can involve changes in the size and number of PSU's, as well as changes in the enzyme levels of the dark reactions. These changes reflect in the shape of the *P v I* curves. The combination of mechanisms involved is species-specific and several patterns of *P v I* curve variation with growth irradiance have been identified (Prézelin and Sweeney, 1979; Richardson *et al.*, 1983). The variability in these patterns is such that there is no definite trend of variation of α^B and P^B_m with growth irradiance, although for P^B_m the most common pattern is that of a decrease at low growth light

intensity (Richardson *et al.*, 1983). Thus the effect of light history can be variable in mixed phytoplankton populations.

Harrison and Platt (1980) observed a minimal effect of light on P^B_m in natural assemblages. Pennock and Sharp (1986) observed no effect of light on P^B_m in the Delaware estuary and concluded that the mixed conditions of the water column most likely prevented light adaptation taking place (i.e. changes in the light field were faster than the time required for physiological adjustments to take place). This is likely the case in an environment like Southampton Water, where the semi-diurnal destabilization of the water column resulting from the tidal incursion is likely to hinder photoadaptational responses during most of the year.

The allometry of carbon specific photosynthesis and growth rates is a well documented feature (Williams, 1964; Eppley, 1966; Taguchi, 1976; Banse, 1976, 1982; Schlesinger *et al.*, 1981; Blasco *et al.*, 1982; Geider *et al.*, 1986; Langdon, 1988). Some results from natural plankton assemblages show higher rates of chlorophyll specific rates of photosynthesis with decreasing cell size (Malone, 1977a; Malone and Neale, 1981; Furnas, 1983), but the size dependence of carbon to chlorophyll ratios and chlorophyll-specific metabolic rates, is not always evident from empirical data (Parsons *et al.*, 1961; Banse, 1976; Taguchi, 1976; Durbin, 1977; Chan, 1980; Blasco *et al.*, 1982; Cole *et al.*, 1986).

Banse (1976) pointed out that variations in growth rate and associated metabolism with growth conditions would tend to obscure the size-dependance of physiological processes, and this may explain the lack of definite trend with size observed in mixed plankton populations (Cole *et al.*, 1986) and in laboratory cultures when growth conditions are not optimal for all species under study (Banse, 1976).

However, it is apparent that the comparison of chlorophyll normalized growth and photosynthetic rates gives more inconsistent results than when carbon-specific metabolic rates are compared. This is likely to reflect the species-specific variations in ratios of chlorophyll to accessory pigments, and numbers and sizes of PSU's.

Recent studies comparing α^B and P^B_m of picoplankton with larger size classes also evidence these discrepancies (see Table 1.5.2). In a number of investigations, in agreement with results from the present

study in Southampton Water, α^B appeared to be higher for the picoplankton size fraction than for larger phytoplankters (Platt *et al.*, 1983; Smith *et al.*, 1985; Joint and Pomroy, 1986; Howard and Joint, 1989), in some studies no consistent trend was found (Furnas and Mitchell, 1988) and in others larger phytoplankton appeared to be more efficient at subsaturating light level (Legendre *et al.*, 1987; Magazzú *et al.*, 1987). Likewise, assimilation numbers were found to be higher for picoplankton in some investigations (Platt *et al.*, 1983; Smith *et al.*, 1985; Furnas and Mitchell, 1988; Howard and Joint, 1989), but not in others (Takahashi and Bienfang, 1984; Herbland and Le Bouteiller, 1981; Magazzú *et al.*, 1987; Ray *et al.*, 1989).

As pointed out by Joint and Pomroy (1986) and Howard and Joint (1989) there are indications of poor extraction of chlorophyll from cyanobacteria (Stauffer *et al.*, 1979) and thus care should be exercised when comparing metabolic rates normalized to chlorophyll *a* in picoplankton. Also, the carbon fixation rate in the picoplankton fraction does not represent only photoautotrophic uptake. Bryan (1979) showed that dark CO_2 uptake rarely exceeded 1% of the rate of primary production in the total plankton community in Southampton Water (i.e. this means it is generally lower than 15% of CO_2 uptake in the light by the <1μm fraction). It is less clear how significant bacterial uptake of algal exudates is in Southampton Water. Thus, at least in the present study, the higher α^B and P^B_m in the picoplankton fraction, should not necessarily be interpreted as a physiological consequence of phytoplankton size. In fact, on some occassions, values of P^B_m and α^B for the smaller than 1μm were somewhat higher than the theoretically derived physiological maximum attainable values i.e 25μgC μgchl $a^{-1}h^{-1}$ for P^B_m (Falkowski, 1981) and 0.102μgC μgchl a^{-1} $h^{-1}(\mu Em^{-2}s^{-1})^{-1}$ for α^B(Platt and Jassby, 1976). Some values of α^B reported by Howard and Joint (1989) from a study in the North Sea were also considerably higher than the theoretical maximum value.

In the other two size fractions (>3(5)μm and 1-3(5)μm) the parameters α^B and P^B_m did not show a consistent trend with size, as observed in other studies for various size classes (Cole *et al.*, 1986; McCarthy *et al.*, 1974; Durbin *et al.*, 1975). Joint and Pomroy (1986) compared photosynthetic parameters in three size classes (>5μm, 1-5μm and 0.2-1μm) in the Celtic Sea in summer and found that α^B and P^B_m were

generally higher for the 0.2-1μm fraction, but both parameters were relatively similar for the two larger size-classes. In this study dark-incubated values were used as blanks. Size-fractionation was performed post-incubation, however, the authors suggested from ancillary measurements that bacterial activity was only of the order of 10% of the carbon fixation in the 0.2-1μm.

Mean α^B and P_m^B for the picoplankton size-fraction obtained in the present study compare well with values reported in the literature (Table 1.5.2). Although there are far less estimates for temperate coastal and estuarine waters, values for both parameters, particularly for α^B seem to be lower in open ocean polar, tropical and subtropical waters than in temperate coastal and estuarine waters. Further investigation is required to test this trend.

Table 1.5.2. α^B and P_m^B estimated in various oceanographic provinces for the picoplankton and larger size fractions. Values are means of all observations ; the range is given in parenthesis.

Location	Filter pore size	P_m^B >	P_m^B <	α^B >	α^B <
[a]Arctic	1	0.5	1.0	0.008	0.017
[b]Arctic	1	-	-	(0.012-0.022)	0.007
[c]Mid Atlantic	1	(0.2-0.9) 0.5	(0.2-1.0) 0.7	(0.003-0.013) 0.007	(0.005-0.020) 0.014
[d]Equatorial Atlantic	3	11.2	2.5	-	-
[e]Southern ocean	1		(0.1-2.6)	-	-
[f]Porcupine Sea Bight 10m	>5,<1	(1.9-5.5) 3.1	(3.5-5.9) 4.7	(0.008-0.012) 0.010	(0.023-0.044) 0.033
20m		(0.4-1.8) 1.2	(1.0-3.4) 2.2	(0.004-0.015) 0.008	(0.021-0.038) 0.028
[f]Celtic Sea 10m		(2.4-4.9) 3.9	(3.2-6.4) 4.4	(0.011-0.023) 0.017	(0.016-0.056) 0.032
25/30m		(1.3-2.5) 1.9	(2.0-7.7) 4.4	(0.005-0.026) 0.016	(0.018-0.079) 0.050
[g]Coral Sea surf.	2	(2.4-3.7) 2.8	(5.1-8.2) 6.3	(0.004-0.007) 0.006	(0.006-0.017) 0.013
bottom	2	(0.3-0.7) 0.6	(0.6-2.1) 1.2	(0.005-0.028) 0.017	(0.005-0.014) 0.009
[h]Strait of Messina	1	(3.5-12) 9.0	(1.4-3.1) 2.1	(0.017-0.026) 0.023	(0.009-0.015) 0.012
[i]North Sea	1	(1.3-5.8) 2.9	(1.9-11) 6.0	(0.028-0.089) 0.046	(0.045-0.630) 0.198
[j]Chesapeake bay	3	5.2	2.5	-	-
[k]Southampton Water	>3,<1	(2.9-15.6) 6.0	(2.3-28) 9.9	(0.011-0.105) 0.036	(0.037-0.155) 0.082

Notes :
P_m^B in µgC µgchl a^{-1} h^{-1}; α^B in µgC µgchl a^{-1} h^{-1} $(\mu Em^{-2}s^{-1})^{-1}$.
W m^{-2} were converted to $\mu Em^{-2}s^{-1}$ by multiplying by 5 (Richardson *et al.*, 1983).
[a]Smith *et al.* (1985); [b]Legendre *et al.* (1987); [c]Platt *et al.* 1983; [d]Weber and El Sayed (1987); [e]Herbland and Le Boutellier (1981); [f]Joint and Pomroy (1986); [g]Furnas and Mitchell (1988); [h]Magazzú et al. (1987); [i]Howard and Joint (1989); [j]Ray *et al.* (1989); [k]the present study.

1.5.1.8.Size-structure of the phytoplankton community along the longitudinal axis of the estuary from Redbrige to Calshot Spit

The salinity distribution along the transect sampled on September 3rd 1990, is in accordance with previous reports (Dyer, 1970; Phillips, 1980). In the region between Redbridge and Marchwood Channel the vessel had to follow a meandering dredged channel and consequently sampling covered a somewhat large horizontal space range in this region. The patchiness in salinity observed in this region is thus likely to reflect the lateral variability in salinity noted by Dyer (1970) further down the estuary and ascribed to a peculiar water circulation pattern affected by the topography.

The nutrient concentration range and distribution are in reasonable agreement with typical conditions for this time of the year (Phillips, 1980). In Southampton Water nitrate and silicate have been reported to behave conservatively, whereas phosphate and ammonium show unconservative behaviours, mainly due to inputs from sewage effluents (Phillips, 1980). The seasonal distribution determined in the present study suggested removal of nitrate and phosphate by primary producers during periods of chlorophyll *a* maxima and re-supply either by effluent discharges or through regeneration within the estuary during the summer. Thus the conservative behaviour of nitrate needs to be qualified with seasonal references. At the time of the survey along the longitudinal axis of the estuary, the biological activity was still considerable (indicated by chlorophyll *a* concentration of *ca.* 10mg m^{-3} at NW Netley) and, although it is difficult to observe removal or addition of the nutrients without being able to determine the theoretical dilution line, the plot of nitrate+nitrite concentration *versus* salinity suggests a slight removal of nitrate+nitrite at salinities over 25ppt. For phosphate, removal at salinities over 25ppt seems clearer, although in the Marchwood Channel area (salinities between 29.1 and 32.7ppt) a source of input, likely to be the Slowhill Copse and Millbrook sewage effluent discharges (see Savari *et al.* (1991) for updated effluent discharge points in Southampton Water), can also be identified.

The distribution pattern of the overall phytoplankton community chlorophyll *a* concentration along the transect showed lowest chlorophyll *a* values at low salinity, between Redbridge and Marchwood,

increasing as salinity increased and reaching a maximum at around mid estuary between salinities of 29 and 34.2ppt (Marchwood Channel to around Bed 31+32 buoy), with a sharp decrease thereafter, as the mouth of the estuary (the Solent) was approached. The pattern is in good agreement with observations of the regional variation of annual primary production reported by Bryan (1979) for this estuary and similar patterns for chlorophyll *a* distribution have been described for other estuaries, e.g. Chesapeake and Delaware Bays (Harding *et al.*, 1986).

A decrease in phytoplankton water column integrated areal production towards the upper limits of the estuary, despite enhanced nutrient levels, is generally explained in terms of increased turbidity and thus a reduction in light availability, e.g. Delaware estuary (Pennock and Sharp, 1986), Chesapeake Bay (Harding *et al.*, 1986), Bristol Channel (Joint and Pomroy, 1981), Fourleague Bay (Randall and Day, 1987), San Francisco Bay (Cole and Cloern, 1984). Parallel spatial variations of chlorophyll *a* concentration have also been reported (Pennock and Sharp, 1986; Harding *et al.*, 1986), but the distribution of the phytoplankton standing stock is not always correlated with production rate (Cloern, 1987; Randall and Day, 1987).

A number of reasons were given by Bryan (1979) to explain the low production in the upper reaches of the Test estuary: e.g. the occurrence of rapid currents and high loading of suspended matter, coupled to rapid tidal and salinity changes. There is little information in the literature on the distribution of suspended sediments and current velocities in the upper reaches of the Test sub-estuary. Longitudinal current velocities show large vertical and lateral variations in Southampton Water (Dyer, 1973). Typical current velocities range between 10-60cm s^{-1} (Dyer, 1973; Crawford and Purdie, in press) and high velocities of up to 100cm s^{-1} in bottom waters have been recorded (Dyer, 1973). However, there are no clear indications of variability in current velocities with distance from the head of the estuary).

In this low salinity region (7 to 18ppt) the salinity did not increase steadily with increasing distance from the mouth, but chlorophyll *a* was positively correlated with salinity ($r=0.506$, $n=15$, $p<0.1$). Pennock and Sharp (1986) observed a decrease in production

from the riverine region down to the turbidity maximum zone in the Delaware estuary and ascribed this feature to the decrease in light level in the turbidity maximum and to osmotic stress of freshwater phytoplankton populations. These conclusions were based on the decline in chlorophyll *a* levels and numbers of freshwater phytoplankton and the decrease in chlorophyll specific production that was noted at the beginning of the mesohalinic region. Blanc (1969) not only noted a decrease in chlorophyll levels, but also observed massive death and disintegration of freshwater phytoplankton in the dilution zone of the Grand Rhône. Similarly, Fast and Kies (1990) also observed a sharp decline of chlorophyll *a* concentration from salinities of 0.4 to 15ppt (180 to 6$\mu g\ l^{-1}$) in the Elbe estuary. These authors further noted a great increase in the phaeophytin to chlorophyll *a* ratio with increasing salinity from freshwater to about 15ppt, and this was taken as evidence for disintegration of freshwater phytoplankton due to osmotic stress.

In the present study in Southampton Water the phaeopigment to (chlorophyll *a* + phaeopigment) ratio (%) exhibited a marked increase (from about 20 to 55%) from salinities of 13ppt down to salinities of 7ppt (the lowest recorded). Relative levels of degraded forms of chlorophyll *a* can increase as a consequence of grazing by zooplankton and from inputs of detrital material, e.g. re-suspension of bottom sediments, and from death of phytoplankton. It is unlikely that variations in grazing rate occurred over such a short distance in the estuary. Also a localized water circulation effect leading to the resuspension of detrital material from the sediment is unlikely to provide an answer, given the uneven distribution of salinity. Disintegration of freshwater algae can provide an explanation for these elevated percentages of phaeopigment. Unfortunately, information on the chlorophyll *a* levels in the riverine region and the pattern of variation in numbers of freshwater algae, which could provide further evidence to support this hypothesis are lacking.

Some freshwater algae show a degree of tolerance to salinity. Foerster (1973) found viable freshwater phytoplankton in the estuary showing their maximum primary production rate in the 0.5 to 10ppt salinity region. Jackson *et al.* (1987) also reported a large population of the freshwater diatom *Cyclotella atomus* at salinities

between 0 and 8ppt in the Tamar estuary, but they also observed dead cells at salinities higher than 8ppt. In general, freshwater phytoplankton do not seem capable of growing well in the estuary (Rice and Ferguson, 1975; Brand, 1984).

It is thus likely that a combination of various factors including light limitation by turbidity, and osmotic stress of freshwater phytoplankton can explain the low chlorophyll *a* levels in the low salinity region of the Test estuary.

At intermediate salinities, as light availability augments, and nutrients are still in excess to phytoplankton demands, phytoplankton biomass increases reaching a maximum at around midway down the estuary. A reduction in chlorophyll *a* seaward of this point, also observed in other studies in this estuary (Bryan, 1979; Kifle, 1989; Antai, 1990), has been explained in terms of increased flushing (Bryan, 1979), based on the findings by Collins (1978) of a seaward increase in the rate of exchange of estuarine and offshore water on each tidal cycle. Nutrient levels were also lowest at the lower limits of the estuary, but by this time of the year levels of nitrate+nitrite and phosphate had risen all along the estuary above the "critical value" (*sensu* Fisher *et al.*, 1988) and were unlikely to be limiting for primary production.

Size fractionated chlorophyll *a* distribution with salinity evidenced that the patterns of variation in the larger than 5μm and 1-5μm fractions were broadly similar to that described for the total chlorophyll *a*. For the <1μm, however, the drop in chlorophyll *a* concentration in the proximities of the lower limits of the estuary (seaward of around buoy Bed 31+32) was not apparent.

In agreement with this observation, numbers of PE-containing picocyanobacteria were low at low salinities and increased in an exponential-like relationship with salinity towards the seaward end. Numbers of PE-containing picocyanobacetria and chlorophyll *a* concentration in the 1-0.2μm size fraction, however, were only weakly correlated. Other workers have also noted that the link between chlorophyll *a* concentration and cell counts is not always evident for the small size classes (Li *et al.*, 1983). If the rate of exchange with seawater inflowing from the Solent increases towards the lower reaches, this did not result in an impoverishment of the

picophytoplankton fraction, but rather enriched it. A similar distribution pattern has been shown in the dilution zone of the Changjiang river, highest numbers of *Synechococcus* spp. being associated with the intrusion of the saline waters of the Taiwan current (Vaulot and Xiuren, 1988).

The effect of salinity on chroococcoid cyanobacteria has been little studied. Shapiro and Haugen (1988) failed to observe a clear relationship between PE-containing *Synechococcus* spp. numbers and salinity on a seasonal basis in Boothbay Harbor, but the salinity range was quite narrow (around 28 to 33ppt). Waterbury *et al.* (1986) found PE-containing *Synechococcus* spp. to have an obligate requirement for high amounts of ions and Haas and Pearl (1988) (cited in Ray *et al.*, 1989) found no PE-containing cyanobacteria below 10ppt in the James River estuary. In contrast, high levels of PE-containing cyanobacteria have been found in areas of the Baltic Sea at salinities of 7ppt and lower. PE-lacking strains of marine *Synechococcus* can grow well at low salinities, even in freshwater (Waterbury *et al.*, 1986), but these have rarely been enumerated, because most workers have only used blue excitation filters and PC-rich strains are not visible when excited with light in this wavelength band (Wood *et al.*, 1985). Waterbury *et al.* (1986) did not find PC-rich cyanobacteria in Woodshole Harbor (see Ray *et al.*, 1989); Campbell *et al.* (1983) recorded a ratio of PC/PE rich strains of 0.02 in Great South Bay, but Ray *et al.* (1989) have recently found PC-rich strains in a concentration 8 times that of PE-containing strains in the Lower York River estuary. PE-lacking picocyanobacteria stratins were not enumerated in the present study due to the lack of an appropiate filter combination on the epifluorescence microscope.

In the present study in Southampton Water eukaryotic picophytoplankters were also higher at higher salinities, i.e. in the outer area of the estuary. Kuosa (1988) also observed substantially more elevated concentrations of both eukaryotic and prokaryotic picophytoplankton in the open sea and outer archipelago stations in an inner archipelago-open sea gradient in the Gulf of Finland, Baltic Sea during June. However, salinities were low (lower than 7ppt), and salinity distribution did not seem to be a primary factor controlling picophytoplankton distribution. Picophytoplankton numbers appeared to

be inversely correlated with a eutrophication gradient, lowest numbers coinciding with highest chlorophyll *a* values. In contrast, Jochem (1988) found PE-containing picocyanobacterial abundance increased from the outer Kiel Bight to the inner eutrophicated Kiel Fjord in the Baltic Sea. Larsson and Hagstrom (1982) did not find a definite pattern of variation in abundance in relation to a eutrophication gradient in Stockholm archipelago (Baltic Sea).

In the present study conducted in Southampton Water on September 1990, when the chlorophyll *a* biomass in the various size fractions was expressed as a percentage of the total phytoplankton chlorophyll *a*, it was apparent that the organisms larger than 5μm were dominant along the entire transect (minimum contribution of 65% to a maximum of about 95%). Thus, in general, the trend of variation of the percentage contribution along the entire transect of this larger fraction was expectedly similar to that of the total chlorophyll *a* concentration, i.e. minimum values were found at the lowest salinity values (head of estuary), increasing rapidly downstream to reach a plateau of maximum values up to a point around Buoy Bed 31+32, after which the percentage decreased considerably in the offshore direction. From the two smaller fractions the 1-5μm fraction was more important than the <1μm at all sampling locations. The variation of the contribution to overall phytoplankton chlorophyll *a* standing stock along the transect in these two smaller fractions was the reverse of that of the dominant >5μm fraction, and was inversely correlated with total chlorophyll *a*.

This result is in very good agreement with previous reports that the relative importance of smaller phytoplankton decreases with increasing phytoplankton biomass, and thus shows a decrease from the open ocean to coastal waters and estuaries (see Joint 1986; Fogg, 1986; Ray *et al.* 1989; Chavez, 1989 and table 1.2.1 in section 1.2.1) and that in temperate coastal waters picophytoplankton biomass is least significant in relation to the biomass of larger phytoplankton during the winter-spring and summer-autumn transition blooms (Durbin *et al.*, 1975; Throndsen, 1978; Malone, 1977a; Joint *et al.*, 1986; Maita and Odate, 1988; Shapiro and Haugen, 1988; Jochem, 1989).

Results from both surveys, the seasonal at NW Netley and Calshot Spit and the longitudinal transect, showed that in Southampton Water the small size fractions showed a higher proportion of

phaeopigment than active chlorophyll *a* than the largest fraction. This suggests that the small size fractions include relatively more detrital particles than the large fraction. This feature was also noted in a study in the equatorial Atlantic (Herbland *et al.*, 1985). Herbland *et al.* (1985) argued, however, that the possibility of chlorophyll *b* interference (if significant numbers of small green algae were present), in the fluorometric determination of phaeopigments could not be ruled out. Taxonomic specifications of the picoflora are lacking in the present work in Southampton Water, and chlorophyll *b* levels were not measured. Leakey (1989), reported chlorophyll *b* concentrations to be negligible (0.02-0.12mg m^{-3}) in relation to the total chlorophyll *a* concentration. However, no indications of the size distribution of the chlorophyll *b* were given.

1.5.1.9.Factors affecting spatio-temporal variations in the size structure of the phytoplankton community: an overview

It is apparent that the size structure of the marine phytoplankton community follows definite patterns of spatio-temporal variability and it should thus be possible to relate this variability to specific environmental parameters. Picophytoplankton appear to make the most significant contribution to overall phytoplankton biomass in open ocean oligotrophic waters (Li *et al.*, 1983; Platt *et al.*, 1983; etc.) and in summer stratified temperate coastal and shelf waters (Larsson and Hagstrom, 1982; Joint *et al.*, 1986; Maita and Odate, 1988). These are waters with well developed pycnoclines, i.e. waters of low frequency of water column destabilization, as a result of which, surface ambient nutrient concentrations are low. In contrast, large phytoplankton cells are usually dominant during spring and autumn bloom events (Mc Carthy *et al.*, 1974; Durbin *et al.*, 1975; Malone, 1977a; Throndsen, 1978; Joint *et al.*, 1986; Jochem, 1989), i.e. under transient pycnoclines that offer high nutrient and relatively well lit conditions.

The physiological consequences of variations in phytoplankton cell size have been extensively discussed (Fogg, 1986; Raven, 1986). The minimized package effect of photosynthetic pigments in smaller cells (Kirk, 1983) possibly overrides the restrictions in the volume

available to house the chromophores, thus confering on smaller cells a higher efficiency of photon absorption (Raven, 1986). Also, half saturation constants for nutrient uptake have been suggested to be an increasing function of cell size (Caperon and Meyer, 1972; Friebele *et al.*, 1978). In contrast, smaller cells theoretically have more severe leakage losses (Raven, 1986). The net result of the combination of these features is that carbon specific growth rates are generally higher for smaller cells under subsaturating levels of light and nutrients. Raven (1986) has pointed out that the small volume imposes a constrain on the amount of rate limiting catalysts, due to the volume occuppied by imcompletely scalable components of the phototrophic cells, which could thus result in reduced maximum specific growth rates (μ_{max}, growth rates at saturating conditions of light and nutrients). However, this author also points out that other restrictions on μ_{max} can apply to larger cells derived from self/shading-package effect, reduced membrane area and increased DNA content. From empirical data Raven (1986) found variations in the μ_{max}-cell size relationship between taxonomic groups and size ranges. In general, μ_{max} has been found to be an inverse function of cell size (Banse, 1976; Schlesinger *et al.*, 1981; Geider *et al.*, 1986; Langdon, 1988).

Thus smaller cells have probably faster carbon specific growth rates than larger cells under subsaturating and saturating light and nutrient conditions; however, they do not outcompete larger cells under all environmental conditions. Results from both the study in Southampton Water and from the study in North Sea waters showed clearly that picoplankton increase their relative significance in diminishing spatio-temporal eutrophication gradients, and this is a generalized pattern observed in other studies (Larsson and Hagstrom, 1982; Joint *et al.*, 1986; Chavez, 1989; Maita and Odate, 1988; Raimbault *et al.*, 1988; Jochem, 1989). This means that small phytoplankton cells rarely attain the large concentrations larger cells can achieve under favourable light and nutrient conditions. Large accumulations of phytoplankton biomass occur as a result of transient imbalances between growth and loss processes (i.e. advection and grazing) (Legendre, 1990). Thus for smaller organisms growth remains more persistently in balance with the loss processes than for

larger cells. Factors controlling the biomass accumulation rate thus become more important than size specific differences in biomass specific growth rates (Cole *et al.*, 1986). Losses through sedimentation are minor in small cells (Smayda, 1970) and picoplankton have been suggested to be controlled by predation rather than by sinking (Takahashi and Bienfang, 1983). Moreover, it has already been pointed out that in systems dominated by small cells production is balanced by grazing (Smith *et al.*, 1986). Malone (1980) argued that the closer coupling between production and grazing could be explained in terms of the lower specificity of predators of smaller cells. This may be the case when comparing microphytoplankton with nanophytoplankton as Malone did. However, it is not so clear that picophytoplankton can be grazed by such a wide variety of organisms. Evidence suggests that picophytoplankton are largely consumed within the microbial loop (Smith *et al.*, 1986; Kudoh *et al.*, 1990). Data indicates that in the marine environment nanoheterotrophs, mainly microflagellates, are maintained at significant concentrations throughout the year, even during winter periods (Levasseur *et al.*, 1984; Kifle, 1989), possibly because bacteria also maintain their numbers during the winter (Antai, 1990). It can then be hypothesized that when growth conditions for phytoplankton become favourable during the winter-spring transition, picoplankton growth is more readily suppressed by the pool of overwintering grazers. This has been shown in trophodynamic modelling studies (Murray, 1991). For larger phytoplankton, however, there seems to be a greater decoupling between production and grazing. Herbivorous zooplankton take about one generation time to build up in response to a substantial increase in phytoplankton biomass (see Legendre and Le Fèvre, 1989) because growth rates of large herbivorous zooplankton are one order of magnitude lower than the growth rates of phytoplankters, and lag time intervals between phytoplankton peaks in biomass and grazing peaks are not uncommon (e.g. North Sea (Fransz and Gieskes, 1984); English Channel (Wafar *et al.*, 1984)).

Peaks of picocyanobacteria in summer may be explainable as transient imbalances between growth rates and grazing rates at times when the temperature-enhanced growth rates of picophytoplankton can outpace nanoheterotrophic grazing rate and/or the increased food

concentration for microflagellates (i.e. increased bacterial concentration in summer) results in a release in grazing pressure on picophytoplankton. Thus (directly or indirectly) temperature maybe a more decisive factor for biomass accumulation for smaller than for larger phytoplankton. However, these imbalances do not generally appear to be significant enough to result in blooms of major proportions, not even when light and nutrients are sufficient for larger organisms to bloom, as in Southampton Water. Exceptions have been noted in coastal and estuarine temperate waters during exceptional meteorological conditions, when certain small sized phytoplankton species have outcompeted larger forms and given rise to blooms of major proportions, e.g. *Aureococcus anophagefferens* in Narragansett Bay in 1985 (Cosper *et al.*, 1989).

Results obtained in the present study in Southampton Water suggested that variations in overall phytoplankton biomass and primary production are mainly controlled by the variations in biomass of the larger cells, possibly microplankton, and thus the relative contribution of the different size classes, i.e. the size structure of the phytoplankton community, is primarily influenced by the concentration of the larger forms. Results from other studies have also suggested these conclusions (Furuya and Marumo, 1983; Gonzalez *et al.*, 1988; Odate and Maita, 1989). Factors limiting accumulation of larger cells thus appear to be the primary determinants of an increase in the relative significance of small phytoplankters.

During summer months in temperate shelf and coastal waters levels of new nutrients are low because of depletion during the spring bloom and the slow rate of replenishment from bottom waters due to thermal stratification. The requirement of increased concentrations of nutrients by larger cells (Raven, 1986) has been suggested to be the decisive factor for the dominance of small cells in these waters (Maita and Odate, 1988). Under these stratified conditions as explained in Margalef's (1978) conceptual model, motile cells, mainly flagellates, tend to dominate, since non-motile organisms will tend to sink out of the euphotic zone (Smayda, 1970). These considerations also apply to stratified open ocean oligotrophic waters. Here the deep water column (i.e. absence of shelf) hinders more severely the entrainment of new nutrients and seed cells of large diatoms. Chavez

(1989) has observed the same lack of large diatoms in deep-sea upwelling areas and has thus suggested that the absence of seed cells due to the absence of a continental shelf is probably more decisive than the nutrient limitation in determining the low level of large diatoms in oceanic waters.

In shelf and coastal temperate regions, during the winter-spring and summer-autumn transitions the covariance of high nutrient concentrations and adequate light levels provide conditions for phytoplankton growth and larger cells can give rise to blooms of major proportions. Diatoms have higher rates of photosynthesis and growth than dinoflagellates, mainly because of higher chlorophyll/protein ratios (Chan, 1980) and lower respiration to photosynthesis ratios (Harris, 1978) and under conditions that allow a reasonable degree of turbulence i.e. preventing large non-motile cells from sinking out of the euphotic zone, such as in these transient pycnoclines, they dominate over the dinoflagellate populations (Margalef, 1978).

The larger nutrient storage capacity of large diatoms has been suggested could also play a role during spring and autumn blooms (Malone, 1980). Large diatom cells that sink below the euphotic zone could store nutrient reserves that can be very useful to outcompete smaller cells in the first two generations when resuspended due to vertical mixing, for example by storm events during autumn. Storage capacity could also set the stage for bursts of growth of large diatoms in those instances prior to the initiation of the spring bloom when illumination conditions may still not be so favorable, allowing nutrient uptake, but little growth. Geider *et al.* (1986), however, have suggested that storage capacity plays a minor role in the predominance of larger forms in these transitional blooms.

Estuarine ecosystems are systems with high input of mechanical energy (i.e. freshwater flow, tidal currents) and are potentially subject to frequent sequences of destabilization and restabilization of the water column. The alternation of mixed/stratified conditions promotes primary production (Legendre, 1981) and estuaries are potentially highly productive environments. It is thus not uncommon that phytoplankton have more extended production periods in some estuaries, like in Southampton Water, with major phytoplankton blooms

occurring during the summer (Kifle, 1989; Antai, 1990). These can be associated with spring/neap tidal or strong winds/calm weather transitions (Crawford, 1991). In Southampton Water the summer blooms appear to be dominated by large motile organisms, mainly by the phototrophic ciliate *Mesodinium rubrum*. This may be explained as stratification being intense and rapid upon stabilization of the water column, so that the higher sinking rates of the diatoms would preclude their accumulation. However, it seems more plausible that silica limitation, resulting from consumption during the spring bloom and lower rate of replenishment due to the negligible input of silicic acid in effluent discharges and also the slow rate of dissolution of silicon from the frustules of diatoms (Spencer, 1975), could be the limiting factor for the diatoms growth during periods of low river runnoff. The significance of small cells within the overall microflora becomes greater during winter and autumn, when it is likely that light becomes more limiting to larger than smaller phytoplankton cells.

1.5.2. Studies in the North Sea

1.5.2.1. Picophytoplankton biomass: cyanobacteria abundance and chlorophyll a concentration

Concentrations of PE-containing cyanobacteria recorded in the present study are in reasonable agreement with numbers reported in other studies in North Sea waters (Geider, 1988; Howard and Joint 1989). The mean concentration measured by Geider (1988) (1.9×10^6 cell l^{-1}) was somewhat lower, but sampling was restricted to areas of the east coast of England, where both, in the present study, and in that reported by Howard and Joint (1989) lowest values were recorded in relation to other areas in the North Sea. As observed in the seasonal survey carried out in Southampton Water, and reported in a number of other studies (Krempin and Sullivan, 1981; Hag and Fogg, 1986; Waterbury *et al.*, 1986; Jochem, 1988; Shapiro and Haugen, 1988; Odate, 1989), picocyanobacteria cell concentration shows a marked seasonality in temperate coastal waters, with peaks of abundance during the summer. Geider's study was carried out in October, and this can be the

reason for his lower values, in comparison to the values measured by Howard and Joint (1989) in July. Concentrations found in the present study in March in areas of the Southern Bight compare better with Geider's autumn numbers.

The vertical distribution pattern showed no evidence of a preferential concentration of picocyanobacteria deep in the euphotic zone, not even under the generally stratified conditions of the water column prevailing in locations sampled in Central North Sea waters. This finding is in accordance with previous reports for the North Sea (Howard and Joint, 1989), and reinforces current views that picoplankton do not accumulate exclusively at low light depths (Waterbury *et al.*, 1979; Krempin and Sullivan, 1981; Hag and Fogg, 1986; Howard and Joint, 1989), and thus it questions the genotypical definition of the picoplankton as shade adapted flora.

The range of chlorophyll *a* in the <3μm fraction compares well with values determined during summer in Southampton Water. Numbers of picocyanobacteria were lower in the latter study, but much of the chlorophyll *a* in the <3μm fraction could be attributable to eukaryotic algae. Overall phytoplankton community chlorophyll *a* concentration was lowest in areas of the eastern coast of England. This spatial variation, however, was not apparent for the chlorophyll *a* in the <3μm fraction. On average, the <3μm fraction accounted for *ca.* 20% of the total phytoplankton chlorophyll *a* concentration, with highest values reaching 57% in June/July 1989. Gieskes and Kraay (1984) reported that at a station in central North Sea during July, 50% of the fluorescence was related to particles <5μm in near surface waters. HPLC measurements allowed chemotaxonomic identification of Chrysophyceae and Primnesiophyceae as major taxa for these microflagellates. Geider (1988) found that, on a cell number basis, the majority of the autotrophic nanoplankton (2-20μm) were cells ≤4μm in the eastern North Sea in October. More than 80% of these cells were unidentified flagellates and monads and about equal biovolumes and biomasses were determined in the nanoplankton and microplankton (20-200μm) size classes. Given the large proportion of organisms in the 0.2-5μm fraction, the selection of the filter pore size for fractionation (1, 2, 3 or 5μm) can be important in determining the biomass attributable to picoplankton and/or small nanoplankton, and the 50% value for

phytoplankton smaller than 5μm is probably in good agreement with the figure of 20% obtained in the present study for the <3μm fraction. The value of 20% appears rather conservative in comparison to the 65% estimated in the Mediterranean Sea (Raimbault *et al.*, 1988) for the same size fraction, although, in the latter study it was found that the percentage contribution was reduced to about half at chlorophyll *a* levels in excess of 1μg l^{-1}. Results compare well with estimates obtained in the outer area of Kiel Bight, Baltic Sea (Jochem, 1989) and they are also in accordance with values determined in Southampton Water during low overall chlorophyll *a* biomass in summer. Measurements from both surveys, in Southampton Water and in the North Sea, showed that, in general, the contribution of the small phytoplankton decreased as overall phytoplankton concentration increased and this is a recurrent pattern observable in small and large scale spatial gradients as well as in temporal cycles (Throndsen, 1978; Raimbault *et al.*, 1988; Chavez, 1989; Jochem, 1989; and others). In accordance with this observation the percentage contribution to total chlorophyll *a* concentration in two measurements undertaken during the cruise in March showed similar values to those obtained in June/July, reflecting the similarity in the overall phytoplankton community chlorophyll *a* concentration determined during the two cruises. A comparison with values in Table 1.2.1 illustrates the decrease in the significance of the small phytoplankton from tropical and subtropical oceanic waters to temperate neritic waters.

1.5.2.2. Primary production rates

Estimates of daily rates of carbon uptake integrated for the mixed layer (mean 212mgC $m^{-2}d^{-1}$) appear to be substantially lower than the primary production rates determined also from ^{14}C measurements by Gieskes and Kraay (1984) in July in the area of the Oyster Ground (Central North Sea): 1057mgC $m^{-2}d^{-1}$. Gross primary production rates determined from oxygen evolution measurements in the present study yielded an average value of 469mgC $m^{-2}d^{-1}$ (using a conventional PQ of 1.25 for growth on ammonia (Davies and Williams, 1984). This order of discrepancy between the ^{14}C and oxygen methods are not uncommon and results are discussed in detail in relation to the PQ in section

1.5.2.4. Despite the discrepancy, the oxygen data are useful in that they provide an additional evidence that primary production rate in the present study was not as high as rates estimated by Gieskes and Kraay (1984) in the Oyster Ground. In waters around Denmark, Steemann Nielsen (1964) estimated mean carbon uptake rates of 297 and 258mgC $m^{-2}d^{-1}$ in summer in the areas of the Great Belt and the Kattegat respectively. Lancelot and Billen (1984) reported a mean carbon production rate of 584mgC $m^{-2}d^{-1}$ in Belgian coastal waters from measurements in June.

The percentage contribution by the <3μm fraction to overall carbon fixation rate was estimated to be about half (9.8%) of the contribution to total chlorophyll *a* biomass during the June/July survey. There are other reports in which the <1μm (Legendre *et al.*, 1987; Magazzú *et al.*, 1987) and the <3μm size fractions (Herbland and LeBouteiller, 1981; Weber and El Sayed, 1987) have been reported to make a substantially higher contribution to the chlorophyll standing stock than to primary production, thus resulting in lower photosynthetic parameters, i.e. initial slope of the P *v* I curve normalized to chlorophyll *a* (α_B) and assimilation number (P^m_B). Other results in the North Sea (Howard and Joint, 1989) and elsewhere (see Table 1.5.2 in section 1.5.1.7) have shown the <1μm size class to have higher α_B and P^m_B. However, as discussed in section 1.5.1.7 for the <3μm (the present study in Southampton Water) and the 1-5μm size fraction (Joint and Pomroy, 1986) these parameters were not very different from the >3 and >5μm fractions respectively. Thus the small nanoplankton and the picoplankton can show different patterns. Herbland and LeBouteiller (1981) suggested from their data that the <3μm size fraction would be mainly constituted by inactive photosynthetic organisms or particles of detritus. However, in contrast to results obtained by Herbland *et al.* (1985) in the Equatorial Atlantic and in the present study in Southampton Water, the percentage phaeopigment was not higher for the <3μm fraction in samples from the North Sea. Thus there was no indication that detrital particles were more numerous in the <3μm fraction.

A source of discrepancy between the chlorophyll *a* and carbon uptake data in the present study may be the difference in the filter types used in June/July. As discussed in a previous section,

cellulose-ester filters are reported to retain particles much smaller than their stated pore size (Sheldon, 1972) and may have retained phytoplankton of smaller size than the Nuclepore filters. Unfortunately appropiate microscopic checks in the 3μm cellulose-ester filter and the 3μm Nuclepore filter filtrates were not performed. However, in the few cases in which Nuclepore filters were used for ^{14}C fractionation the contribution to community primary production was similar or higher than to total chlorophyll *a* standing stock and in the few measurements that were carried out in March when Nuclepores were used for both fractionations, the contribution to carbon uptake rate was higher than to chlorophyll *a*. Thus rates of fractionated carbon uptake must be viewed in the context of the the filter type used in each case. It is unfortunate that these measurements had to be done on existing stocks of filters in the department due to budget limitations and that consistency in the use of Nuclepore filters throughout the study could not be maintained. Due to this uncertainty no attempt has been made to normalize the primary production rates to chlorophyll *a* concentration in data from the June/July 1989 research cruise.

A comparison of values of initial slope of the P *v* I curve by using dark incubated values and zero times as blanks showed the former procedure yielded estimates ≥90% of the latter on five occasions out of eight, when the parameter adopted values higher than 0.85×10^{-4} μmolC $l^{-1}h^{-1}(\mu Em^{-2}s^{-1})^{-1}$. At lower values discrepancies were around 20-25% on two occassions. In one case, experiment 10, the difference was around 50%. Thus in most cases the adoption of the dark or zerotime blanks did not appear to make a great difference in the primary production rate estimates.

At the depth of sampling concentrations of inorganic nitrogen, phosphate and silicate were below levels suggested as critical points for growth limitation (Fisher *et al.*, 1988). In accordance with this observation light saturated rates of primary production in the >3μm fraction appeared to be correlated ($p<0.01$) with a composite parameter N x chl, where N is the ammonia concentration (μM) and chl is the chlorophyll *a* concentration (mg m^{-3}), suggesting phytoplankton in this size range were likely to be nutrient limited. This was not apparent for the <3μm fraction, which would indicate a better adaption of

smaller phytoplankton to growth under low nutrient concentrations as seemed to suggest also the data from Southampton Water. However, in the absence of ancillary evidence, this remains as a hypothesis.

1.5.2.3. Respiration rate

Rates of respiration measured in whole samples (i.e. without pre-screening) are regarded as plankton community respiration rates. However, in practice, there is an upper limit to the size of planktonic organism studied, mainly imposed by the small volume of the incubation bottle (60 and 125ml), in relation to the low density of the larger forms of plankton, i.e. mainly macrozooplankton.

Plankton community rates of respiration measured during June/July in the southern and central North Sea (mean 0.2μmolO_2 $l^{-1}h^{-1}$) are in good agreement with rates determined in other studies in the area of the Oyster Ground (Central North Sea) during July (Baars and Fransz, 1984; Gieskes and Kraay, 1984) and fall within the general range of respiration rates reported for temperate coastal and shelf waters, as reviewed by Williams (1984).

Mixed layer integrated daily rates of respiration exceeded those of gross primary production, i.e. net primary production was negative, at all sampling locations except for one sampling site where the mixed layer was very shallow. Except in two locations the depth of the euphotic zone was shallower than the depth of the surface mixed layer, so the respiration/production ratio for the entire water column can be assumed to be even higher. Similar situations have been reported in other studies in the southern and central North Sea during summer (Joiris *et al.*, 1982; Gieskes and Kraay, 1984). Tijssen and Wetsteyn (1984) from measurements of *in situ* variations in oxygen concentration estimated a positive net production from May to September of 0.26gC $m^{-2}d^{-1}$. Discrepancies between estimates of oxygen production from incubated and non-incubated techniques have been reported (Tijssen, 1979; Gieskes *et al.*, 1979; Daneri, unpublished data), which may be attributable to biases associated with sampling different water masses and/or oxygen exchanges between the sea and the atmosphere during sampling, when following a particular water mass for *in situ* measurements, and/or containment effects (Venrick *et al.*,

1977) associated with bottle techniques.

Size fractionated respiration rate estimates suggested around 80% of the plankton community rates of respiration could be attributed to organisms passing through a 3μm filter. Rates of respiration in pre-screened samples should be taken with some caution, since changes in incubation conditions following screening have been reported to enhance rates of respiration in filtrates (Salonen and Kononen, 1984; Hopkinson *et al.*, 1989). Other studies, however, have not shown this effect (Williams, 1981; Harrison, 1986). The difference in the contribution to chlorophyll *a* biomass (mean 11.4(SD 4.7)%) and to the rate of respiration (mean 83.2(SD 12.5)%) by the <3μm fraction and the absence of a significant correlation between respiration rate and chlorophyll *a* concentration suggests that small heterotrophic organisms (possibly bacteria and microflagellates) were the major contributors to the plankton community respiration rate in the present study (Iriarte *et al.*, 1991). Werf and Niewland (1984), in contrast, reported from ETS activity measurements, that the fraction smaller than 50μm was responsible for a small proportion of the plankton community rates of respiration in the Oyster Ground in July. Baars and Fransz (1984), from measurements in concentrated zooplankton samples, however, estimated zooplankton respiration to account for 16% of the respiration rate measured in whole, non-manipulated samples in the same area in July, even though summer is reported as the period of highest grazing activity by zooplankton (Fransz and Gieskes, 1984). In contrast to Steele's (1974) model, zooplankton respiration is often reported as a minor component of plankton community rates of respiration (Andersen and Jacobsen, 1979; Baars and Fransz, 1984; Bell and Kuparinen, 1984; Harrison *et al.*, 1987; Williams, 1981) and size fractionated respiration measurements suggest microheterotrophs as major planktonic oxygen consumers (Williams, 1981; Kuparinen, 1984; Harrison, 1986; Schwaerter *et al.*, 1988), although at exceptionally high algal biomass concentrations, i.e. >5μgchl a l^{-1}, autotrophic respiration may dominate (Packard, 1979; Kuparinen, 1984; Schwaerter *et al.*, 1988; Iriarte *et al.*, 1991). These results are in agreement with current views that the microheterotrophs are major consumers of photosynthetically fixed organic carbon (Azam *et al.*, 1983; Linley *et al.*, 1983) and highlight the importance of a microbial loop in the

trophodynamics of the pelagic ecosystem (Williams, 1981).

A comparison of data for gross primary production and respiration rates in an area of the southern North Sea from March to October 1989 including the March and June/July data from the present study (Purdie *et al.*, in prep.) suggested highest rates of primary production to occur in spring, but rates of respiration to be higher during summer. It has already been pointed out that in the North Sea an imbalance occurs between the peaks in primary production and grazing by herbivorous zooplankton caused by the temporal decoupling between the maximum rate of activity of phytoplankton and zooplankton (Fransz and Gieskes, 1984). If the size fractionated results from the present study are valid, the decoupling would also apply to the microheterotrophic activity, suggesting that most of the spring primary production sediments to the bottom or is exported from the southern North Sea, unlike other hypothesis suggesting that the spring bloom production would be largely consumed within the microbial loop (Lancelot and Billen, 1984). The high percentage oxygen saturation values in surface waters during spring also suggests that the organic carbon derived from the spring phytoplankton bloom is not largely consumed in spring (Purdie *et al.*, unpublished data).

1.5.2.4. Photosynthetic quotients

Based on the stoichiometry of the photosynthetic reaction and the works of Ryther (1956), the molar ratio of oxygen produced to carbon dioxide assimilated (PQ) has conventionally been assumed to range between 1.1 and 1.35, average of 1.25. Most of the empirical values of PQ have been obtained by comparison of oxygen production measurements using either the Winkler assay or electrochemical sensors and the ^{14}C derived CO_2 fixation measurements. The disagreements between the O_2 and the ^{14}C approaches have rarely been found to be explainable in terms of this conventional PQ (Peterson, 1980; Williams and Robertson, 1991; see also Table 2.4.1). In the past the discrepancies have been attributed amongst other factors to a number of possible analytical biases associated with the ^{14}C technique, such as contamination of ^{14}C stocks with trace metals (Carpenter and Lively, 1980). Nowadays, however, recommendations for trace metal

clean techniques appear to be implementd in most laboratories. A major and still unresolved problem is the difficulty of interpretation of what is measured by each of the two approaches. There is still great uncertainty as to whether the ^{14}C technique measures gross or net primary production or something in between. This will greatly depend on the duration of the experimental incubation and the relative composition of the trophic components of the sample. Strickland (1960) considered that during incubations of a few hours the loss of ^{14}C in respiration was negligible. The work of Hobson *et al.* (1976) suggested that approximately 30 hours was required before isotopic equilibrium of ^{14}C:^{12}C was reached. More recently, however, Harris and Piccinin (1983) have observed that with incubations of 15 minutes ^{14}C uptake was equivalent to gross photosynthesis but only at maximum growth rates. At low growth rates ^{14}C uptake was close to net photosynthesis, suggesting a more readily transference of ^{14}C from photosynthetic to respiratory pathways at these low growth rates.

Williams *et al.* (1979) suggested that the gross/net problem could only account for about 30% of the discrepancy. However, rates of algal respiration can sometimes account for a large proportion of photosynthesis (>50%) (Humphrey, 1975; Falkowski and Owens, 1978). Also, although the majority of the data on rates of extracellular release of carbon by algae derived from ^{14}C measurements suggest excretion to be a minor (≤10%) component of total inorganic carbon uptake (Williams and Yentsch, 1976; Mague *et al.*, 1980; Falkowski *et al.*, 1985; Joint and Pomroy, 1981; Marlow *et* al., 1989; Zlotnik and Dubinsky, 1989), there are also reports of higher rates, i.e. 50% (see Fogg, 1983 and references therein). Furthermore, studies on the transference of photoassimilated carbon to bacteria generally imply higher rates of extracellular release of carbon by phytoplankton, around 40% (Cole *et al.*, 1982; Brock and Clyne, 1984), and most of the material incorporated by bacteria is subsequently respired (60%) (Payne, 1970).

With the light and dark oxygen technique there are basically two difficulties : a) the failure to account for the rates of photorespiration, leading to an underestimation of gross photosynthesis and b) the uncertainty of whether the rates of dark respiration measured in the dark are comparable to the rates of dark

respiration in the light. In contrast to most of the earlier investigations, in which dark respiration seemed to be inhibited in the light (see Burris, 1980 and references cited), more recent studies suggest that dark respiration is little or not affected in the light (Falkowski and Owens, 1978; Grande *et al.*, 1989a; Grande *et al.*, 1989b; Bender *et al.*, 1987) or substantially increased in the light (Stone and Ganf, 1981; Lancelot and Mathot, 1985; Bender *et al.*, 1987)

It has been shown that the source of inorganic nitrogen used by the algae can affect the PQ (Cramer and Myers, 1948; Williams *et al.*, 1979). Williams *et al.*(1979) proposed to separate the PQ into two components, the "carbon PQ", which is dependent on the ratio of glucids, lipids, proteins and nucleic acids produced and is considered to range between 1.1 and 1.35, average of 1.25 (Ryther, 1956) and the "nitrogen PQ" which is determined by the state of reduction of the inorganic nitrogen source utilized. When using nitrate, the reduction of this molecule will yield two extra molecules of oxygen per atom of nitrogen incorporated. Thus, for algae growing on nitrate, the PQ will greatly depend on the carbon to nitrogen assimilation ratio.

It has been suggested (Davies and Williams, 1984) that an overall PQ can be calculated from

$$PQ=PQ_c + 2/(C:N)$$

where

PQ_c stands for the "carbon PQ" (*ca.* 1.25)

C:N is the molar assimilation ratio of carbon to NO_3-nitrogen

From this expression, for values of C:N close to 3:1 overall PQ's in the region of 2 can be expected. A number of recent works that have made the allowance for the "nitrogen PQ" seem to suggest that ^{14}C uptake rates are in good agreement with oxygen production rates as measured with the light and dark bottle technique (Williams *et al.*, 1983; Sakamoto *et al.*, 1984; Bell and Kuparinen, 1984; Platt *et al.*, 1987). Other workers claim there is a good approximation, around 30% deviation (Grande *et al.*, 1989b; Bender *et al.*, 1987). Others, however, report values of apparent PQ that cannot be explained in terms of the nitrogen PQ alone, reaching values well above 2-3 (Table

Table 1.5.3. Summary of apparent PQ's in natural plankton assemblages and monoalgal laboratory cultures.

Location	Apparent PQ	Reference
Mixed plankton population		
Plastic spheres	1.40-3.30	McAllister *et al.* (1961)
Southampton Water (UK)	2.01	Bryan (1979)
Lake Frederiksborg Slotssø (Denmark)	<1.0-4.00*	Andersen and Sand Jensen (1980)
Coastal waters off Hawaii	0.92-1.66	Williams *et al.* (1983)
Lake Erken (Sweden)	1.47-1.75	Bell and Kuparinen (1984)
English Channel	1.60-4.50	Holligan *et al.* (1984)
Lake Constanz (Switzerland)	0.60-1.98	Sakamoto *et al.* (1984)
Coast of Finland	0.60-2.40*	Kuparinen (1985)
Lake Kinneret (Israel)	1.00-5.00*	Megard *et al.* (1985)
Experimental tanks	ca. 1	Bender *et al.* (1987)
Canadian Arctic	1.30-1.80	Platt *et al.* (1987)
Lakes Huron and Michigan (Canada)	1.58-4.90	Fahnenstiel and Carrick (1988)
English Channel	1.20-3.70	Garcia (1989)
Bedford Basin	0.95-3.20	Grande *et al.* (1989b)
Monoalgal cultures		
A range of species	0.90-1.90	Eppley and Sloan (1965)
Dunaliella sp.	1.10-2.25	Williams *et al.* (1979)
Selenastrum capricornutum	1.14-8.00	Andersen and Sand Jensen (1980)
Pavlova lutheri and *Glenodinium sp.*	1.00-1.80	Burris (1981)

* : derived from graph

1.5.3). Laws (1991), however, after a detailed examination of the biochemical composition of phytoplankton and the chemical equations for the production of the phytoplankton cell constituents, has recently suggested that, for growth on nitrate the PQ lies around 1.4, and for growth on ammonium the PQ takes the value of 1.1.

Holligan *et al.* (1984) found that the apparent PQ's were 35 to 50% higher than derived from the above considerations of the nitrogen source and suggested that some other biochemical explanation may be responsible for the discrepancy. There are indications of increased PQ's to levels as high as 5 to 8 at low photon flux densities

(Andersen and Sand Jensen, 1980; Megard *et al.*, 1985; Fahnenstiel and Carrick, 1988). A higher affinity of enzymes for reduction of nitrate to ammonium than the carboxylating enzymes for the photoreductants at low irradiances (Kessler, 1959; Hatori, 1962; Priscu, 1984) has been suggested as an explanation (Megard *et al.*, 1985). However C:N assimilation ratios estimated by Megard *et al.* (1985) were not as low as would have been expected from these considerations. Andersen and Sand Jensen (1980) made no account for the possible influence of nitrate reduction and argued that at low photon flux densities $^{14}CO_2$ fixation would underestimate gross photosynthesis due to assimilation of respired $^{12}CO_2$ in preference to the dissolved ^{14}C-bicarbonate.

The production of "unconventional metabolites" can yield elevated PQ's, e.g. methane 2, dimethylsulphide 2.75, methylamine growing on nitrate 3.5 (Williams and Robertson, 1991). But these compounds are not currently considered to be major products of algal metabolism, and thus cannot provide an explanation for the observed high PQ's. However, metabolic processes in algae have yet to be more thoroughly investigated for a definite rejection of this alternative. Methane metabolism in the sea, for example, is now believed to be more important than previously considered (Sieburth, 1987). Laws (1991), however, argues that the biochemical composition of phytoplankton is suggestive of PQ's in the range 1.1-1.4.

Other hypothetical explanations of elevated values of PQ discussed by Williams and Robertson (1991) include a decoupling of nitrate reduction and carbon dioxide fixation. This would lead to an accumulation of ammonia or nitrite, but the accumulation of ammonia would eventually result in a suppression of nitrate assimilation due to the preferential use of ammonia instead of nitrate (McCarthy *et al.*, 1974; Paasche and Kristiansen, 1982) and large accumulations of nitrite have not been observed.

Also, oxygen liberated from photochemical reactions unrelated to carbon synthesis (Laane *et al,* 1985) may be quantitatively more important than currently believed.

PQ results from the present measurements in the North Sea showed that in general terms gross oxygen production rates and carbon uptake rates were reasonably well correlated (r^2= 0.81, n=42, $p<0.001$). Data in the literature tend to confirm this observation

(Bryan, 1979; Williams *et al.*, 1979; Holligan *et al.*, 1984; Bell and Kuparinen, 1984; Kuparinen, 1985; Williams and Purdie, 1991).

From the nutrient data it can be seen that ammonium was in excess to nitrate (2 to 3 times) in experiments 2, 5, 6 and 10. The concentrations of both nitrogenous compounds were roughly similar in experiment 4 and nitrate was more abundant than ammonium in experiment 3. Thus it seems likely that phytoplankton was growing primarily on ammonium in all experiments but perhaps in experiment 3.

From the theoretical considerations of the effect of the inorganic nitrogen source alone (Williams *et al.*, 1979; Davies and Williams, 1984) a value of PQ close to 1.25 (or a value close to 1.1 according to Laws, 1991) should have been expected in those experiments where phytoplankton was growing on ammonium. Kuparinen (1985) from a productivity study in the coast of Finland on a seasonal basis also failed to observe a close relationship between apparent PQ's and nitrate/ammonium levels. Highest apparent PQ's were found in summer, when phytoplankton was growing on ammonium, coinciding with an enhanced heterotrophic activity, Holligan *et al.* (1984) in a study in the English Channel during summer also failed to explain their apparent PQ's solely in terms of the oxidation state of inorganic nitrogen assimilated.

The effect of irradiance in the present study appeared to conform with the general view that at low photon flux densities PQ's tend to be higher (Megard *et al.*, 1985; Holligan *et al.*, 1984; Fahnenstiel and Carrick, 1988; Andersen and Sand Jensen, 1980). If in most experiments phytoplankton was growing primarily on ammonium, it is unlikely that explanations of a preferential reduction of nitrate instead of CO_2 at low light (Megard *et al.*, 1985; Fahnenstiel and Carrick, 1988) would apply. An underestimation of carbon uptake due to a preferential uptake of respired $^{12}CO_2$ in relation to dissolved ^{14}C-bicarbonate (Andersen and Sand Jensen, 1980) may have contributed to the apparent enhancement of the PQ's at low light. Another possible explanation could be that at low growth rates the ratio of respired ^{14}C:^{12}C is increased (Harris and Piccinin, 1983), which is somewhat in contrast with the previous hypothesis. However apparent PQ's were as high as 3 at 100% incident irradiance levels.

Photoinhibition occurred in most experiments to some extent and

this was aprent both in the oxygen and carbon *P v I* curves . Thus it seems that the decline in photosynthetic rate was largely due to photodestructive processes rather than a photorespiration derived effect. Failure to account for the rates of photorespiration with the oxygen technique does not seem to have had an appreciable effect on the PQ in the present study.

Apparent PQ's were calculated without including the extracellularly released organic carbon. Excretion seemed to be higher, as a proportion of total carbon uptake, (20%) at the lowest light levels (5.2% incident irradiance). Overall, as measured with the ^{14}C technique, excretion was estimated to account for about 10% of the carbon uptake. However, if there is a substantial uptake of exudated organic carbon by bacteria, the rates of excretion, as measured with the ^{14}C technique, can be largely underestimated (Cole *et al.*, 1982). This would not greatly affect estimates of gross carbon uptake, since bacteria would be retained in the 0.2μm filters, unless bacterial respiration of photosynthetically fixed carbon was significant.

The rates of respiration were high in relation to the rates of photosynthesis as indicated from the ratio of respiration to light saturated rate of photosynthesis : 19.4 to 91.6 (average of 46.5). If all the measured respiration was based on the consumption of photosynthetically fixed carbon, under isotopic equilibrium conditions, gross carbon uptake could be underestimated by *ca.* 50% (much higher at lower than saturating irradiance levels). From size-fractionated respiration rate measurements it was estimated that 70 to 100% of the respiration rates were attributable to organisms passing 3μm filters. In terms of chlorophyll *a* the contribution of the smaller than 3μm fraction was only 19.6%. Assuming that rates of respiration per unit of chlorophyll *a* are roughly the same for phytoplankton greater and smaller than 3μm and that respiration in the larger than 3μm fraction is mostly algal, the proportional contribution of autotrophs and heterotrophs to the overall rate of respiration in the smaller than 3μm can be estimated (Bell and Kuparinen, 1984). On average 79% of the community respiration was attributable to the microheterotrophs. The question is how much of this respiration involved oxidation of photosynthetically derived radio-labelled organic carbon. An important source of organic ^{14}C

available to the microheterotrophs is the pool of extracellularly released organic carbon compounds (EOC) by the algae (Iturriaga, 1981). Estimates of EOC taken up by bacteria range from 40% (Cole *et al.*, 1982; Moller Jensen, 1983) to 90-100% (Wiebe and Smith, 1977). Data derived from measurements of the amount of radio-labelled organic carbon in the dissolved phase i.e. the filtrate, suggest exudation to be a minor proportion of the carbon uptake rate in phytoplankton, ≤ 10% (Williams and Yentsch, 1976; Mague *et al.*, 1980; Falkowski *et al.*, 1985; Joint and Pomroy, 1981; Zlotnik and Dubinsky, 1989), although rates as high as 40% have been reported (see Fogg, 1983; and references therein). However, estimates of excretion rate that have taken into account the proportion of EOC transferred to bacteria are in general higher. Larsson and Hagstrom (1982) determined excretion to be 16% of carbon uptake rate in a eutrophicated Baltic inlet. Moller Jensen (1983) found excretion to account for 8 to 35 % in a Danish estuary. Brock and Clyne (1984) estimated that an annual mean of 41% of the photosynthetically fixed carbon by phytoplankton was released as EOC in Lake Mendota (USA). Cole *et al.* (1982) gave estimates of 45% of true exudation rate in Mirror Lake (USA).

An alternative pathway of transference of photoassimilated carbon to bacteria is DOM released as a byproduct of zooplankton feeding , either from the sloppy feeding (Furhman *et al.*, 1980) or diffusion from faecal pellets (Jumars *et al.*, 1989). It is estimated that 20 to 50% of photosynthetic carbon can reach the bacteria this way (Jumars *et al.*, 1989). With the ^{14}C technique it is not possible to distinguish between the $DO^{14}C$ originated from these processes and the $DO^{14}C$ leaked from active phytoplankton, i.e. excretion. The time course for the ^{14}C to appear in the faecal pellets and then be taken up by bacteria or microflagellates and respired is probably too long to account for a significant underestimation of gross carbon uptake by the ^{14}C method. Information on zooplankton standing stock and or activity is lacking from the present study to evaluate the possible effect of the sloppy feeding. Baars and Fransz (1984) estimated that calanoid copepods comprised 80% of the total biomass of herbivorous zooplankton in the Oyster Grounds in the central North Sea in a study conducted in 1981. As discussed in a previous section, in July phytoplankton was composed mainly by the dinoflagellate *Ceratium* and

small (<3μm) microflagellates (Gieskes and Kraay, 1984). *Ceratium* was also observed to be abundant in samples collected during the present study. Baars and Franz (1984) argue that this food spectrum is not probably adequuate for copepods. These authors further estimated that zooplankton did not make a major contribution to community respiration rates.

Bacteria have been shown to have much lower rates of net production than phytoplankton. Moller Jensen (1983) estimated bacteria to respire 20 to 50% of incorporated carbon. Payne (1970) gave an average estimate of 60% and Iturriaga (1981) determined that 95% of the low molecular weight products taken up by bacteria are respired.

If we assume a rate of excretion of 40% of gross carbon uptake in the present study, about 30% should have been passed to bacteria, since the apparent excretion rate was only 10%. This would imply that 75% of EOC was transferred to bacteria, a percentage within the expected range (Wiebe and Smith, 1977). A rate of bacterial respiration of 50% of the carbon taken up would result in estimates of 15% of primary production consumed in respiration by bacteria. Assuming a lower rate of transference of EOC to bacteria, such as 40% (Moller Jensen, 1983), would mean that only 7% of primary production was transferred to bacteria and bacterial respiration of EOC would account for 3.5% of gross photosynthetic carbon uptake. This means that an amount of carbon equal to 25-36% of primary production, consumed by the microheterotrophs is of no direct active photosynthetic origin. Adding up the proportions of gross carbon uptake respired by the autotrophs, which was estimated to be at least 10%, by bacteria, which from the above considerations can account for 3.5 to 15%, and the proportion excreted i.e. 10%, a total of 23.5 to 35% of carbon incorporation could have been underestimated with the ^{14}carbon technique. A figure of around 30% is commonly assumed to be a maximum of underestimation due to respiratory and excretory losses (Williams *et al.*, 1979). If excretion rate or herbivory activity was not so high, underestimations due to bacterial respiration losses would obviously be even less important.

If underestimation of carbon uptake due to respiration accounted for an important proportion of the discrepancy between the oxygen production and carbon uptake rates, a correlation between

R/P_{max} and PQ should be expected. However, this was not evident in the current work. Nevertheless, it was noted that PQ's were better correlated with incubation time . The longer the incubation time, the greater the pool of labelled carbon available to bacteria and phytoplankters themselves, and thus the higher the ratio of $^{14}CO_2$:$^{12}CO_2$ respired. A significant correlation ($r=0.918$, $n=6$, $p<0.01$) was found between PQ's and a composite parameter of $0.165t + 0.55n$, where **t** is the incubation time and **n** is the ratio of the ambient concentrations of nitrate and ammonium (Figure 5.1.1). The coefficients of the composite parameter were estimated as follows: 0.165 is the slope of the regression of PQ *versus* incubation time (hours) for experiments where n was similar; 0.55 is the slope of the regression line of PQ *versus* the nitrate to ammonium concentration ratio ($\mu M:\mu M$) for experiments of similar duration respectively.

Although the quantitative approach to the analysis of possible factors affecting the PQ can be regarded in many instances as rather crude and somewhat speculative, it is clear that the respiratory activity of the microheterotrophic population needs to be taken into consideration in the present study and ought to be borne in mind in studies under similar conditions of enhanced microheterotrophic activity. However, respiratory and excretory carbon losses could only account for 35% of the discrepancy between the two techniques. In those experiments in which deviations were of the order of up to 60%, the remaining 25% has to be explained from considerations of algal metabolism that are probably as yet poorly understood (Williams and Robertson, 1991) or others related to the principle of the techniques. These results are useful in terms of confirming that discrepancies between the oxygen and ^{14}C technique can be large and that the use of ^{14}C data may not be advisable to obtain real values of PQ needed to convert oxygen results to carbon flow rates.

1.5.2.5.Comparison of excretion rates in the North Sea and in Southampton Water

The quantification of the rates of extracellular release of organic matter in phytoplankton has always suffered from analytical difficulties (Williams, 1990). Identified methodological errors

include incomplete removal of unassimilated inorganic ^{14}C, contamination of ^{14}C-bicarbonate stocks with ^{14}C-labelled organic compounds, rupture of cells during filtration (Sharp, 1977). Appropiate checks and adequate control of vacuum help alleviate these problems (Mague *et al.*, 1980). A major difficulty with the standard method, however, is the problem of low radioactivity, i.e. estimates are based on counts that are usually not greatly in excess of background counts, thus resulting in a low precision technique (McKinley *et al.*, 1976). This was apparent in the present study, where at times background dpm were higher than sample dpm, yielding meaningless negative values of excretion.

Despite these difficulties and the discrepancies in the magnitude of the estimates (Sharp, 1977; Fogg, 1983), it is now generally accepted that the extracellular release of organic matter is a normal function of healthy phytoplankton cells (Mague *et al.*, 1980; Fogg, 1983; Bjørnsen, 1988). Fogg (1983) argued that except as a mechanism of overflow when the carbon fixation rate exceeds the rate of incorporation into new cell material, excretion has no biological advantage to phytoplankton cells. Bjørnsen (1988) argues, however, that exudation in unstressed cells can be explained as a passive diffusion process, rather than an active release.

The majority of data from laboratory culture studies suggests that excretion represents a relatively minor loss (≤10% of total carbon fixation) in healthy, unstressed phytoplankton (Sharp, 1977; Mague *et al.*, 1980; Falkowski *et al.*, 1985; Marlow *et al.*, 1989; Zlotnik and Dubinsky, 1989), increasing under conditions of extreme light and temperature that tend to depress photosynthesis (Belay and Fogg, 1978; Verity, 1982c; Zlotnik and Dubinsky, 1989) and upon brusque changes in growth conditions that can result in cultural shock (Sharp, 1977).

Data from studies in the natural environment show PER (percentage of extracellular release) to be variable, with values up to 50% in oligotrophic waters and at high irradiances near the water surface and at the bottom of the euphotic zone (see review by Fogg, 1983). Other results, however, suggest no substantial differences of PER between oligotrophic and eutrophic waters (Larsson and Hagstrom, 1982; Lancelot, 1983).

Results from studies on the transference of photosynthetically produced organic carbon to bacteria generally imply higher rates of extracellular release by phytoplankton, evidencing potential underestimations with the ^{14}C technique (Lancelot, 1979). These estimates suggest true algal exudation rates of around 40% (Cole *et al.*, 1982; Brock and Clyne, 1984).

Data obtained in the present study in the North Sea during June and July agree with the generalized estimates of excretion accounting only for around 10% of the total carbon fixation (Williams and Yentsch, 1976; Mague *et al.*, 1980; Joint and Pomroy, 1981), with values of PER raising at the lowest light level, primarily as a result of a decrease in the rate of particulate production, rather than as a result of appreciable increases in the absolute rate of dissolved production. Estimates for the North Sea, except during *Phaeocystis* spp. occurrences, are in the range 0-16%, as reviewed by Reid *et al.* (1990).

The elevated R/P_{max} ratios suggest, though, that in the present study heterotrophic activity was substantial in relation to primary production and the high percentage of total respiration (80%) in the 3μm filtrates, implies bacterioplankton as major contributors to the overall heterotrophic activity. Clearly a 10% excretion would be insufficient to support an 80% of respiration rate in the <3μm fraction. This means that either the bacteria were growing primarily on substrates other than algal exudates or that the rates of excretion were severely undermeasured due to the bacterial uptake. In general, extracellularly released organic carbon represents an important input to the pool of dissolved organic carbon in the marine environment (Riemann and Sondergaard, 1984) and underestimation of excretion due to bacterial uptake may be of the order of 68% (Lancelot, 1979). However, during summer, grazing by herbivorous zooplankton is at its peak in the North Sea (Fransz and Gieskes, 1984) and release of organic carbon from the sloppy feeding of zooplankton could account for some of the DOC available for bacteria.

The spring to summer survey in Southampton Water yielded a wide range of PER (1.3 to 43%) and the mean (18%) was higher than that obatined from measurements in the North Sea. No consistent trend with

primary production rate or biomass was found and the lack of precision of the technique may be partly responsible for the variability in the PER, particularly in measurements during low algal biomass periods. A large proportion of the variability could also be atributed to variations in species composition. Values of around 40% were associated with the presence of fragile organisms, such as the colonial *Phaeocystis* spp.. PER values of up to 40% in the Ems estuary (Colijn, 1983) and 80% in Belgian coastal waters (Lancelot, 1983) during blooms of *Phaeocystis* spp. have later been re-interpreted as representing largely rates of secretion into the colonial matrix (Reid *et al.*, 1990) and true excretion has been suggested to be <10%. Rupture of colonies and subsequent solubilization of the mucilaginous substances is thus primarily responsible for these large values of PER during the occurrence of this algae.

CHAPTER TWO

THE EFFECT OF LIGHT AND TEMPERATURE ON PHOTOSYNTHESIS, RESPIRATION AND GROWTH OF *Pycnococcus provasolii*

In this chapter results of the laboratory studies conducted with the picoplanktonic Chlorophyceae *Pycnococcus provasolii* Guillard are presented. The chapter is structured in the following five sections: a brief introduction to the study, a literature review of the physiological responses of phytoplankton to irradiance and temperature, description of the methodology adopted, presentation of the results and discussion of the results.

2.1. INTRODUCTION

Studies with natural phytoplankton assemblages suggest that the picophytoplankton show defined spatio-temporal distribution and productivity patterns (Li *et al.*, 1983; Platt *et al.*, 1983; Murphy and Haugen, 1985; Hag and Fogg, 1986; Odate, 1989). A need has thus been identified to study the growth response of picophytoplanktonic isolates to environmental parameters, under laboratory controlled conditions, to provide a reliable physiological basis for interpreting these biomass and productivity patterns in natural assemblages.

To date culture work on picophytoplankton has been almost exclusively devoted to studying aspects of the photosynthetic and growth response of the cyanobacterium *Synechococcus* spp. (e.g. Morris and Glover, 1981; Barlow and Alberte, 1985; Barlow and Alberte, 1987; Kana and Glibert, 1987a,b). A few investigations have studied eukaryotic members of the picoplankton in culture (e.g. Glover *et al.*, 1987). This is mainly because phycoerythrin-containing picocyanobacteria of the genus *Synechococcus* are readily recognizable under the epifluorescence microscope, due to their characteristic pigmentation and small size (*ca.* 1μm in diameter) and have now been identified as ubiquitous and important components of the picophytoplankton. For the identification of most of the eukaryotic

picophytoplankters, however, electron transmission microscopy is required, and thus most of the reports on cell counts of eukaryotic picoplankton lack taxonomic specifications. Additional difficulties are the fragility and the poor success of current fixation techniques with many of these eukaryotic picoplanktonic cells (Murphy and Haugen, 1985). For this reason there is as yet little information on how representative the particular isolates that have recently been brought into culture are of the picophytoplankton in the marine environment.

The high chlorophyll *b:a* ratios systematically found at the depth of the chlorophyll *a* maximum in open ocean environments (Jeffrey, 1976; Gieskes *et al.*, 1978; Vernet and Lorenzen, 1987) are suggestive of a relative abundance of phytoplankton containing chlorophyll *b* as accesory pigment, i.e. chlorophytes and prasinophytes. More recently, pigment analysis by high performance liquid chromatography (HPLC) used as a chemotaxonomic tool has revealed more clearly prasinophyte-like organisms as a potentially important group of the oceanic picoplankton (Hooks *et al.*, 1988).

The vertical distribution and photosynthetic pattern of picophytoplankton in stratified oligotrophic waters of the open ocean is suggestive of a capacity to grow efficiently deep in the euphotic zone, i.e. in low light environments (Li *et al.*, 1983; Platt *et al.*, 1983; Murphy and Haugen, 1985; Prézelin *et al.*, 1986). In recent years much effort has been placed on investigating the photophysiology of *Synechococcus* spp., results suggesting that the strains investigated display positive photoadaptation to low irradiance (Barlow and Alberte, 1985; Kana and Glibert, 1987a,b), but possibly also a great plasticity to adjust to a wide array of light levels (Kana and Glibert, 1987a,b). Some evidence has been put forward to suggest that in the open ocean eukaryotic picoplankton have their maxima below the peak of the picocyanobacteria (Murphy and Haugen, 1985), a pattern that has been ascribed to differences in their response to light quality; eukaryotic algae having higher photosynthetic efficiency under blue-violet and blue wavelengths than prokaryotic algae (Wood, 1985; Glover *et al.*, 1987). However, as yet little is known about the photoadaptational response of individual isolates of the oceanic eukaryotic picoplankton flora.

Picocyanobacterial abundance has also been shown to be

positively correlated with temperature, both on a latitudinal basis (Murphy and Haugen, 1985) and on a temporal basis in temperate regions (Hag and Fogg, 1986; Shapiro and Haugen, 1988; Odate, 1989). Few studies have attempted to relate temperature to eukaryotic picophytoplankton abundance. Similarly to picocyanobacteria, they have been shown to peak in late summer in Funka Bay, Japan (Odate, 1989). However, the effect of temperature on the growth physiology of picophytoplankton has not been adressed in any detail.

From this review it is clear that little is currently known of the influence that temperature and irradiance have on eukaryotic picophytoplankton physiology. It was therefore considered that original and useful information that would aid in the understanding of the spatial distribution of the picophytoplankton could be gained from investigating the growth and photosynthesis response of an oceanic picoplanktonic isolate, to acclimation at various conditions of photon flux density, photoperiod and temperature. A comparatively recently isolated green algal strain (Ω48-23), now positioned taxonomically in the species *Pycnococcus provasolii* Guillard, of the class Micromonadophyceae (Guillard *et al.*, 1991), has been claimed to be a very common picoplanktonic form in oceanic waters (Campbell *et al.*, 1989). Due to its characteristic distribution at the depth of the pycnocline and its efficient growth under blue and blue/violet light (Glover *et al.*, 1987) the organism was considered interesting to investigate particularly aspects of photophysiology. Thus the following measurements were undertaken with *P. provasolii* Guillard under laboratory controlled conditions of temperature and illumination in batch culture:

I. Measurement of growth rate, as determined from three different biomass indicators: cell number , chlorophyll and carbon at different irradiance, photoperiods and temperatures.
II. Measurement of the rates of photosynthesis of algae grown at a given combination of irradiance, photoperiod and temperature and exposed for short periods of time (3 hours) to a wide range of irradiance levels.

2.2. LITERATURE REVIEW

2.2.1. Physiological responses of phytoplankton to irradiance

Photon flux density is exponentially attenuated with depth in the water column due to absorption and scattering of light photons by the water molecules and suspended particles. Photosynthetic organisms are thus almost exclusively restricted to the euphotic zone. This is comprised by all layers in the water column in which photoautotrophic production exceeds heterotrophic consumption on the time-scale under investigation (Tett, 1990). It has classically been considered as the portion of the water column from the surface down to a depth of 1% surface incident irradiance level, however, there are now indications that it can be a deeper layer, since phytoplankton are suggested can grow at extremely low irradiances (less than $1\mu Em^{-2}s^{-1}$) (Raven, 1986). An important factor controlling the light regime that phytoplankton cells are exposed to is the hydrodynamics of the water column. In a well mixed euphotic layer cells are displaced by the turbulent motion of the water and subject to a continuously changing light field. In highly stratified waters some phytoplankton can control their relative position in the water column and are not always randomly distributed. In the latter case it is clear that adaptation to a certain light level can be an advantage. In near surface waters, where light is not limiting, the conditions appear *a priori* ideal for phytoplankton growth, although an excessively high irradiance can also cause photochemical damage to the photosynthetic apparatus (Belay and Fogg, 1978). However, other factors co-vary with light in the water column, mainly the nutrient concentration and to a lesser extent the temperature, so there may be some advantages for primary producers growing at depths of low photon flux density. Additionally, adaption to living at different depths can allow a niche segregation, reducing the competitive pressure among phytoplankters. It is not clear whether photoadaption can be important in a mixed water column (Falkowski, 1980). This will greatly depend upon the time scale of the mixing process and the time scale of the adjustments in the cell in response to the changes in the light field. Significant variations in irradiance are also experienced by species that migrate vertically in the water column, such as many dinoflagellates (Holligan, 1985) or

phototrophic ciliates (Lindholm, 1985). Environmental illumination conditions can be very variable and knowledge of the response of phytoplankton to irradiance is a vital tool in understanding their vertical distribution patterns.

All phytoplankton species have a genetically determined range of photon flux densities over which growth and photosynthesis can occur, i.e. they are genotypically adapted to a range of photon flux densities. In some species this range is narrow and there are species known to be genotypically shade adapted, e.g. *Amphidinium carterae* (Samuelsson and Richardson, 1982), or genotypically high light adapted, e.g. *Gonyaulax polyedra* (Rivkin *et al.*, 1982b). However, most phytoplankters exhibit a remarkable tolerance to wide variations in irradiance, they are able to counteract to various degrees the negative effects of sub- or supra-optimal growth irradiances by phenotypic adjustments that can involve a combination of structural, behavioral, physiological and biochemical changes. It is in the sense of phenotypic adjustments to growth under a certain level of irradiance that the term adaptation will be used in the present research.

In the early stages of the research on light-shade adaption of unicellular algae, Steemann Nielsen and Jørgensen (1968) suggested that there are essentially two types of adaptative response: The "*Chlorella*" type where a cell responds to changes in irradiance by changing its pigment content; and the "*Cyclotella*" type where a cell adapts primarily by changing the rate of the dark reactions. Evidence to date shows that phytoplankters usually undergo a combination of changes at the cellular level and these can be summarized as follows:

a) Changes in the photosynthetic apparatus

i)Pigment concentration and relative composition

One of the most commonly reported responses in marine phytoplankton to decreased photon flux density is an increase in chlorophyll *a* content, observed in all major phytoplankton classes: diatoms (Chan, 1980; Falkowski and Owens, 1980; Perry *et al.*, 1981; Verity, 1981; Falkowski *et al.*, 1985; Langdon, 1987), dinoflagellates (Chan, 1980; Rivkin *et*

al., 1982a; Falkowski *et al.*, 1985; Langdon, 1987; Coats and Harding, 1988), chlorophytes (Steemann Nielsen and Jørgensen, 1968; Senger and Fleischhacker, 1978; Falkowski and Owens, 1980; Perry *et al.*, 1981), chrysophytes (Falkowski *et al.*, 1985) and cyanobacteria (Vierling and Alberte, 1980; Raps *et al.*, 1983; Barlow and Alberte, 1985; Kana and Glibert, 1987a). A generalized pattern appears to be an exponential decrease in pigment content per cell with increasing irradiance (Steemann Nielsen and Jørgensen, 1968; Falkowski and Owens, 1980 among others). However, there appear to be significant taxonomic differences in the magnitude of this change with increases in irradiance.

Physiological adjustments have also been reported to involve changes in the ratio and content of accessory pigments. Increased cellular contents of peridinin under decreased light conditions have been measured in dinoflagellates such as *Glenodinium* spp. (Prézelin, 1976) and *Prorocentrum mariae-lebouriae* (Coats and Harding, 1988). Similar trends have been observed in certain phycobilliproteins in species of red algae and cyanobacteria. *Porphyridium cruentum* had 7.3 times as much phycoerythrin when grown at $0.68 Wm^{-2}$ ($3.4 \mu Em^{-2}s^{-1}$) as cultures grown at $11.4 Wm^{-2}$ ($57 \mu Em^{-2}s^{-1}$) (Brody and Emerson, 1959). Vierling and Alberte (1980) recorded increases in phycocyanin in low light grown *Anacystis nidulans*. Kana and Glibert (1987a) reported changes in both phycocyanin and phycoerythrin in *Synechococcus* WH7803, with phycoerythrin increasing in cells grown under, $30 \mu Em^{-2}s^{-1}$ up to 20 times the concentration of those adapted at $700 \mu Em^{-2}s^{-1}$. In addition to changes in content, Falkowski and Owens (1980) also observed disproportionate increases in chlorophyll *b* for *Dunaliella tertiolecta* and chlorophyll *c* for *Skeletonema costatum* relative to chlorophyll *a* with decreasing growth irradiance. Prézelin (1976) also observed decreases in the ratio of peridinin/chlorophyll *a* and chlorophyll *a/c* in *Glenodinium* spp. at low growth photon flux density. Total pigment content does not increase solely to provide more photosynthetic potential at low light levels. For example high irradiances can cause photooxidation of chlorophylls. Carotenoids and billiproteins are known to play a photoprotective role and increases in the cellular content of these pigments may also occur at very high irradiances (Dohler *et al.*, 1976; Ley and Butler, 1980; Barlow and Alberte, 1987).

Increased cellular pigmentation under lowered light conditions can be interpreted in terms of the photosynthetic unit (PSU) and in recent years much effort has been focused on investigating whether cells adapt by changing the size or number of PSU's. The PSU is the assemblage of pigment molecules (photosystem I, photosystem II and associated light harvesting pigments) that carry out the complete set of light reactions of photosynthesis (Prézelin, 1976). Non proportional changes in pigments associated with light harvesting components, relative to chlorophyll molecules of reaction centers, are interpreted as reflecting changes in the size of the PSU; increases in size occur with relative larger increases in the pigments of the light harvesting component. Equimolar changes in the pigments associated with the light harvesting components and the chlorophylls of the reaction centers are considered to reflect changes in the number of PSU's.

Increases in the number of PSU's with shade adaptation have been measured for the cyanobacterium *Microcystis aeruginosa* (Raps *et al.*, 1983) and the chlorophyte *Dunaliella tertiolecta* (Falkowski and Owens, 1980). Examples of phytoplankters increasing the size of the PSU's are the diatoms *Chaetoceros* spp. and *Skeletonema costatum* (Perry *et al.*, 1981) and the dinoflagellate *Glenodinium* spp. (Prézelin, 1976). There have also been reports of species suggested to encompass both, changes in the number and size of PSU's (Kana and Glibert, 1987a; Coats and Harding, 1988).

ii)Morphological changes in the photosynthetic apparatus

Little attention has been paid to ultrastructural aspects of photoadaption in marine phytoplankton. Coats and Harding (1988) have reported that, in the dinoflagellate *Prorocentrum mariae-lebouriae*, low light grown cells show reduced starch content, larger chloroplasts, higher number of thylakoids per lamella and greater photosynthetic membrane surface area than high light grown cells. The inverse relationship between changes in starch content and increased chloroplast volume and elaboration of photosynthetic structure was interpreted as storage reserves used as carbon source for production of additional photosynthetic machinery.

iii) Changes in the activity of dark enzymatic reactions

There is evidence to suggest that the carboxylating activity of the enzyme Ribulose-1,5-biscarboxylase-oxygenase (RUBISCO) decreases in low light grown cells in some phytoplankton species (Beardall and Morris, 1976; Senger and Fleischhacker, 1978; Rivkin *et al.*, 1982a). Beardall and Morris (1976) were the first to suggest that this was an adaptative response, since, under conditions of limiting growth irradiance, a decrease in the level of enzymatic machinery required for maximum utilization of saturating photon flux densities results in an energy save. In all three works quoted above, reduction in RuDP carboxylase levels was accompanied by a decrease in light saturated rates of photosynthesis (P_{max}) normalized on a cell number basis.

iv) Cellular size and elemental composition

Other responses observed in some phytoplankters involve changes in cell size and cellular carbon and nitrogen content. *Isochrysis galbana, Prorocentrum micans and Thalassiossira weisflogii* reduced their cell volume and cellular carbon content following growth at low irradiances (Falkowski *et al.*, 1985). The same trend was observed for *Skeletonema costatum, Olisthodiscus luteus and Gonyaulax tamarensis* (Langdon, 1987). Falkowski and Owens (1980) also measured decreases in cellular volume in shade adapted cells of *Skeletonema costatum and Dunaliella tertiolecta,* although they recorded higher carbon contents.

Some species have been shown to vary the relative elemental composition with growth irradiance, evidencing lower C:N ratios at low photon flux density, e.g. *Isochrysis galbana, Thalassiosira weisflogii, Skeletonema costatum, Dunaliella tertiolecta* (Falkowski and Owens, 1980), *Olisthodiscus luteus* (Langdon, 1987). In others the C:N ratio remains invariant with growth photon flux density, e.g. *Leptocylindricus danicus* (Verity, 1981), *Prorocentrum micans* (Falkowski *et al.*, 1985).

These adjustments ultimately result in a maximization of the rates of gross photosynthesis and/or minimization of the loss

processes i.e. respiration and excretion at a given photon flux density.

Much effort has been devoted to investigating adaptative changes to irradiance through the quantitative description and interpretation of the photosynthesis-irradiance curves (P v I) of algae grown at different light levels. There are basically two ways of constructing these curves and one should be aware of their different meaning.

a) Photosynthesis *versus* a range of light intensities at which the cells have been exposed for short term incubation periods (generally 3-4 hours). Adaptative responses can be inferred from a comparison of curves from algae preconditioned to different photon flux densities.

b) Photosynthetic performance, defined as the rate of photosynthesis at which the cells have been growing, *versus* growth irradiance. Photoadaption cannot be observed from these curves alone, but they are most useful for comparison with growth *versus* irradiance (μ v I) curves and for interespecific comparisons.

The general shape of the P v I curve (*sensu* (a)) is an initial almost linear segment (photoenhanced region) that bends towards the horizontal approaching saturation asymptotically. Saturation can be extended over a plateau region which sometimes is followed by a declining segment without a uniquely defined shape (photoinhibited region) (Figure 2.2.1).

The initial linear portion can be interpreted as the primary photochemical process being so slow, that the catalysts that participate in the dark (non-photochemical) reactions of photosynthesis can supply the substrates needed for, and transform the intermediates formed, by the primary process, without depletion of the former or accumulation of the latter (Rabinowitch, 1951). In some cases instead of a linear segment a sigmoid shape has been observed, which may be interpreted in terms of competition for reductant power at very low light intensities, particularly when cells are growing on nitrate (Megard *et al.*, 1985).

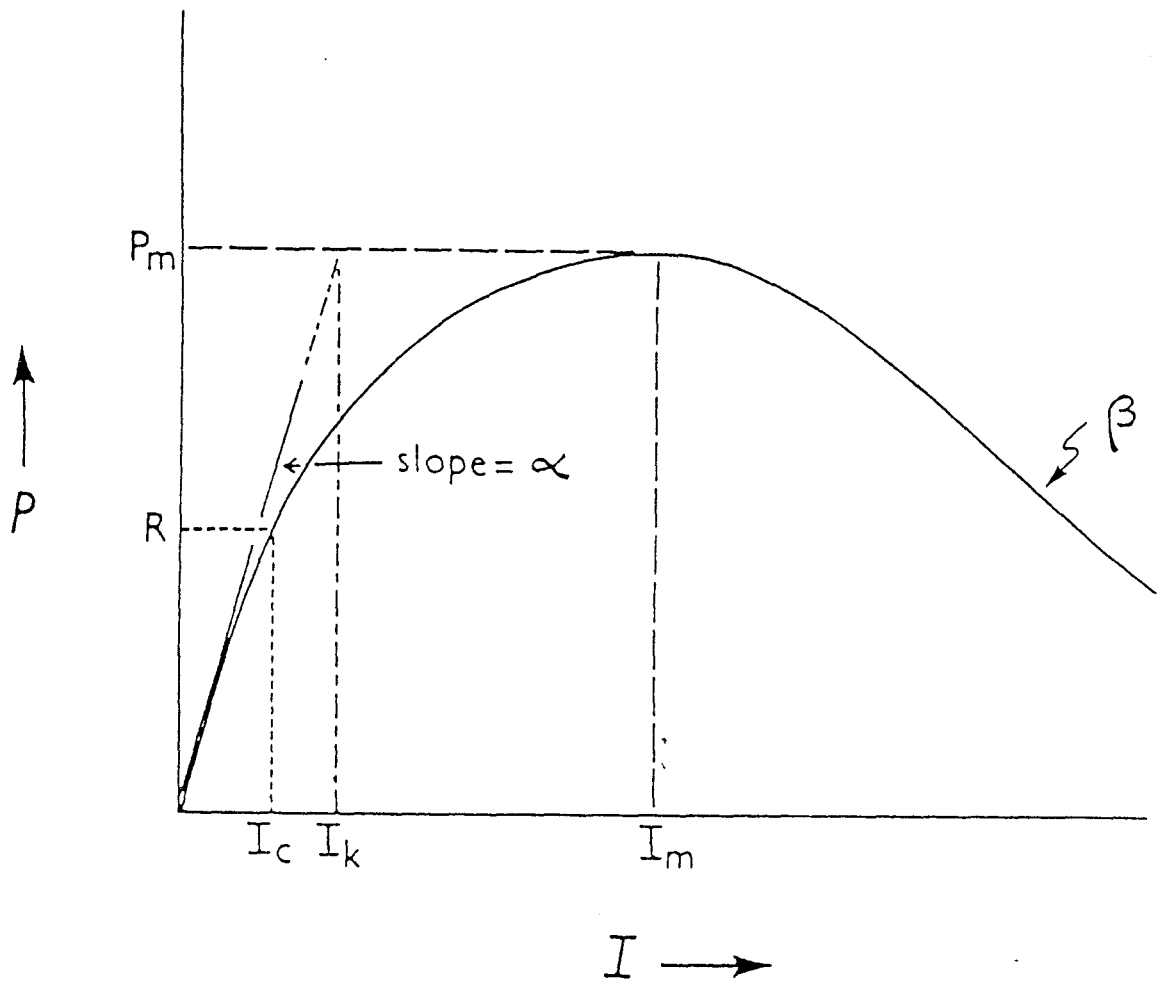

Figure 2.2.1. Schematic diagram of the photosynthesis *versus* irradiance (P *v* I) curve, showing parameters used to represent the relationship (from Figure 2.3 in Garcia, 1989).

Saturation is thought to be the result of multiple catenary processes, but possibly one of the most important limiting factors may be the speed of enzyme mediated processes in the dark reactions of photosynthesis.

Photoinhibition is caused by processes of photochemical damage to the photosynthetic apparatus. The precise mechanism of photosynthetic photoinhibition is not yet known. Some of the changes detected are inactivation of reaction centers, both photosystem I (Harvey and Bishop, 1978) and photosystem II (Samuelsson and Richardson, 1982) and morphological changes, such as chloroplast shrinkage (Kiefer, 1973). Also, photooxidative reactions through diversion of the energy of excited chlorophyll molecules into activation of unspecific oxidative reactions can be responsible for lowering the rates of photosynthesis (Belay and Fogg, 1978). It has been suggested that light damage may actually occur over the plateau region as well, but here repair mechanisms taking place simultaneously would be able to keep pace with the photodestructive processes (Platt and Gallegos, 1980). Also, it is important to note that in P v I curves derived from oxygen production measurements it is not possible to distinguish between a real photoinhibitory effect and an apparent decline in the rate of gross photosynthesis caused by photorespiration, a process that cannot be accounted for with the ordinary light and dark bottle technique.

The parameters that are generally used for a quantitative description of P v I curves are the following:

- α (alpha= initial slope): the slope of the initial linear segment of the curve.
- P_{max}: the light saturated maximum rate of photosynthesis, often called assimilation number when expressed as mgC mgchla^{-1} h^{-1}.
- β (beta): the negative slope of the curve at supra-saturating irradiance.
- I_k (saturation irradiance): the irradiance at the intersection of an extension of the initial slope with P_{max}.
- I_b: a parameter indicative of the susceptibility to photoinhibition, representing the irradiance at which the rate of photosynthesis is reduced to $0.37P_{max}$.

- I_c (compensation irradiance): the irradiance at which net photosynthetic rate equals zero.

An important point is the choice of the biomass basis on which the rates of photosynthesis are expressed. Very often chlorophyll specific rates are used, however, usually changes in cellular chlorophyll content occur between growth photon flux densities as a means of photoadaption, so a cellular basis or other biomass indicator that does not vary with growth photon flux density should be utilized to observe adaptation at the cellular level. For interspecific comparisons a cellular basis may not be the best choice due to differences in cell size and cellular carbon content. A combined analysis of the P v I curves in terms of chlorophyll with those on a cell number or other biomass basis that does not vary with growth irradiance can be useful in inferring the possible mechanisms or adjustments involved in the adaptative response, as discussed below. In natural asssemblages chlorophyll is often taken as a reliable means of estimating phytoplankton biomass, however, interpretation of light/shade adaptation from chlorophyll normalized P *v* I curves is not always straightforward, since cells can alter their pigment contents according to the incident light levels.

Steemann Nielsen and Hansen (1959) and Ryther and Menzel (1959) studied the sun/shade adaption of natural populations of phytoplankton producing P v I curves where photosynthesis was normalized to P_{max}=100. Increased values of α and lower I_k values (lower requirement for light saturation) from deep populations were interpreted as characteristics of shade adaptation. Much emphasis was placed on I_k as an indicative parameter of adaption as suggested by Talling (1957). Yentsch and Lee (1966) and later Beardall and Morris (1976), when reviewing the concept of light adaptation, have pointed out that a reduced I_k value can be the result of decreased values of P_{max} and hence may not necessarily be interpreted as an enhanced ability to utilize lower irradiances. Curves normalized to 100 do not allow this distinction and should perhaps be avoided when interpreting photoadaptational responses. An increased α value at low growth irradiance can be taken as an unequivocal indication of increased

efficiency of utilization of light at low photon flux density. Beardall and Morris (1976) concluded that from the data they had examined there was little evidence to suggest that growth at low irradiances enhanced the ability to utilize these low light levels, but the general response to growth at low irradiance was a reduced capacity to utilize saturating levels, although they themselves found that *Phaeodactylum tricornutum* cells preconditioned to low photon flux densities showed an increased value of α. Data is now available to support the contention that many phytoplankters show positive photoadaption to low irradiance, with enhanced initial slopes when grown at low photon flux densities (Richardson *et al.*, 1983; Langdon, 1988). This may sometimes be accompanied by lower rates of light saturated photosynthesis (P_{max}) and/or higher susceptibility to photoinhibition (i.e. lower values for I_b), which can be considered as a reduced ability to utilize high irradiances. A reduced ability to utilize high irradiances and an enhanced ability to utilize low light intensities, however, are not mutually exclusive characteristics.

It has been suggested that from the characteristics of the P v I curves (Prézelin and Sweeney, 1979; Richardson *et al.*, 1983) it can be inferred whether cells are changing the number or size of PSU's. Increasing the size of the PSU involves an increase in the light harvesting capacity, enhancing the flow rate of photons to the reaction centers. This results in an increase of photosynthetic efficiency at low photon flux density (α) expressed on a per cell or other biomass unit that remains invariant with growth irradiance. The same result is achieved by increasing the number of PSU's, since even if the PSU's are light limited, increasing the number of them should increase the photosynthetic output of the cell proportionately. Armond and Mooney (1978) and Prézelin and Sweeney (1979) further suggest that if the amount of O_2 per PSU remains constant, increasing the number of PSU's also results in enhanced photosynthetic capacity (P_{max}).

Expressed on a per chlorophyll basis Prézelin and Sweeney (1979) predict no differences in α nor P_{max} values for low and high light preconditioned cells when increases in the number of PSU take place, whereas for increases in the size of PSU's at low growth photon flux density they have suggested lower α and P_{max} values for low

light grown cells. The latter can be explained by the fact that losses of energy in photon transference from light harvesting pigments to reaction centers are likely to be greater in larger PSU's (Richardson *et al.*, 1983).

Literature in relation to the magnitude of the rates of excretion and respiration and the effect that environmental factors can exert on them is still sparse and controversial (Williams, 1990; Geider and Osborne, 1989), but there are some indications that growth irradiance has an influence.

Excretion rate has been suggested to increase significantly in stressed phytoplankton (Sharp, 1977). It has been reported to be enhanced at very low and very high photon flux densities (Mague *et al.*, 1980). However, the general indication from ^{14}C technique derived measurements is that, in healthy growing cells under laboratory controlled steady state conditions, excretion is a minor process (less than 10% of gross photosynthetic rate) (Sharp, 1977; Harris, 1978; Mague *et al.*, 1980; Falkowski *et al.*, 1985; Marlow *et al.*, 1989; Zlotnik and Dubinsky, 1989).

Riley's (1946) assumption that algal dark respiration rate is 10% of the maximum photosynthetic rate has been shown to be an oversimplification. Burris (1977) and Humphrey (1975) found photosynthesis to respiration ratios to range from 1 to 10 for a number of algae examined. Falkowski and Owens (1978) reported a mean value of dark respiration as 25% of gross P_{max}. There are indications that light-shade adaption can have a bearing on the dark respiration rates. For a variety of phytoplankton species rates of respiration have been observed to decline as cells become shade adapted (Falkowski and Owens, 1980; Falkowski *et al.*, 1985; Verity, 1982a; Langdon, 1987). Respiration can be viewed both as a process generating ATP, reductant power and carbon skeletons for biosynthetic purposes and also a biosynthate oxidizing (consuming) process. Thus the shade adaption to lowering rates of respiration can be seen, at least partially, as a positive adjustment under conditions in which cells have lower expectations for photosynthetic activity (i.e. lower energetic demands) and less availability for substrates for respiration. Many authors have emphasized the positive correlations

observed between growth rates and dark respiration rates (Laws and Caperon 1976; Bannister, 1979; Laws and Bannister, 1980; Verity, 1982a; Falkowski *et al.*, 1985; Langdon, 1987).

Photorespiration is the light dependent production of CO_2 and consumption of O_2 from the biosynthesis and metabolism of glycolate (Burris, 1980). O_2 and CO_2 compete for the active sites of the RUBISCO enzyme . At high partial tensions of O_2 and CO_2 the activity of the RUBISCO enzyme is channeled preferentially towards the oxygenase activity. It is a process well known and described in terrestrial plants (Tolbert, 1971). Indirect evidence suggests that photorespiration is a process occurring also in phytoplankton, but the exact mechanism and the rates at which it occurs are still unknown (Harris and Piccinin, 1977; Burris, 1980).

The result of maximizing photosynthesis and minimizing loss processes at a given irradiance is an enhancement of growth rate. For a hypothetically non adapting cell the relationship between growth rate and irradiance (μ v I) would be positive and linear, up to a point, beyond which factors other than the light energy available for photosynthesis are responsible for limiting the rate of growth. At very high light levels excess energy may result in a decrease in the rates of growth. By physiologically adapting to the high and low light levels the effect of irradiance can be reduced. The degree of adaptation shown by phytoplankton can be variable; ideally, if adaption was optimal, the μ v I relationship would be constant and maximum.

Recently interest has focused on examining which of the factors potentially affecting the growth-irradiance relationship are responsible for the interspecific differences. Falkowski *et al.* (1985) on examining the growth-irradiance relationship of three phytoplankton species suggested that interspecific differences in growth rate at a given irradiance were primarily attributable to differences in the optical absorption cross section normalized to chlorophyll *a* and differences in chlorophyll to carbon ratios. Langdon (1987), comparing three different species, found that the interspecific differences in the μ v I relationship were associated with the carbon to chlorophyll ratio at the limits as growth rate approaches zero, the respiration

rate at zero growth rate and the carbon content at maximum growth rate.

Comparing the various algal classes, Richardson *et al.* (1983) concluded that all classes except the Chlorophyceae are able to grow under very low light and, that taxonomically they can be ranked in preference to light (from lowest to highest) as Cyanophyceae< Dynophyceae<Bacillariophyceae<Chlorophyceae. Chan (1978) reported that dinoflagellates did not require higher photon flux density than diatoms to saturate growth. Langdon (1988) in his review of the subject found that dinoflagellates exhibit such a diversity of responses to irradiance that it is not possible to generalize about them. For the remaining algal classes, apart from the cyanobacteria, which have been investigated in less detail, Langdon's conclusions were similar to those of Richardson *et al.* (1983): diatoms are the best adapted to grow at low light and Chlorophyceae, Haptophyceae and Chrysophyceae are intermediate in their response to low photon flux density.

As discussed in the previous chapter, phytoplankton cell size is also a parameter influencing the photosynthetic response to irradiance. In smaller cells, the package effect of photosynthetic pigments is reduced in comparison to larger cells, thus increasing the absorption cross section normalized to chlorophyll (Kiefer and Mitchell, 1983; Kirk, 1983). This factor possibly overrides the restrictions in the volume available to house chromophores in small cells and confers them a higher efficiency of photon absorption (Raven, 1986). In laboratory controlled culture studies the photosynthetic efficiency (α) has been shown to decrease with increasing phytoplankton cell size (Taguchi, 1976; Geider *et al.*, 1986; Glover *et al.*, 1987), suggesting a competitive advantage of cells in the picoplankton size range over larger ones under light-limiting conditions.

Studies on mechanisms of photoadaptation in picophytoplankton are almost exclusively restricted to strains of the cyanobacterium *Synechococcus* sp.. Phycoerythrin-containing *Synechococcus* clone WH7803 has been shown to be able to adapt positively to low irradiance. Cells of this cyanobacterium grown at low photon flux density show enhanced

α and P_{max} per cell in comparison to cells grown at high irradiance (Barlow and Alberte, 1985; Kana and Glibert, 1987a,b). Both these studies suggested that at growth irradiances below *ca.* $100\mu Em^{-2}s^{-1}$ low light photoadaption was accomplished primarily by increasing the number of PSU's with decreasing irradiance. Barlow and Alberte's study suggested that at high growth light levels, although the number of PSU's were lower, PSU's were larger than at low growth photon flux densities. In contrast, results obtained by Kana and Glibert suggested that PSU size was rather constant and maximal in cells grown at irradiances below $100\mu Em^{-2}s^{-1}$ and they observed an increase in the size of PSU with decreasing irradiance from about 700 to $100\mu Em^{-2}s^{-1}$.

These two studies further disagreed in the photosynthetic response of this strain of *Synechococcus* at high growth irradiances. Barlow and Alberte (1985) suggested that high light (higher than $100\mu Em^{-2}s^{-1}$) grown cells showed substantially lower rates of photosynthesis at all irradiances in comparison to cells grown at low photon flux densities. Kana and Glibert (1987b) showed high light grown cells could photosynthesize at near maximal rates, showing no photoinhibition of photosynthesis, when cells had been acclimated to high irradiances by gradually increasing the photon flux density.

Little is known about the eukaryotic component of the picophytoplankton in terms of adaptative response to photon flux density. Geider and Osborne (1986) studied the effect of irradiance on the parameters of the P *v* I curve on the small marine chlorophyte *Nannochloris atomus* ($1.45 pgC\ cell^{-1}$). The authors observed a decrease in the C:chl *a* ratio with decreasing irradiance. This was accompanied by a decrease in the chlorophyll *a* absorption cross section and in α normalized to chlorophyll *a* with decreasing growth photon flux density, which the authors interpreted as an intracellular self shading of pigments associated with high cellular chlorophyll *a* contents. Photosynthetic pigment analysis at various growth irradiances have also been carried out with the oceanic chlorophyte *Pycnococcus provasolii* (clone Ω48-23). Low light grown cells showed a moderate increase in the chlorophyll *b* to *a* ratio and a slight increase in the prasinoxanthin to chlorophyll *a* ratio in comparison to those grown at high photon flux densities (Hooks *et al.*, 1988).

2.2.2. Physiological responses of phytoplankton to temperature

In the aquatic environment it is often difficult to identify the direct effect of temperature on the ecology of phytoplankton because of the co-variation of light availability. From laboratory algal culture studies, however, there is considerable data on the effect of temperature on the physiology of phytoplankton (Li, 1980; Raven and Geider, 1988; Davison, 1991).

Few studies have addressed the question of thermal adaptation of photosynthesis, that is whether the photosynthetic response of a cell at a given temperature is dependent on the temperature experienced during growth, or, in other words, whether phytoplankton growing at lower temperatures gain ability to perform better at these lower temperatures than when grown at higher temperatures. Morris and Glover (1974) argued that studies showing "positive thermoadaptation" are biased by the changes in the rates of photosynthesis during batch growth. These authors found no such evidence when they compared the rates of photosynthesis in early-mid exponential phase of *Nitzchia closterium*, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. However, although data are sparse for phytoplankton, there seem to be indications now that the photosynthesis *versus* temperature relationship depends on the growth temperature (Li, 1980; Davison, 1991). Here a brief summary on the general trends of physiological response of phytoplankton to temperature is given. Since there are not many studies with phytoplankton that have dealt with thermoadaptation and this aspect was not investigated in the present study with *Pycnococcus provasolii*, the question of thermoadaptation has not been specifically addressed.

Photosynthesis

A number of studies have shown that temperature affects the parameters of the photosynthesis *versus* irradiance relationship. Maximum rate of photosynthesis normalized to chlorophyll *a* content (i.e. assimilation number) has been shown to increase with temperature (Morgan and Kalff, 1979; Post *et al.*, 1985; Verity, 1981) up to a plateau region beyond which it starts to decline (Li, 1980; Collins and Boylen, 1982). Saturation of photosynthesis is known to be limited primarily by the rate of activity of dark reaction enzymes, which are

temperature dependent. In natural populations assimilation number does not normally reach a plateau and increases linearly or exponentially with temperature (Harrrison and Platt, 1980; Li, 1980). This is probably due to a combination of factors such as species and size specific differences in assimilation numbers and that other factors (e.g. nutrients, light) may be at sub-optimal levels. The efficiency of utilization of low photon flux densities (α) appeared to be independent of temperature for the cyanobacterium *Oscillatoria agardhii* (Post *et al.*, 1985), but increased with temperature in *Leptocylindricus danicus* (Verity, 1981), *Skeletonema costatum* (Cloern, 1979) and *Nannochloris americana* (Miller and Kamykowski, 1986). Verity (1981) argues that the former pattern conforms with the contention that the initial slope represents a rate constant of a photochemical reaction that is independent of temperature, whereas the latter suggests an enzymatic control. These enzymes are likely to be enzymes of photophosphorylation and electron transport system (Davison, 1991). As a consequence of the low temperature limited ability of algae to use light, low temperature grown algae are more susceptible to photoinhibition (Geider, 1987; Davison, 1991). A further effect appears to be a decrease of the compensation irradiance for photosynthesis at low temperatures (Davison, 1991).

Respiration

Temperature induced increases in the rates of respiration have been reported for both marine (Morgan and Kalff, 1979; Verity, 1982a) and freshwater (Scherer *et al.*, 1981 cited in Robarts and Zohary, 1987) phytoplankton in culture, with Q_{10} values ranging from 2 to 4.

Increased rates of respiration at higher temperatures have also been observed in natural populations (Kuparinen, 1984; Robarts and Zohary, 1987; Iriarte *et al.*, 1991), but in most of these cases the measured respiration rate was not solely attributable to phytoplankton, but to a planktonic community of both autotrophic and heterotrophic organisms. Also, in the field, a direct influence of temperature on respiration is not easy to establish. A greater availability of organic substrates for respiration generally coincides with warmer periods, and is likely to have a stronger influence on the rates of plankton community respiration.

Excretion

Studies on the temperature response of excretion rates are sparse. Data available in the literature suggests interspecific differences on the effect of temperature. Rates of excretion in *Leptocylindricus danicus* and *Isochrysis galbana* have been found to be unaffected by temperature (Verity, 1982c; Zlotnik and Dubinsky, 1989). *Chlorella vulgaris* and *Synechococcus*, however, showed enhanced rates of extracellular release of organic carbon as temperature increased up to a point beyond which further increases in temperature resulted in decreases in the rates of excretion. Excretion as a percentage of total carbon fixation was minimum at the optimum temperature and increased at sub-optimal and supra-optimal temperatures in these two organisms. Similar results were obtained by Watanabe (1980) with natural populations. Rates of extracellular release of carbohydrates in *Katodinium dorsalisulcum* (McLaughlin *et al.*, 1960) were found to be inhibited at low temperatures and glycolic acid excretion in *Porphyridinium cruentum* (Dohler, 1972) reached a maximum rate at 15°C, decreasing thereafter.

Cellular carbon, chlorophyll, nitrogen and protein content

Geider (1987) examined the relationship between carbon to chlorophyll *a* ratio (C:Chl *a* = θ) in phytoplankton of different taxonomic groups from data available in the literature. The conclusion was that C:Chl *a* decreases exponentially with increasing temperature at constant light level. The author suggested this trend could be explained in various ways:

i) Cells grown at low temperatures generally show increased cellular lipid content. This could be viewed as an increase in the ratio of membrane lipid to protein content helping to maintain the fluidity of the thylakoid membranes. However, the observed increases in lipids are not probably sufficient to fully account for the increases in the C:Chl *a* ratio.

ii) An increase in the amount of a catalyst can help maintain the rate of a given reaction at lower temperatures. A reallocation of carbon resources into catalysts of dark reactions at the expense of light harvesting pigments can take place.

iii) Since algal cells are considered to be more susceptible to photoinhibition at low temperatures, increases in C:Chl *a* could be the result of an increment in components used for repairs of photodestructive processes. A reallocation of carbon resources as in ii) could also contribute to avoidance of photooxidative damage.

Cellular chlorophyll *a* and carbon content, however, does not display a universal response pattern with temperature, which suggests there are interespecific differences in the relative contribution of the mechanisms that can induce changes in C:Chl *a*.

For *Dunaliella tertiolecta* increases in C:Chl *a* with declining temperature could be explained as increases in cellular carbon content (Morris and Glover, 1974) and, since cellular chlorophyll content remained relatively constant, a reallocation of carbon resources at the expense of light harvesting pigments would not be the main mechanism. Other studies have also shown cellular increases in carbon and nitrogen content with decreasing temperature (Jørgensen, 1968; Goldman, 1977). Increases in cell volume (Morgan and Kalff, 1979; Meeson and Sweeney, 1982) or dry weight (Morris and Glover, 1974) at low temperature have also been reported. In some studies these have been associated with increases in protein content (Jørgensen, 1968), but not in others (Morgan and Kalff, 1979). Yoder (1979), however, reported that temperature had no effect on the carbon and nitrogen biomass of *Skeletonema costatum* and that chlorophyll per cell was lower at lower temperatures. In this case a reallocation of carbon resources could explain the changes in the C:Chl *a* ratio.

Growth

The general pattern is that growth rates of phytoplankton increase with temperature up to an optimum temperature beyond which they decline sharply (Eppley, 1972; Canale and Vogel, 1974). Typical values of Q_{10} for growth rate of phytoplankton are in the region of 2 (Raven and Geider, 1988). The optimum temperature and the range over which growth can occur is highly variable amongst species. Observations have been made on the interaction of temperature with light and there are reports on the effect of temperature on the parameters of the growth *versus* light curves. Light saturated growth rate (μ_{max}) for a given phytoplankton species has been shown to

increase with temperature (Eppley, 1972; Cloern, 1977; Yoder, 1979; Meeson and Sweeney, 1982; Verity, 1982b; Post *et al.*, 1985). Eppley (1972) concluded that temperature sets an upper limit for growth rate of phytoplankton in the sea and this maximum growth rate increases exponentially as temperature increases. The initial slope of the growth *versus* irradiance curves (α_μ) has also been found to have a strong temperature dependence, increasing temperature resulting in a more efficient utilization of low photon flux density (Verity, 1982b), although beyond an optimum temperature, α_μ can also decline (Yoder, 1979). The photon flux density at which maximum growth rate is attained is generally reported to be higher at higher temperatures (Smayda, 1969; Meeson and Sweeney, 1982), although in some species the highest temperature tested also caused the saturation irradiance to decrease (Durbin, 1974; Cloern, 1977; Morgan and Kalff, 1979; Yoder, 1979). The compensation irradiance for growth, i.e. the photon flux density at zero growth rate does not appear to exhibit a unique and definite trend of variation with temperature. For *Ceratium furca* (Meeson and Sweeney, 1982) and *Cryptomonas erosa* (Morgan and Kalff, 1979) the compensation irradiance was lower with decreasing temperature, whereas *Gonyaulax polyedra* (Meeson and Sweeney, 1982) showed a V shaped response. Verity (1981) found this parameter to be very low and constant for *Leptocylindricus danicus* and Yoder (1979) failed to observe a clear pattern for *Skeletonema costatum.* These variations in trends are likely to be explainable, at least partially in terms of differences in the Q_{10} for photosynthesis and for respiration.

2.3. MATERIAL AND METHODS

2.3.1. Culturing the organism

2.3.1.1. The organism under study

The organism used in this study is a comparatively recently isolated oceanic, coccoid, usually non-motile (i.e. rarely flagellated), chlorophyte algae of 1.5 to 4μm in diameter. Originally it was designated clone Ω48-23 by L.S. Murphy and was positioned in the class Prasinophyceae (Foss *et al.*, 1984). Recently a new genus i.e. *Pycnococcus* and a new species i.e. *Pycnococcus provasolii* have been created to accommodate this and other newly isolated clones of picoplanktonic green algae (Guillard *et al.*, 1991), and suggested to belong to the class Micromonadophycea (Campbell *et al.* 1989; Guillard *et al.*, 1991). Cells are spherical, solitary and olive-green in colour, containing chlorophyll *b* and the carotenoid prasinoxanthin as major accesory pigments (Foss *et al.*, 1984; Hooks *et al.*, 1988). They divide by binary cell division and the chloroplasts contain a characteristic pyrenoid (Guillard *et al.*, 1991). The clone was originally isolated by L.S. Murphy in July 1980 in slope waters of the Atlantic at 38°19.5'N and 69°34.5'W. The pycnocline area of the northwestern Atlantic and Gulf of Mexico waters has been identified so far as its general habitat (Guillard *et al.*, 1991).

The original source of the isolate used in this work was obtained from Dr. Graham Dixon from the School of Biological Sciences, University College of Swansea.

2.3.1.2. Nutritional medium and maintenance conditions

In the present study the algae was grown in natural seawater (salinity ≥33ppt) enriched with Guillard's f/2 nutrient recipe without added silicate (Guillard, 1980). To prevent contamination from other organisms and maintain a unialgal culture, the culture medium was sterilized and all sampling from experimental flasks was performed under a sterile air hood. However, cultures were not axenic and contained some bacteria. Seawater was autoclaved for 20 minutes at 121°C and 15p.s.i. When cool, nutrient solutions were added (according

to Guillard's f/2 recipe) aseptically by filtration through a 0.2μm filter with a sterile disposable syringe filtering system (Sartorius Minisart). To maintain sterility, all flasks were stoppered with cotton bungs.

For maintenance purposes the organism was kept in 250ml conical pyrex glass Erlenmeyer flasks containing 200ml of medium in temperature controlled incubation cabinets (model Gallenkamp cooled incubators) set at 10, 15 and 20°C. These cabinets were provided with small fluorescent tubes that provided a maximum illumination of *ca.* 60$\mu Em^{-2}s^{-1}$. The lights were connected to a timer, so that the photoperiod was also adjustable according to experimental requirements (continuous light or 12:12h light:dark cycle).

Maintenance cultures were subcultured every 10 to 15 days by adding a small aliquot (*ca.* 1ml) to a flask containing 200ml of sterile medium.

2.3.2. Growth rate measurements

Rates of cell growth were measured at 6 to 8 different photon flux densities, ranging from 3 to 540$\mu Em^{-2}s^{-1}$, each at three temperatures (10, 15, 20°C). Growth rate was determined as the rate of increase in three biomass indicators: cell number, chlorophyll *a* and particulate organic carbon. Cultures for growth rate experiments were incubated in 2litre conical pyrex glass Erlenmeyer flasks containing 1.5 litre of medium and incubated in water tank incubators. These incubators consisted of glass water tanks illuminated from below by a series of fluorescent tubes that yielded a maximum photon flux density of 400 and 540$\mu Em^{-2}s^{-1}$ (For a more detailed description see Garcia, 1989). At 20°C temperature was regulated by circulating water pumped with a thermostatic heater/circulator (Grant FH15) from a reservoir where water was cooled using two coiled coolers. At 10 and 15°C a refrigerated circulator (Neslab Cool flow CFT-33) was used. A range of irradiances were obtained using neutral density screens, by placing a combination of layers of mosquito netting underneath the experimental vessels. All flasks were wrapped in aluminium foil to avoid influence of the room light. Incubation photon flux density (PAR) was determined using a quantum meter Model Q.101 provided with a cosine collector

sensor (Macam Photometrics Ltd.), by placing the sensor on the bottom of an empty pyrex glass beaker. Cells were mainatined in suspension by shaking the flasks periodically (i.e. twice daily).

Aliquots for the determination of cell counts (1 to 10ml), chlorophyll *a* and b concentration (5 to 25ml) and particulate organic carbon (10 to 75ml) were removed from the flasks once daily.

2.3.2.1.Cell enumeration

The use of preservatives was avoided (Murphy and Haugen, 1985) and cells were counted within a short interval (0.5hours) after sampling. Cell enumeration was carried out with an epifluorescence microscope. Details of enumeration procedure and calculation of cell concentration are given in section 1.3.1.3. It was found not to be essential to use a polycarbonate Nuclepore filter and to count cells from cultures cellulose nitrate filters were used.

2.3.2.2. Chlorophyll analysis

Duplicate aliquots of 5 to 25ml (depending on density of the culture) were filtered on to 25mm Whatman GF/F filters at low vacuum (less than 10cmHg). The filters were then folded, wrapped in aluminium foil and stored frozen at -20°C until analysis was carried out within less than two weeks. Chlorophyll was extracted by homogenization of the filter in a small amount (*ca.*5ml) of 90% Analar grade acetone. The homogenate was transferred to a plastic centrifuge tube and left in the dark for half an hour to allow for complete extraction. The extract was centrifuged for 5 minutes at 4000rpm and the supernatant decanted into a 10 or 20ml volumetric flask, which was then made up to the mark with 90% acetone.

In the early stages of the project chlorophyll *a* was analyzed fluorometrically using an AMINCO fluoro-colorimeter provided with a C5560 Corning blue excitation filter and a C5264 emission filter. Values were corrected for phaeopigments by the acidification method, taking readings before and after acidification with two drops of 10% HCl (Parsons *et al.*, 1984a). A solution of chlorophyll *a* extracted from *Anacystis nidulans* (Sigma Ltd.) was made up in 90% Analar grade

acetone and standardized spectrophotometrically using Lorenzen's (1967) equations. Chlorophyll *a* and phaeopigment *a* concentrations were calculated as described in Parsons *et al.* (1984a). (Note: in the present study, the terms phaeopigment *a* and phaeopigment *b* are used to designate the degraded forms of chlorophyll *a* and chlorophyll *b* respectively).

This method yielded higher phaeopigment concentrations in culture studies than might be expected for an exponentially growing population. It was then suspected that the chlorophyll *b* concentration could be high enough to lead to an apparent high concentration of phaeopigments and a concomitant underestimation of chlorophyll *a*, because phaeopigment *b* has an emission peak close to that of chlorophyll *a*. Gibbs (1979) estimated that the undegraded chlorophyll *a* is underestimated by an amount equal to that of chlorophyll *b* present if corrected for phaeopigment, and if uncorrected, it is overestimated by 30% of the chlorophyll *b* value. When chlorophyll *b* is present in low concentration the uncorrected estimate of chlorophyll *a* is not seriously affected.

From these considerations it was judged it could be useful to quantify chlorophyll *b* as well as chlorophyll *a* in the present study. A possible available method was the spectrophotometric trichromatic analysis for the determination of chlorophylls *a*, *b* and *c*. However, in view of the enhanced sensitivity (*ca.* two orders of magnitude) of the fluorometric techniques over the spectrophotometric method, the possibility of a fluorometric determination of chlorophylls *a* and *b* was investigated. Loftus and Carpenter (1971) used a combination of three emission filters (Corning glass) in a Turner III fluorometer and gave equations to calculate chlorophylls *a*, *b* and *c*. Boto and Bunt (1978) used the same approach, but using selected monochromatic excitation and emission wavelengths with an spectrofluorometer. This reduced the overlapping between the emission spectrum of each pigment to a greater extent than with broad band emission filters. A similar approach to that used by Boto and Bunt (1978) was employed in the present work. As recommended by these authors, it was considered necessary to carry out a calibration for the instrument used, a Perkin Elmer model LS5 luminescence spectrometer equipped with a Xenon discharge lamp (9.9W) and F13 Monk Gillieson type monochromators. The

slit width combination used was 10mm for both excitation and emission.

Purified extracts of chlorophylls a and b were obtained from Sigma Ltd.. Chlorophyll *a* was an extract from *Anacystis nidulans* and chlorophyll *b* was an extract from spinach. Extracts were diluted in 90% Analar grade acetone and scanned in the spectrofluorometer to obtain excitation and emmision wavelength peaks. A comparison of peaks of excitation and emission found by several authors is presented in Table 2.3.1. Peaks of excitation of both chlorophyll *a* and chlorophyll *b* appeared to be somewhat lower than in other studies, but the resons for this were not clear.

Table 2.3.1. Comparison of excitation and emmision peak wavelengths.

Reference	Chl *a*		Ph *a*		Chl *b*		Ph *b*	
	ex	em	ex	em	ex	em	ex	em
Gibbs (1979)[a]	437	676	420	672	467	659	442	666
Boto and Bunt (1978)[b]	435	667	390	667	470	651	435	657
This study[c]	424	671	400	673	453	654	430	658

[a] Extracts from *Abutilon* spp.
[b] Extracts from *Melaleuca alba*
[c] Extract for chlorophyll *a* from *Anacystis nidulans* and for chlorophyll *b* from spinach.

Assuming that the relative fluorescence at given excitation and emission wavelengths is the sum of relative fluorescence values of each of the pigments at those selected wavelengths, a series of equations can be derived (Boto and Bunt, 1978):

$$H(424,671) = k(424,671,a)Ca + k(424,671,pa)Cpa + k(424,671,b)Cb + k(424,671,pb)Cpb$$

$$H(453,654) = k(453,654,a)Ca + k(453,654,pa)Cpa + k(453,654,b)Cb + k(453,654,pb)Cpb$$

After acidification

$$H(400,673) = k(400,673,pa)C'pa + k(400,673,pb)C'pb$$

$$H(430,658) = k(430,658,pa)C'pa + k(430,658,pb)C'pb$$

where

$H(x,y)$: is the relative fluorescence emission at x and y excitation and emission wavelengths respectively

C_z: is the concentration of chlorophyll z

C_{pz}: is the concentration of the phaeopigment z

C'_{pz}: is the sum of concentrations of the chlorophyll and the corresponding phaeopigment z

$k(,x,y,v)$: is a coefficient determined as the slope of the linear regression line of the emission response (H) *versus* concentration (C) for each pigment (v) at the corresponding excitation (x) and emission (y) wavelengths.

The concentrations of the standard chlorophyll solutions were calculated spectrophotometrically using a Pye Unicam SP6-350 according to the equations given by Lorenzen (1967). Relative fluorescence values were recorded for a series of dilutions of these solutions (1/10, 1/25, 1/50, 1/100) at each selected pair of excitation and emission wavelengths (Table 2.3.2). K coefficients were calculated as the slopes of the regression lines of the relative fluorescence (scale 0.1, i.e. 0-1000) against the concentration of pigment (mg m^{-3}) (Table 2.3.3). The 90% acetone solution was used as a blank and values were corrected for the small fluorescence signal.

In order to check the validity of the equations when both chlorophyll *a* and *b* are present artificial mixtures were prepared with the standard chlorophylls a and *b* solutions and the fluorescence measured at the different wavelengths. Concentrations calculated from the derived fluorometric equations in the mixtures were compared to the concentrations determined spectrophotometrically (Table 2.3.4). Since there were no appreciable amounts of phaeopigments in the standards, values of phaeopigments yielded very low numbers, sometime even negative, so for a comparison only chlorophylls *a* and *b* were considered. The percentage difference error ranged from 0.26 to 13% (in one occassion it reached 22%), and appeared to be higher for the chlorophyll *b* concentration.

Table 2.3.2. Relative fluorescence values of a series of dilutions of the standard solutions of chlorophyll *a* and b in 90% acetone at the corresponding wavelengths of excitation (λ_{ex}) and emmision (λ_{em}).

λ_{ex} λ_{em}	1:10	1:25	1:50	1:100	Blank*
		chlorophyll *a*			
424, 671	540.0	226.6	116.3	58.2	0.2
453, 654	4.9	2.1	1.2	0.7	0.3
After acidification					
424, 671	96.1	38.8	20.0	10.0	0.2
453, 654	3.6	1.6	0.9	0.6	0.3
400, 673	448.8	182.5	93.8	46.5	0.3
430, 658	20.5	8.3	4.3	2.4	0.3
		chlorophyll *b*			
424, 671	29.9	10.6	5.6	3.4	
453, 654	155.8	64.3	33.2	17.5	
After acidification					
424, 671	91.0	37.1	19.3	10.2	
453, 654	26.4	10.8	5.7	3.0	
400, 673	37.9	14.1	7.4	4.4	
430, 658	208.6	85.2	44.3	23.4	

* A solution of 90% acetone was used as blank.

Table 2.3.3. K coefficients obtained from the linear regression analysis of the plots of relative fluorescence *versus* the dilution ratio (r = 0.999, n=4, p=0.001)

	$\lambda_{ex,em}$			
	424,671	453,654	400,673	430,658
chlorophyll *a*	5.7627	0.0503		
chlorophyll *b*	0.6668	3.4235		
phaeopigment *a*	1.0324	0.0362	4.8205	0.2181
phaeopigment *b*	2.0014	0.5789	0.8373	4.5867

Table 2.3.4. Comparison of values of chlorophyll *a* and *b* (mg m^{-3}) estimated by the fluorometric and spectrophotometric methods for various artificial mixtures of standard solutions of these two pigments.

	Approximate chl *a*:*b* ratios						
	10:1	6:1	4:1	4:1	3:1	2:1	4:3
			chlorophyll *a*				
Fluor equat	30.63	26.87	24.81	58.33	52.60	42.93	34.89
Spectro	38.81	27.73	24.65	61.68	55.47	46.22	36.98
%Error	-0.58	-3.10	0.65	-5.43	-5.17	-7.13	-5.65
			chlorophyll *b*				
Fluor equat	3.64	4.48	6.12	14.98	16.10	19.62	23.40
Spectro	2.98	4.48	5.97	14.94	18.17	22.41	26.88
%Error	22.14	0.00	2.50	0.26	-11.43	-12.45	-12.97

A comparison of concentrations of chlorophyll *a*, chlorophyll *b*, phaeopigment *a* and phaeopigment *b* in cultures of *Pycnococcus provasolii*, determined using various fluorometric approaches, is presented in Table 2.3.5. Chlorophyll *a* concentrations determined using broad band excitation and emission filters (i.e. AMINCO fluorocolorimeter) and correcting for phaeopigments were on average 59.6%(SD 3.1) of the chlorophyll *a* concentrations determined using the same procedure without correcting for phaeopigments and 63.5%(SD 4.6) of the values estimated using monochromatic excitation and emission wavelengths and the equations derived in the present study. Phaeopigment *a* concentration determined with the monochromatic wavelength technique was on average only 3.8%(SD 1.1) of values determined with the broad band filter technique. Chlorophyll *a* concentration compared better between the broad band and the monochromatic wavelength techniques when the former was used without correcting for phaeopigments, because the proportion of phaeopigment to chlorophyll was low in the cultures. The difference between these two estimates expressed as a percentage of the value obtained using monochromatic wavelengths, was on average 7.5%(SD 5.8) and there was no definite trend of one set of estimates been consistently higher than the other.

Table 2.3.5. Comparison of values of chlorophyll *a*, chlorophyll *b*, phaeopigment *a* and phaeopigment *b* concentration ($\mu g\ l^{-1}$) as determined from various fluorometric approaches: A= using broad band filters (AMINCO fluorocolorimeter) and correcting for phaeopigments ; B= the same as A, but without phaeopigment correction; C= using monochromatic wavelengths and equations derived in this work.

sample	chl *a*			phaeo *a*		chl *b*	phaeo *b*
	A	B	C	A	C	C	C
1	9.64	17.56	15.75	17.18	0.36	14.23	0.00
2	10.11	17.75	15.96	16.57	0.11	14.54	0.00
3	14.58	24.09	22.38	20.63	0.98	17.18	0.02
4	15.29	24.72	22.73	20.48	0.99	17.29	0.22
5	41.16	69.73	62.57	62.00	2.09	53.96	0.09
6	41.16	69.73	62.16	62.00	1.72	53.61	0.00
7	22.11	36.64	32.85	31.54	1.13	27.68	0.02
8	20.46	35.44	31.67	32.49	0.86	26.91	0.00
9	11.23	19.97	17.66	18.96	0.56	16.59	0.03
10	11.99	21.07	18.53	19.71	0.54	17.39	0.02
11	7.17	13.31	11.67	13.32	0.36	11.46	0.00
12	7.17	13.37	11.71	13.46	0.37	11.46	0.00
13	7.70	12.52	11.17	10.45	0.63	8.26	0.22
14	7.47	12.01	10.76	9.86	0.49	8.00	0.07
15	11.17	18.38	16.08	15.65	0.56	13.48	0.00
16	11.11	18.45	16.21	15.92	0.71	13.53	0.17
17	11.35	19.40	16.99	17.47	0.64	13.59	0.18
18	11.76	19.49	17.06	16.78	0.71	13.66	0.15
19	10.47	17.94	15.29	16.22	0.55	13.36	0.00
20	10.47	17.94	15.50	16.22	0.60	13.64	0.00
21	4.61	6.59	5.92	4.30	0.23	4.23	0.01
22	4.33	6.74	5.90	5.25	0.33	4.23	0.00
23	25.21	43.56	37.25	39.82	1.38	33.87	0.23
24	24.97	43.41	37.29	40.00	1.31	33.48	0.00
25	16.75	26.72	27.88	21.28	1.02	19.53	0.36
26	17.29	27.00	28.40	20.74	1.04	19.94	0.46
27	16.21	25.57	26.97	19.98	0.65	19.62	0.00
28	16.10	25.80	26.50	20.70	1.12	19.45	0.06
29	24.86	39.14	40.45	31.57	1.71	30.85	0.51
30	24.73	39.64	39.91	30.77	1.39	30.78	0.37
31	32.42	52.86	53.45	43.64	2.18	42.34	0.48
32	30.37	49.99	51.60	41.89	1.53	40.69	0.00
33	20.64	33.50	32.68	27.45	0.93	29.49	0.00
34	18.70	32.92	32.62	30.37	1.03	29.36	0.00
35	14.00	24.39	23.64	22.19	0.68	21.56	0.00
36	14.37	24.68	23.95	22.00	1.02	21.73	0.19
37	16.64	26.37	26.99	20.77	0.91	21.05	0.00
38	18.37	29.87	29.93	24.56	1.02	24.05	0.21
39	16.75	28.44	28.50	24.96	0.75	24.74	0.00
40	30.80	51.13	51.16	43.42	1.16	44.40	0.00
41	11.24	20.63	20.23	20.04	0.79	18.67	0.06
42	11.35	20.40	20.06	19.32	0.79	18.36	0.16
43	1.69	2.70	2.68	2.17	0.10	2.12	0.02
44	1.76	2.86	2.81	2.34	0.09	2.17	0.00
45	6.79	11.38	11.36	9.80	0.37	9.45	0.00

2.3.2.3. Particulate organic carbon (POC) and nitrogen (PON) analysis

Duplicate aliquots of between 10 and 75 ml were filtered at low vacuum (less than 10cmHg) onto 25mm diameter Whatman GF/F filters precombusted at 450°C overnight. The filters were then folded, wrapped in precombusted aluminium foil and stored frozen at -20°C prior to analysis. Before analysis, filters were dried in a desiccator containing a small amount of phosphorus pentoxide for 2 to 3 days.

Most of the samples analyzed for POC were done by combustion of the filter in oxygen and measurement of the carbon dioxide produced in an infra-red gas analyzer (Analytical development Ltd., type 200 and 225) as described by Banoub (1970). A series of dilutions of a standard sucrose solution was used to produce a calibration curve. Combusted filters were used as blanks. A typical calibration curve is shown in Figure A.2 in Appendix.

In a later stage of the project the acquirement by the department of Geology of an elemental analyzer provided the opportunity to analyze the samples from growth rate experiments at 10 and 15°C for POC and PON. Dried filters were wrapped in 27mm aluminium disks (Elemental Microanalysis, Ltd.) and pelletized using a small in-house built press. Analysis for carbon and nitrogen was performed in a Carlo Erba model 1108 elemental analyzer. The principle of the technique lies in the flash combustion of the samples wrapped in aluminium, and the subsequent separation of gases produced by gas chromatography, which are then detected by thermal conductivity (Verardo *et al.*, 1990). Acetanilide (Analar) was used as the standard compound against which samples were calibrated. Tin capsules were used for the standard. Standard calibration curves were obtained by plotting the weight of each element against the count area underneath the peak for each element. Examples of standard calibration curves for carbon and nitrogen are given in Figure A.3 and Figure A.4 in Appendix.

2.3.2.4. Calculation of growth rates

Growth rates were calculated as the rate of increase in cell number, chlorophyll *a* and particulate organic carbon concentration. Growth rate was determined as the slope of the regression line

obtained by least squares fit of the semilogarithmic plot of the concentration of the corresponding biomass index against time in hours. Thus obtained specific growth rates (μ) had units of h^{-1}, i.e. logarithm to the base e units of increase per hour (Guillard, 1973).

2.3.2.5. μ versus I curve fitting: parameter calculation

The hyperbolic tangent function has been found to consistently give the best fit to growth *versus* irradiance relationships (Platt and Jassby, 1976; Yoder, 1979; Verity, 1982b; Langdon, 1987), and the following mathematical expression was fitted to the data (Langdon, 1987) in the present study:

$$\mu = (\mu_{max} + \mu_o) \tanh(\alpha_g I/(\mu_{max} + \mu_o)) - \mu_o$$

where

μ = growth rate (h^{-1})

μ_{max} = light saturated growth rate (h^{-1})

μ_o = intercept of the μ *v* I curve, i.e. maintenance respiration rate (h^{-1})

α_g = initial slope of the μ *v* I curve, i.e. growth efficiency ($h^{-1}(\mu Em^{-2}s^{-1})^{-1}$)

I = irradiance ($\mu Em^{-2}s^{-1}$)

The fitting was performed using a non-linear least squares regression computer programme contained in the statistical package 'Statgraphics'. The irradiance for saturation of growth ($I_{\mu k}$) was derived as μ_{max}/α_g (in units of $\mu Em^{-2}s^{-1}$).

2.3.3. Photosynthesis and respiration rate measurements

Rates of photosynthesis and respiration were determined as changes in the concentration of dissolved oxygen using the Winkler titration technique. On some occasions rates of photosynthesis were also measured as rates of $^{14}CO_2$ incorporation.

2.3.3.1. Incubation conditions

In a few experiments aimed to measure photosynthetic performance, growth and subsequent photosynthetic measurements were carried out in the water tank incubators described in section 2.3.2. The algae was grown in 2 litre conical Erlenmeyer flasks and incubated for 3 hours in 60ml Duran glass BOD bottles. Four replicates were fixed at time zero, four were incubated in the dark and a further four were incubated at the corresponding photon flux density.

When photosynthesis was measured over a range of photon flux densities, cultures were grown in 10 litre Duran glass bottles in the afore mentioned water tank incubators and then incubated for photosynthesis measurements in 60 ml Duran glass BOD bottles (for dissolved oxygen measurement) and in 60ml plastic tissue culture bottles (for ^{14}C uptake measurement) for three hours in a light gradient box. Measurements were undertaken at 2 temperatures (10 and 20°C) and at 20°C two photoperiods (continuous light and 12:12 hour L:D cycle) were tested. At 20°C and continuous light both oxygen and ^{14}C techniques were employed. These data were used to derive photosynthetic parameters.

The light gradient box consisted of a rectangular wooden black box 110 cm long and 16 cm wide with a perspex window at one end. Water was circulated through the box from a reservoir of cooled water with a heater/circulator. Light was provided by a 500 Watt Tungsten halogen lamp placed at one end. Bottles were aligned in two rows in the box, and a light gradient produced due to the attenuation by each bottle. A total of 22 oxygen bottles and a further 22 tissue culture bottles could be accommodated. A range of irradiance values of 3 to 2000 $\mu Em^{-2}s^{-1}$ (PAR) was achieved. To minimize overwarming of the bottles nearest to the light source, a 10cm glass box with circulating water was fitted between the lamp and the wooden box. A diagram of the light gradient incubator is presented in Figure 2.3.1.

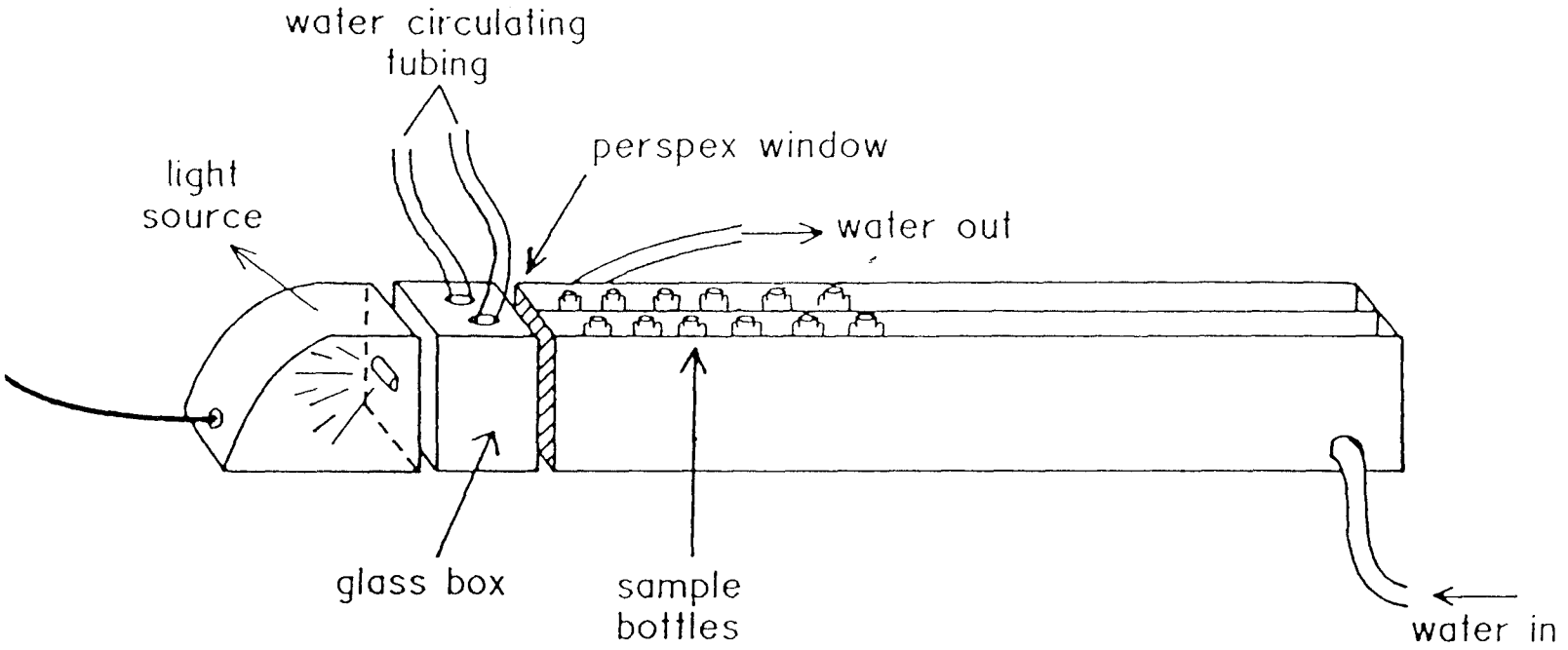

Figure 2.3.1. Diagrammatic presentation of the light gradient incubation system (from Figure 2.2 in Garcia, 1989).

2.3.3.2. Oxygen evolution measurements

A detailed description of the oxygen technique is given in section 1.3.1.4 in chapter one.

2.3.3.3. Carbon uptake determination: the ^{14}C technique

The method used was based on that introduced by Steemann Nielsen (1952). A full description of the procedure adopted is given in section 1.3.1.4 in chapter one. With the monoalgal cultures 47mm diameter, 0.45μm Whatman cellulose nitrate filters were used.

2.3.3.4. P v I curve fitting: parameter calculation

For a detailed description of the procedure adopted see section 1.3.1.4 in chapter one.

2.3.4. Size fractionated respiration rate measurements

To evaluate the impact of the presence of bacteria in the algal respiration rate estimates, size fractionated respiration rate measurements were carried out at three different algal cell concentrations. Culture samples were grown at $360\mu Em^{-2}s^{-1}$ under 12:12h photoperiod and at 20°C in the water tank incubator described in section 2.3.2. When the desired algal concentration was reached, the sample was fractionated by reverse filtration through 1μm Nuclepore filters as described in section 1.3.1.4 and incubated (both the unfractionated and the 1μm filtrate) for measurements of dark respiration rate. Four replicate bottles of each sample were incubated in the dark for 3hours. A further set of four replicate bottles for each sample were fixed at zero time. Oxygen concentration measurements and respiration rate calculations were performed as described in sections 1.3.1.4 in chapter one.

2.3.5. Bacteria cell enumeration

Bacterial cell counts were carried out using an epifluorescence technique, slightly modified from that used for counting

picocyanobacteria (described in section 1.3.1.3 in chapter one). For bacteria cell counting a sensitive DNA-staining fluorochrome, 4'6-diamidino-2phenylindole (DAPI) recommended by Porter and Feig (1980) was used, which fluoresces a bright blue. A stock solution of DAPI (1mg ml^{-1}) was stored at 4°C and from this, a working solution of 0.02mg ml^{-1} was prepared by dilution in 0.2μm filtered 2.5% glutaraldehyde. When filtering the sample for the microscopic slide preparation, and whilst the sample was still in the filter tower, 0.1ml of this working solution was added to each 1ml of sample, yielding a final concentration of 0.002mg ml^{-1}. A period of five minutes was allowed in the dark for the sample to become evenly and brightly stained. For details of the remaining steps of the procedure of cell enumeration and calculation of cell concentration see section 1.3.1.3 in chapter one. The only other difference was the use of Neofluar (x100) in place of the Planapochromat objectives.

2.4. RESULTS

2.4.1. Cellular chlorophyll *a*, chlorophyll *b*, POC and PON content.

Chlorophyll *a* and chlorophyll *b* content

Cellular concentrations of chlorophylls *a* and *b* were measured each day in batch grown cultures of *Pycnococcus provasolii* incubated at light levels between 3 and 540$\mu Em^{-2}s^{-1}$ under 12:12h light:dark cycle and at three temperatures: 10, 15 and 20°C. At 15°C and 20°C cell pigment contents were monitored over a range of cell concentrations between approximately 0.1 and 3-5x10^6cells ml^{-1}. Over the course of 5 to 7 days a lag phase, an exponential phase and the beginning of a stationary phase of growth was observed at growth irradiances higher than 3-10$\mu Em^{-2}s^{-1}$ (Figure 2.4.1, Figure 2.4.2A and Figure 2.4.2B). At 3$\mu Em^{-2}s^{-1}$, growth was monitored for between 29 and 32 days and cells did not reach stationary phase. At all growth irradiances growth rate was observed to decline from exponential rate at cell concentrations of around 1.5x10^6cells ml^{-1}. No clear variations in cellular chlorophyll *a* or chlorophyll *b* content with the growth phase were detected. At 10°C it was decided to monitor growth over a lower range of cell concentrations in order to have a more extended exponential phase and thus have a larger number of points from which growth rates would be derived. Samples were taken daily for 12 to 15 days and cell concentration ranged between 0.1 and 2-5x10^5cells ml^{-1} (Figure 2.4.3). At 3$\mu Em^{-2}s^{-1}$ growth was monitored for 43 days. Cells did not reach the stationary phase at any irradiance level at this temperature.

Chlorophyll *a* and *b* concentrations were determined in duplicate or triplicate samples and maximum deviation from mean of replicate samples was 9%. The mean cellular contents of chlorophyll *a* and chlorophyll *b* of all determinations during batch growth at the corresponding irradiance and temperature are presented in Table 2.4.1. Results are also shown in Figure 2.4.4. Both chlorophyll *a* and chlorophyll *b* decreased exponentially with irradiance at all temperatures tested. Lowest concentrations were determined at 10°C, chlorophyll *a* varying 4.4 fold, between 0.0223 and 0.0979pg $cell^{-1}$ between 360 and 3$\mu Em^{-2}s^{-1}$ and chlorophyll *b* ranging 6 fold between

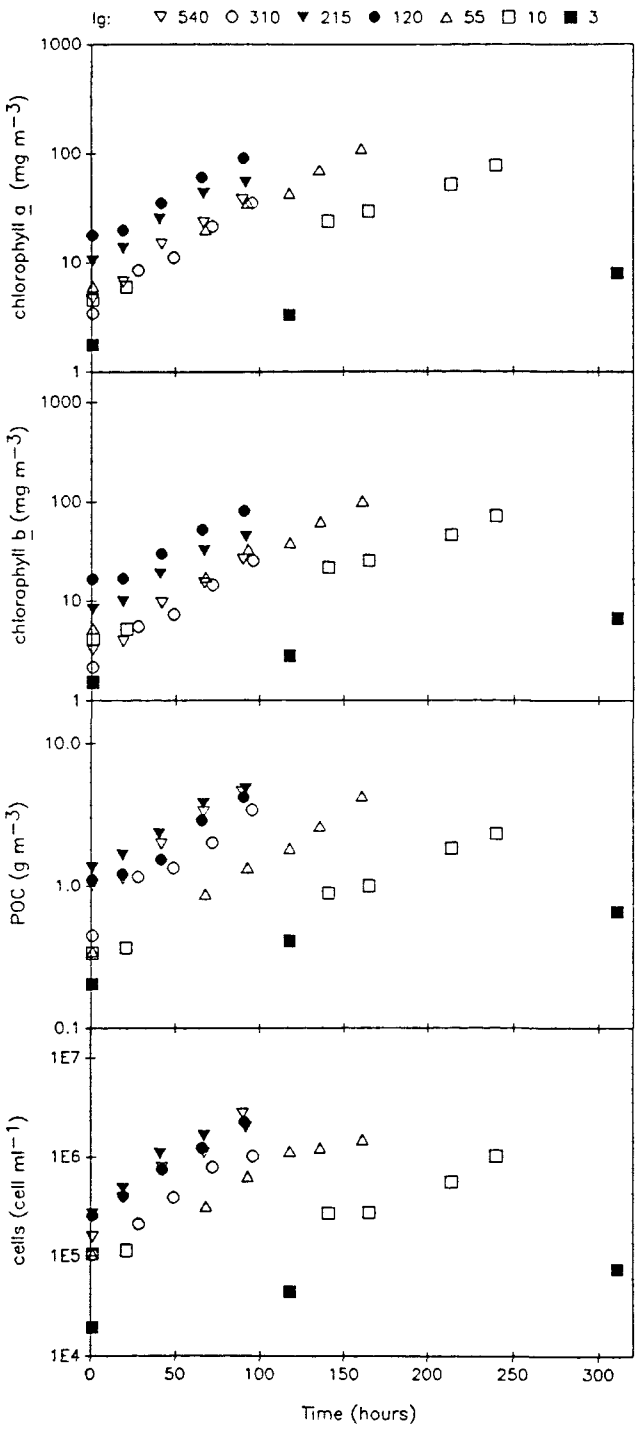

Figure 2.4.1. Growth curves constructed as increases with time in cell number, chlorophyll *a*, chlorophyll *b* and POC concentration at various irradiances (Ig, in $\mu Em^{-2}s^{-1}$) for cells of *Pycnococcus provasolii* grown at 20°C and 12:12h L:D cycle.

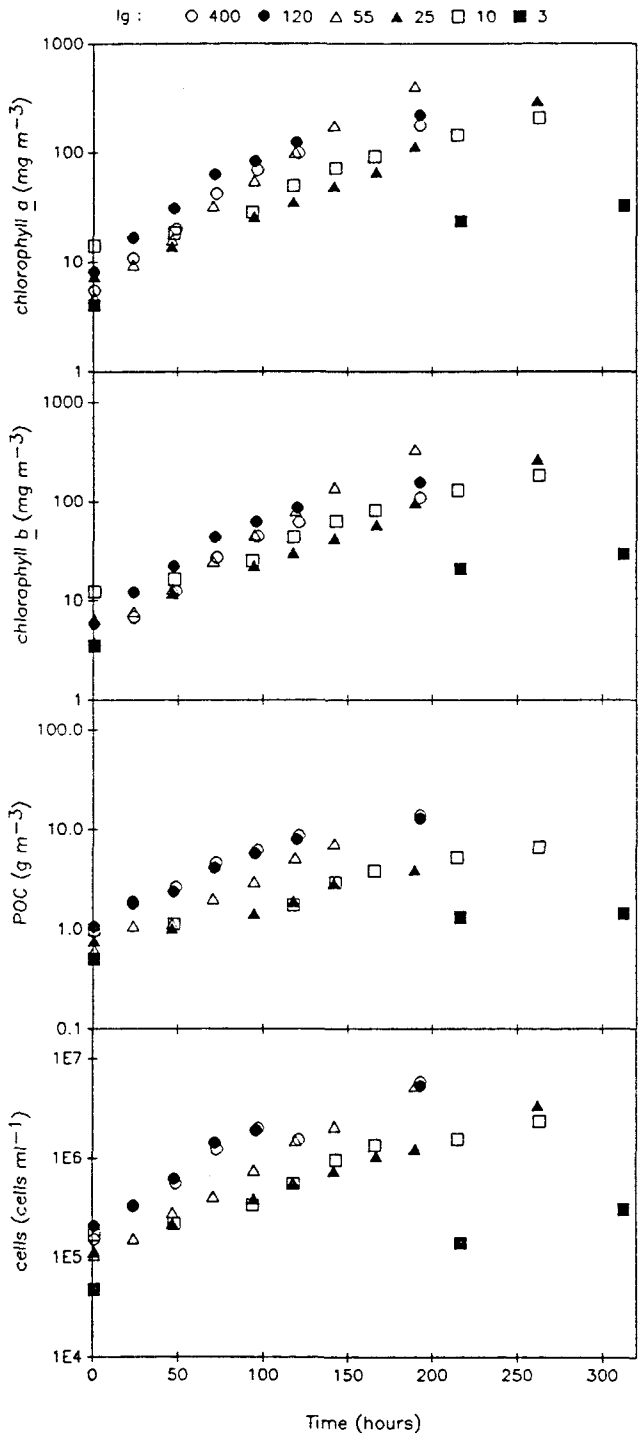

Figure 2.4.2A. Growth curves constructed as increases with time in cell number, chlorophyll *a*, chlorophyll *b* and POC concentration at various irradiances (Ig, in $\mu Em^{-2}s^{-1}$) for cells of *Pycnococcus provasolii* grown at 15°C and 12:12h L:D cycle.

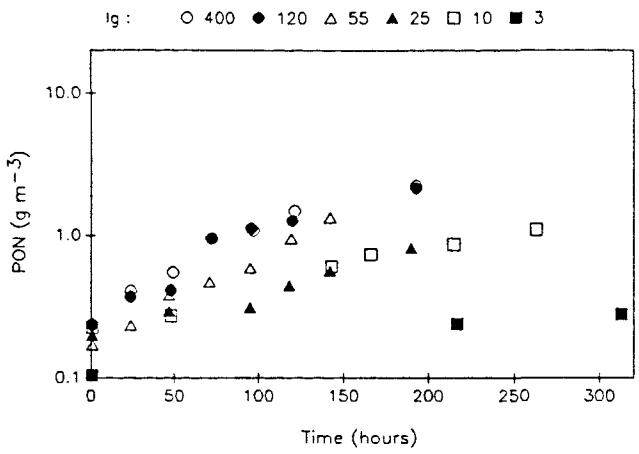

Figure 2.4.2B. Growth curves constructed as increases with time in PON concentration at various irradiances (Ig, in $\mu Em^{-2}s^{-1}$) for cells of *Pycnococcus provasolii* grown at 15°C and 12:12h L:D cycle.

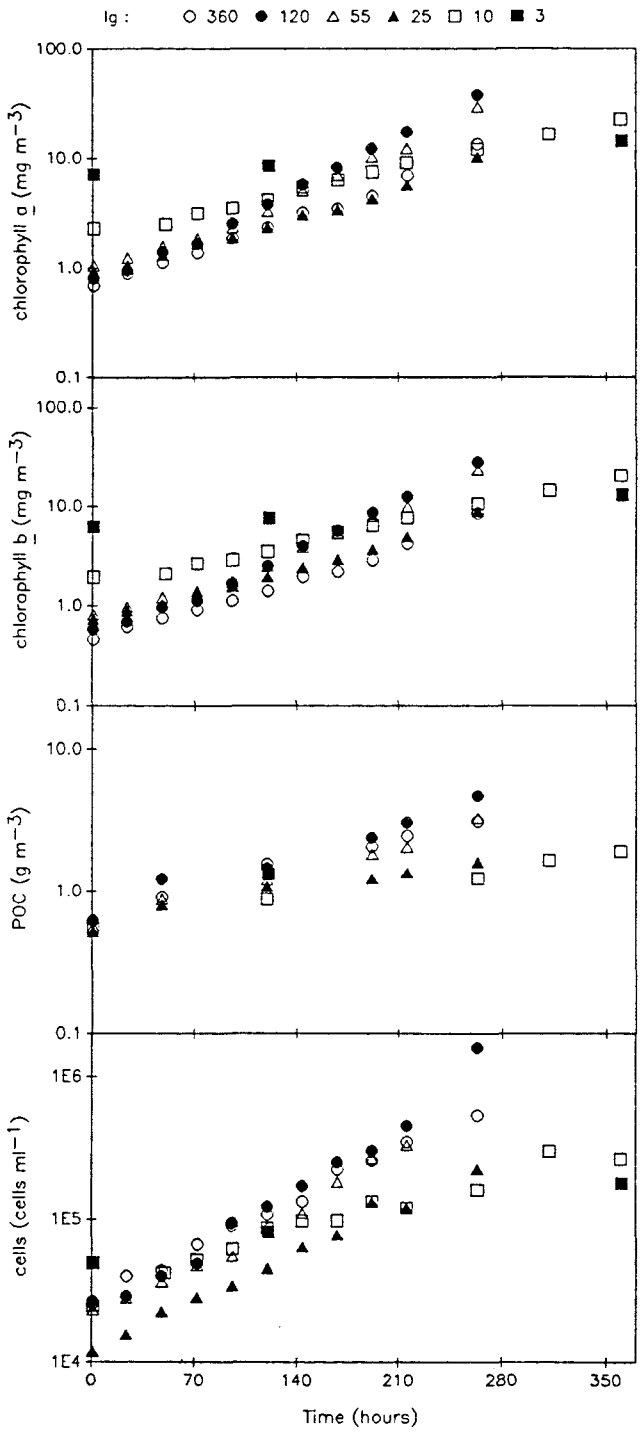

Figure 2.4.3. Growth curves constructed as increases with time in cell number, chlorophyll *a*, chlorophyll *b* and POC concentration at various irradiances for cells of *Pycnococcus provasolii* grown at 10°C and 12:12h L:D cycle.

0.0143 and 0.0859pg $cell^{-1}$ between the same irradiance levels. Highest chlorophyll concentrations were measured at 15°C, chlorophyll *a* varying 3.5 fold between 0.0341 and 0.1183pg $cell^{-1}$ between 400 and 3$\mu Em^{-2}s^{-1}$ and chlorophyll *b* oscillating between 0.0215 and 0.1048pg $cell^{-1}$ (i.e. 4.9 fold) in the same irradiance interval. At 20°C chlorophyll *a* increased 4.9 fold (0.0200-0.0977pg $cell^{-1}$) and chlorophyll *b* ranged 6.4 fold (0.0132-0.0849pg $cell^{-1}$) between 540 and 3$\mu Em^{-2}s^{-1}$. The chlorophyll *versus* irradiance data for cultures grown at all three temperatures could be fitted to an exponential relationship, which took the expression:

$chl = 11.42(\pm 0.53) - 1.53(\pm 0.13)\log_e I$ ($r^2=0.88$, n=20) for chlorophyll *a*

and

$chl = 10.28(\pm 0.46) - 1.48(\pm 0.11)\log_e I$ ($r^2=0.90$, n=20) for chlorophyll *b*

where
chl= chlorophyll *a* or *b* concentration (pg $cell^{-1}$)
I= irradiance ($\mu Em^{-2}s^{-1}$).
Values in parenthesis are standard errors

Clearly growth at low photon flux densities resulted in a proportionately larger accumulation of chlorophyll *b* than chlorophyll *a*, and the chlorophyll *b* to chlorophyll *a* ratio increased exponentially with decreasing irradiance down to a photon flux density (PFD) of 25$\mu Em^{-2}s^{-1}$ at the three temperatures tested. The ratio decreased from around 0.9 to 0.6 (i.e. as a percentage, chlorophyll *b* decreased from around 47 to 38% of the chlorophyll *a* plus chlorophyll *b* concentration) from 3 to 360$\mu Em^{-2}s^{-2}$ (Table 2.4.1; Figure 2.4.4). Below 25$\mu Em^{-2}s^{-1}$ the ratio appeared to remain rather constant.

Phaeopigment *a* and *b* concentrations were also measured daily in these batch grown cultures of *P. provasolii*. Values were in all cases less than 5% of the corresponding chlorophyll concentrations (data are not shown).

Chlorophyll *a* concentration of cells grown at 20°C under continuous light are only available from single measurements during P *v* I experiment incubations. Measurements were undertaken with the Aminco fluoro-colorimeter and chlorophyll *b* was not determined.

Table 2.3.1. Cellular carbon (C), nitrogen (N), chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) content, chlorophyll *b* to *a* ratio (*b*:*a*), carbon to chlorophyll *a* ratio (θ) and carbon to nitrogen ratio (C:N) of cells of *Pycnococcus provasolii* grown at various combinations of irradiance (I_g) and temperature (T^a) under 12:12 L:D cycle (data obtained during batch growth experiments). (Values in parenthesis are standard errors).

I_g	T^a	chl *a* x10^{-2}	chl *b* x10^{-2}	*b*:*a*	C	θ	N	C:N
3	10	9.79(0.86)	8.59(0.80)	0.87	*13.15(-)	134*	-	-
	15	11.83(1.39)	10.48(1.25)	0.88	4.01(-)	34	0.92(-)	5.0
	20	9.77(1.14)	8.49(1.08)	0.86	-	-	-	-
10	10	6.04(0.28)	5.15(0.25)	0.83	*7.20(-)	119*	-	-
	15	8.31(0.29)	7.37(0.26)	0.89	3.75(0.46)	45	0.57(0.05)	7.6
	20	7.64(0.99)	6.82(0.87)	0.89	3.15(0.19)	41	-	-
25	10	5.51(0.35)	4.55(0.26)	0.83	*7.15(-)	130*	-	-
	15	7.05(0.43)	6.12(0.39)	0.86	4.22(0.53)	60	0.75(0.06)	6.5
	20	5.76(0.49)	5.16(0.44)	0.90	2.31(0.18)	40	-	-
55	10	4.08(0.11)	3.15(0.08)	0.77	*6.48(0.27+)	159*	-	-
	15	6.79(0.45)	5.47(0.36)	0.81	4.27(0.39)	63	0.69(0.08)	7.2
	20	5.09(0.48)	4.49(0.51)	0.88	2.31(0.22)	51	-	-
120	10	3.25(0.15)	2.28(0.11)	0.70	*7.30(0.56+)	225*	-	-
	15	4.47(0.19)	3.22(0.14)	0.72	3.76(0.51)	84	0.61(0.13)	7.2
	20	3.85(0.65)	3.31(0.60)	0.85	2.47(0.43)	64	-	-
215	20	2.86(0.27)	2.20(0.24)	0.76	2.56(0.29)	89	-	-
360	10	2.23(0.09)	1.43(0.07)	0.63	*6.42(0.61+)	288*	-	-
400	15	3.41(0.10)	2.15(0.07)	0.63	4.31(0.66)	126	0.66(0.15)	7.6
310	20	3.26(0.24)	2.17(0.16)	0.67	3.08(0.29)	94	-	-
540	20	2.00(0.27)	1.32(0.20)	0.65	2.50(0.30)	125	-	-

I_g ($\mu Em^{-2}s^{-1}$); T^a (°C); chl *a*, chl *b* (pgchl (*a* or *b*) $cell^{-1}$); C, N (pg(C or N) $cell^{-1}$); θ ($g\ g^{-1}$); C:N (at at^{-1}).

- no value

(-) no replicate

* carbon measurements made on low cell concentration samples; algal carbon content considered to be unreliable (see text)

\+ values are standard difference (2 replicates)

Table 2.4.2. Effect of irradiance (I_g) on the cellular chlorophyll *a* (chl *a*) and carbon (C) content and carbon:chlorophyll *a* ratio (θ) of cells of *Pycnococcus provasolii* grown at 20°C under continuous light. Values in parenthesis are standard differences as a percentage of the mean.

I_g	chl *a* ($\times 10^{-2}$)	C	θ
25	7.82(4.5)	2.91(0.6)	37
65	8.03(2.5)	3.73(1.8)	47
140	7.47(12.1)	3.61(3.6)	49
260	2.69(4.7)	3.02(0.0)	113
370	2.32(1.8)	3.30(3.0)	142

I_g ($\mu Em^{-2}s^{-1}$)
chl *a*, C (pg $cell^{-1}$)
θ (g g^{-1})

Estimates of chlorophyll *a* decreased 3.4 fold between 25 and 370$\mu Em^{-2}s^{-1}$, ranging between 0.0232 and 0.0803pg $cell^{-1}$ (Table 2.4.2).

Carbon content

Particulate organic carbon measurements were made on samples taken during the same batch growth experiments as chlorophyll *a* concentrations were measured (Figure 2.4.1, Figure 2.4.2A and Figure 2.4.3). At 15 and 20°C, at the majority of irradiance levels, during the first and second sampling dates, i.e. low cell concentrations and lag phase of growth, carbon per cell values were higher than for later sampling dates. At 10°C a progressive decrease of cell carbon content with time was observed and during the initial stages of growth cell carbon contents were as high as 9 times those recorded at higher temperatures. This was probably due to the presence of bacteria in the cultures and their more significant impact on the particulate organic

carbon concentration at the low algal concentrations at which measurements at 10°C were undertaken. Cell carbon content values estimated for cultures grown at 10°C are therefore considered to be unreliable and cannot be discussed with data from other temperatures.

POC concentration was determined from duplicate or triplicate samples and maximum deviation from mean of the replicate values was 10%. Mean cellular carbon concentrations for cells grown under 12:12h photoperiod (derived from POC measurements) are presented in Table 2.4.1 and Figure 2.4.5. Estimates varied between 2.31 and 3.15pg $cell^{-1}$ at 20°C. Values estimated at 15°C were higher and ranged between 3.75 and 4.31pg $cell^{-1}$. There was no significant variation in carbon content per cell amongst the various light levels at each temperature (Figure 2.4.5). Carbon concentrations at 20°C, under continuous light, determined during incubations for P *v* I measurements ranged between 2.91 and 3.73pg $cell^{-1}$ and no clear pattern of variation with irradiance was observable (Table 2.4.2).

Nitrogen content

Cellular nitrogen content (derived from PON measurements) was determined only in cultures grown at 15°C (Figure 2.4.2B). Values are presented in Table 2.4.1. There was no clear variation with growth irradiance, except for a somewhat higher concentration at $3\mu Em^{-2}s^{-1}$. Concentrations ranged from 0.61 to 0.92pg $cell^{-1}$ (mean 0.7(SE 0.12). The C:N ratio ranged between 5.0 and 7.6 and did not show clear trends of variation with growth irradiance.

Values of cell nitrogen content at 10°C suffered from the problems encountered with measurements at low algal cell concentrations described for the carbon measurements and are not included in Table 2.4.1.

Carbon to chlorophyll *a* ratio

Values of carbon to chlorophyll *a* ratio (θ) (g:g) from cultures grown at 15 and 20°C are presented in Table 2.4.1 and Figure 2.4.5. Values varied between 40 and 125 over a 10-540$\mu Em^{-2}s^{-1}$ irradiance range at 20°C and between 34 and 126 at 15°C over a 3-400$\mu Em^{-2}s^{-1}$ irradiance range. θ from the two temperatures tested was positively correlated with growth irradiance. Estimates from 15 and 20°C could be

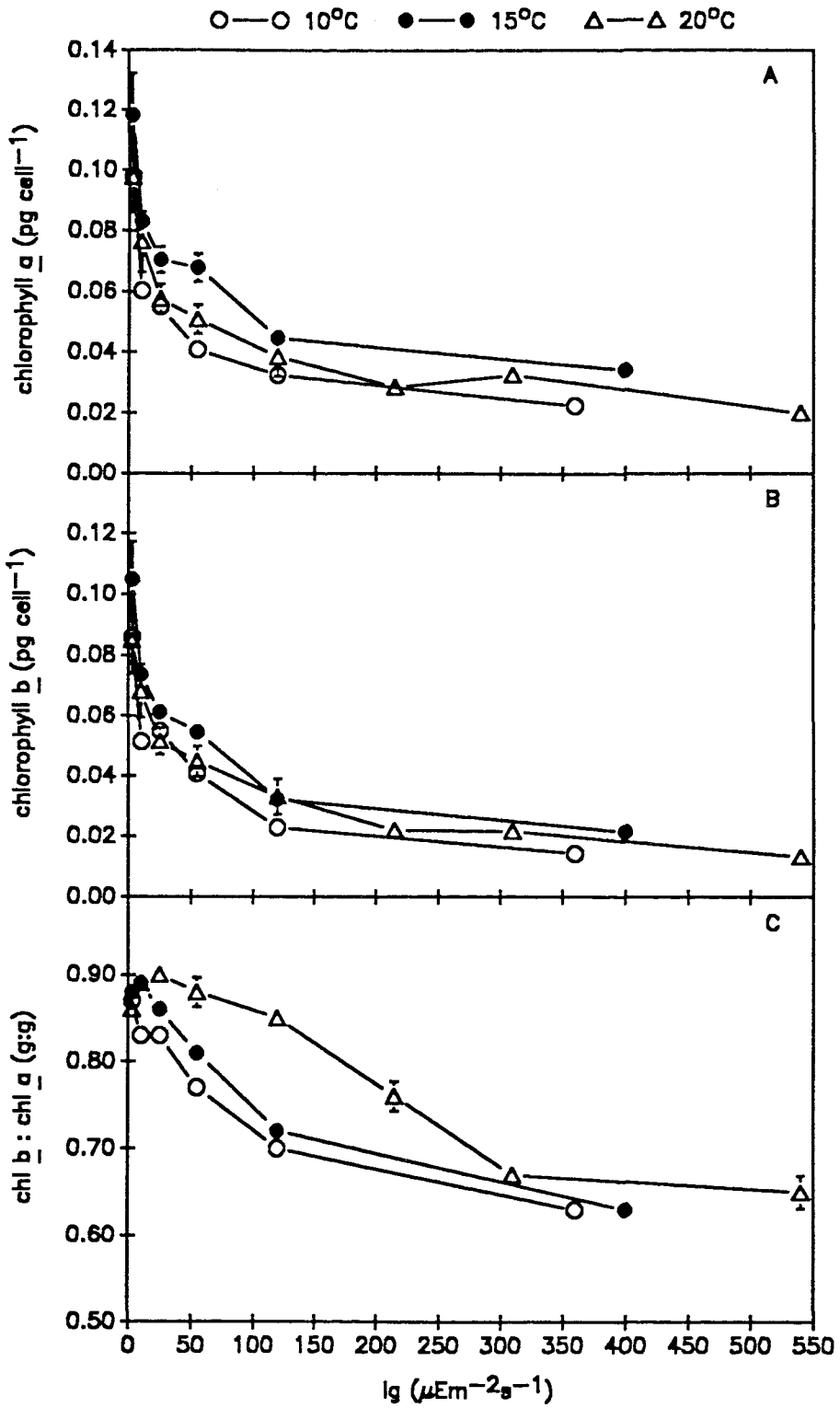

Figure 2.4.4. Effect of growth irradiance and temperature on the cellular (A) chlorophyll *a* and (B) chlorophyll *b* concentrations and (C) the chlorophyll *b* to *a* ratio in *Pycnococcus provasolii* grown under 12:12 L:D cycle. Standard error bars are included when greater than symbol.

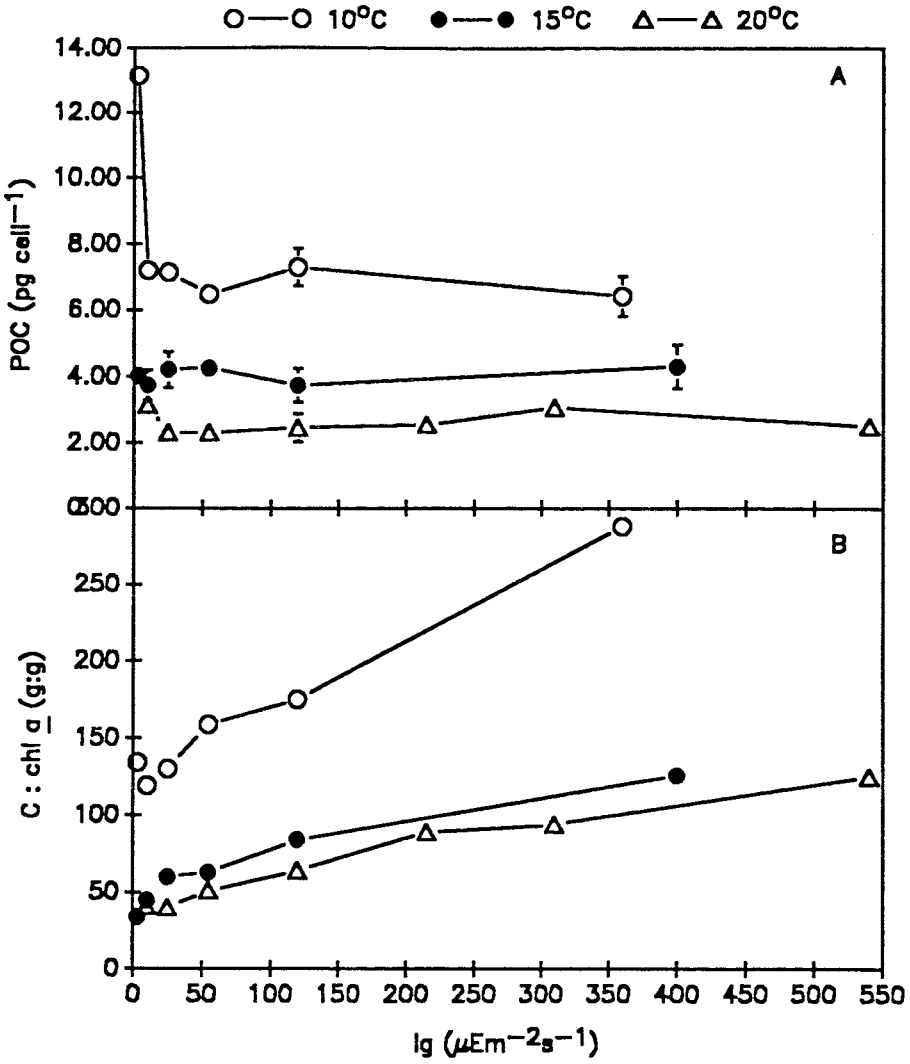

Figure 2.4.5. Effect of growth irradiance and temperature on the (A) cellular carbon content and (B) carbon to chlorophyll *a* ratio in *Pycnococcus provasolii* grown under 12:12 L:D cycle. Standard error bars are included when greater than symbol.

fitted by least square regression analysis to a single line which could be formulated as $\theta=45.7+0.17I$ ($r=0.94$, $p<0.001$), where θ is the carbon to chlorophyll *a* ratio (g:g) and I is the growth irradiance ($\mu Em^{-2}s^{-1}$).

The carbon to chlorophyll *a* ratio data for the 10°C cultures included in Table 2.4.1 are considered to be in error due to problems encountered with estimates of algal cellular carbon content derived from measurements of particulate organic carbon in low algal cell concentration samples.

2.4.2.P v I curves: photosynthetic parameters

The parameters of the *P v I* curves were derived from experiments where cultures were grown at a certain photon flux density and incubated over a range of photon flux densities in a light gradient box. The growth irradiances examined ranged between 11 and 370$\mu Em^{-2}s^{-1}$. Two temperatures were tested, 10 and 20°C, and at 20°C two photoperiods were compared: continuous light and 12:12 hours light:dark cycle. In cultures grown under continuous light conditions at 20°C photosynthesis was measured with both, the oxygen technique and the ^{14}C technique, and *P v I* parameters derived from both methods are presented. Results are shown in Table 2.4.3 and Table 2.4.4 and Figures 2.4.6, 2.4.7, 2.4.8 and 2.4.9. The standard errors of the estimated photosynthetic parameters are presented in Table A.19 in Appendix.

Oxygen based P v I curves

At 20°C under both photoperiods tested the initial slope of the P *v* I curve, i.e. the efficiency of light utilization at subsaturating irradiances (α), normalized to cell number exhibited a tendency to be highest at low to intermediate light levels 25-110$\mu Em^{-2}s^{-1}$ and decreased clearly at growth photon flux densities above 110-140$\mu Em^{-2}s^{-1}$. At 20°C and continuous light α was normalized to cell carbon content as well and this parameter decreased with increasing light level. In contrast, when normalized to chlorophyll *a* concentration, α clearly increased with growth photon flux density at all temperatures and photoperiods tested.

A comparison between continuous light and 12:12h light:dark cycle showed α normalized to cell number to be 1.4 to 1.7 fold α under 24h light dose. Differences were less pronounced for α normalized to chlorophyll *a* concentration.

The pattern of variation of α with temperature was not clear. Values were rather similar for cells grown at 360$\mu Em^{-2}s^{-1}$, but at the other two irradiances tested, 20/30 and 110$\mu Em^{-2}s^{-1}$, 20°C resulted in increased values in relation to 10°C.

Light saturated rates of photosynthesis (P_{max}) showed similar patterns of variation to those of α. At 20°C, under both photoperiods, expressed on a cell number basis, highest values were determined at low to intermediate irradiances (25-110$\mu Em^{-2}s^{-1}$), decreasing at higher irradiances. Values under 12:12h photoperiod at 20°C were 1.8 to 2 times those under 24h light dose. At 10°C the trend of variation of P_{max} per cell with growth photon flux density was not clear.

When normalized to chlorophyll *a* concentration as for α, P_{max} increased with growth irradiance at both temperatures and photoperiods tested: 1.57 fold at 20°C and 12:12h photoperiod from 30 to 360$\mu Em^{-2}s^{-1}$; 2.14 fold at 20°C and continuous light between 25 and 370$\mu Em^{-2}s^{-1}$; 1.47 fold at 10°C and 12:12h photoperiod between 20 to 360$\mu Em^{-2}s^{-1}$.

Estimates of P_{max}, both expressed on a cell number and chlorophyll *a* content were greater (Q_{10} greater than 2) at 20°C than at 10°C under 12:12 L:D cycle. Also, at 20°C growth under 12:12h light:dark cycle resulted in an enhancement of P_{max} in relation to growth under 24h light dose. This increase was more marked when expressed on a cell number basis, but was also clearly apparent when normalized to chlorophyll *a* concentration.

I_k exhibited a general pattern of increase with increasing irradiance, i.e. at low growth photon flux density cells required lower irradiance to saturate photosynthesis. At 10°C values were lower (66 to 110$\mu Em^{-2}s^{-1}$) than at 20°C (151-222$\mu Em^{-2}s^{-1}$). No substantial difference could be observed between photoperiods. I_m also showed a tendency to increase with raising photon flux density and differences between low and high growth photon flux density were much more pronounced than for I_k.

Photoinhibition as indicated from the parameter I_b was apparent

at low growth irradiances at 20°C, the difference being more pronounced under continuous than under light and dark cycle. At 10°C there was hardly any photoinhibition at any growth photon flux density.

Carbon based P v I curves

Values of both α and P_{max} were markedly depressed in relation to estimates derived from oxygen production measurements. The trend of variation with growth photon flux density of P_{max} and α normalized to cell number and carbon content was analogous to that described for oxygen evolution derived values, however the magnitude of variation between low light and high light grown cells appeared to be much greater for carbon fixation rates derived parameters, particularly for α. Thus P_{max} normalized to cell number decreased 1.32 fold from 25 to 370$\mu Em^{-2}s^{-1}$ in terms of oxygen production and 1.92 fold in terms of carbon fixation. α normalized to cell number decreased 1.56 times in terms of oxygen production and 3.31 times in terms of carbon fixation. When normalized to chlorophyll *a* concentration, the 2.55 fold increase observed for P_{max} in terms of oxygen production between growth photon flux densities of 25 and 370$\mu Em^{-2}s^{-1}$ was reduced to 1.96 fold in terms of carbon fixation and the 2.14 fold decrease for α in terms of oxygen production was reduced to 1.01 fold in terms of carbon fixation between the same growth irradiances.

I_k values were similar to those obtained from oxygen measurements and also exhibited an increase with increasing growth photon flux density. I_b values were high, indicating no photoinhibition at any growth photon flux density.

2.4.3. Photosynthetic quotients

Photosynthetic quotients, i.e. molar ratios of O_2 produced to CO_2 fixed (PQ), were derived from algae grown under continuous light from light box incubation P *v* I measurements. Since rates of carbon uptake and oxygen evolution were not measured at exactly the same photon flux density, PQ's were estimated and expressed as a function of irradiance by dividing the equation of the oxygen based by the equation of the carbon based P *v* I fitted curves. Results are

Table 2.4.3. The effect of photon flux density on the photosynthetic characteristics of *Pycnococcus provasolii* grown at 20°C under continuous light.

I_g	P_{max}			α			I_k	I_m	I_b	I_c
	cell $x10^{-8}$	C $x10^{-3}$	chl $x10^{-1}$	cell $x10^{-10}$	C $x10^{-5}$	chl $x10^{-3}$				
				A: Oxygen evolution						
25	2.85	9.74	3.64	2.15	7.37	2.75	132	387	837	8
65	3.28	8.66	4.09	2.51	6.63	3.12	130	391	973	15
140	2.94	8.11	3.93	1.78	4.96	2.39	163	451	751	27
260	2.06	6.78	7.69	1.07	3.53	4.00	191	591	1514	30
370	2.15	6.55	9.29	1.37	4.16	5.90	157	614	4552	30
				B: Carbon fixation						
25	1.30	4.43	1.66	1.06	3.63	1.36	122	459	3128	-
140	1.11	3.07	1.50	0.58	1.61	0.78	190	654	3040	-
260	0.63	2.06	2.34	0.31	1.02	1.15	202	747	4688	-
370	0.76	2.30	3.27	0.32	0.97	1.38	239	847	4462	-

P_{max} : cell (μmol (O_2 or C) $cell^{-1}$ h^{-1})
C (μmol (O_2 or C) μgC^{-1} h^{-1})
chl (μmol (O_2 or C) $\mu g chl\ a^{-1}$ h^{-1})

α : cell ((μmol (O_2 or C) $cell^{-1}$ h^{-1}) ($\mu Em^{-2}s^{-1}$)$^{-1}$)
C ((μmol (O_2 or C) μgC^{-1} h^{-1}) ($\mu Em^{-2}s^{-1}$)$^{-1}$)
chl ((μmol (O_2 or C) $\mu g chl\ a^{-1}$ h^{-1}) ($\mu Em^{-2}s^{-1}$)$^{-1}$)

I_g, I_k, I_m, I_b, I_c ($\mu Em^{-2}s^{-1}$)

Table 2.4.4. The effect of photon flux density on the photosynthetic characteristics of *Pycnococcus provasolii* grown at 10 and 20°C under 12:12h light:dark cycle.

I_g	P_{max} cell $x10^{-8}$	P_{max} chl $x10^{-1}$	α cell $x10^{-10}$	α chl $x10^{-3}$	I_k	I_m	I_b	I_c
			A: 20°C					
30	5.06	4.93	3.33	3.26	151	539	2925	8
110	5.69	8.29	3.56	5.19	159	654	6504	5
360	4.30	12.06	1.93	5.14	222	977	13047	3
			B: 10°C					
20	1.71	2.38	2.57	3.59	66	301	4729	3
110	1.55	3.97	1.68	3.66	108	515	9778	7
360	2.03	5.81	1.84	5.27	110	472	5613	27

P_{max} : cell (μmol (O_2 or C) $cell^{-1}$ h^{-1})
chl (μmol (O_2 or C) μgchl a^{-1} h^{-1})

α : cell (((μmol (O_2 or C) $cell^{-1}$ h^{-1}) ($\mu Em^{-2}s^{-1}$)$^{-1}$)
chl (((μmol (O_2 or C) μgchl a^{-1} h^{-1}) ($\mu Em^{-2}s^{-1}$)$^{-1}$)

I_g, I_k, I_m, I_b, I_c ($\mu Em^{-2}s^{-1}$)

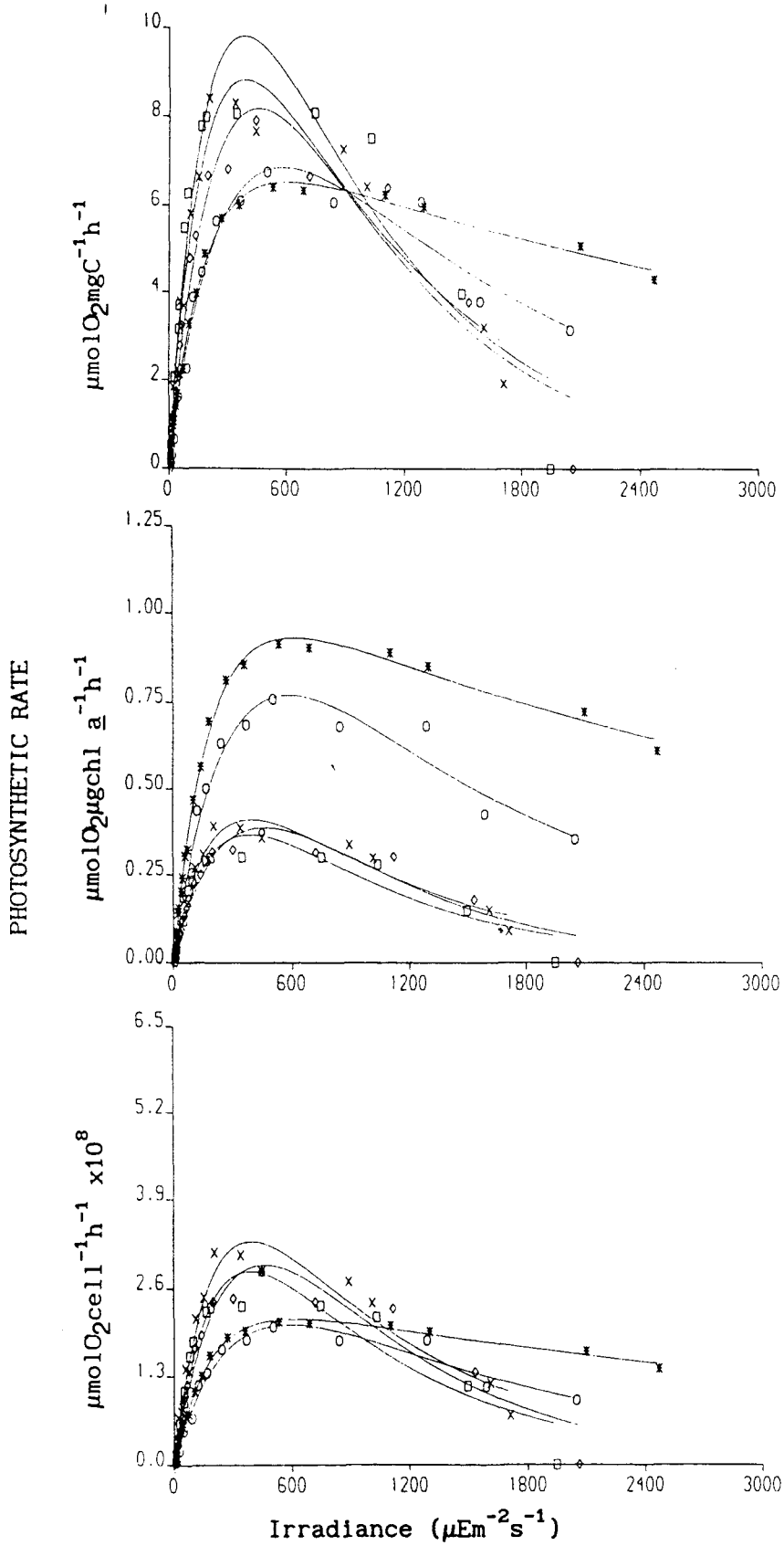

Figure 2.4.6. P *v* I curves derived from gross oxygen production measurements, for cells of *Pycnococcus provasolii* grown at 20°C and continuous light at various growth irradiances: (✱) 370, (o) 260, (◊) 140, (x) 65 and (□) 25 µEm^{-2}s^{-1}.

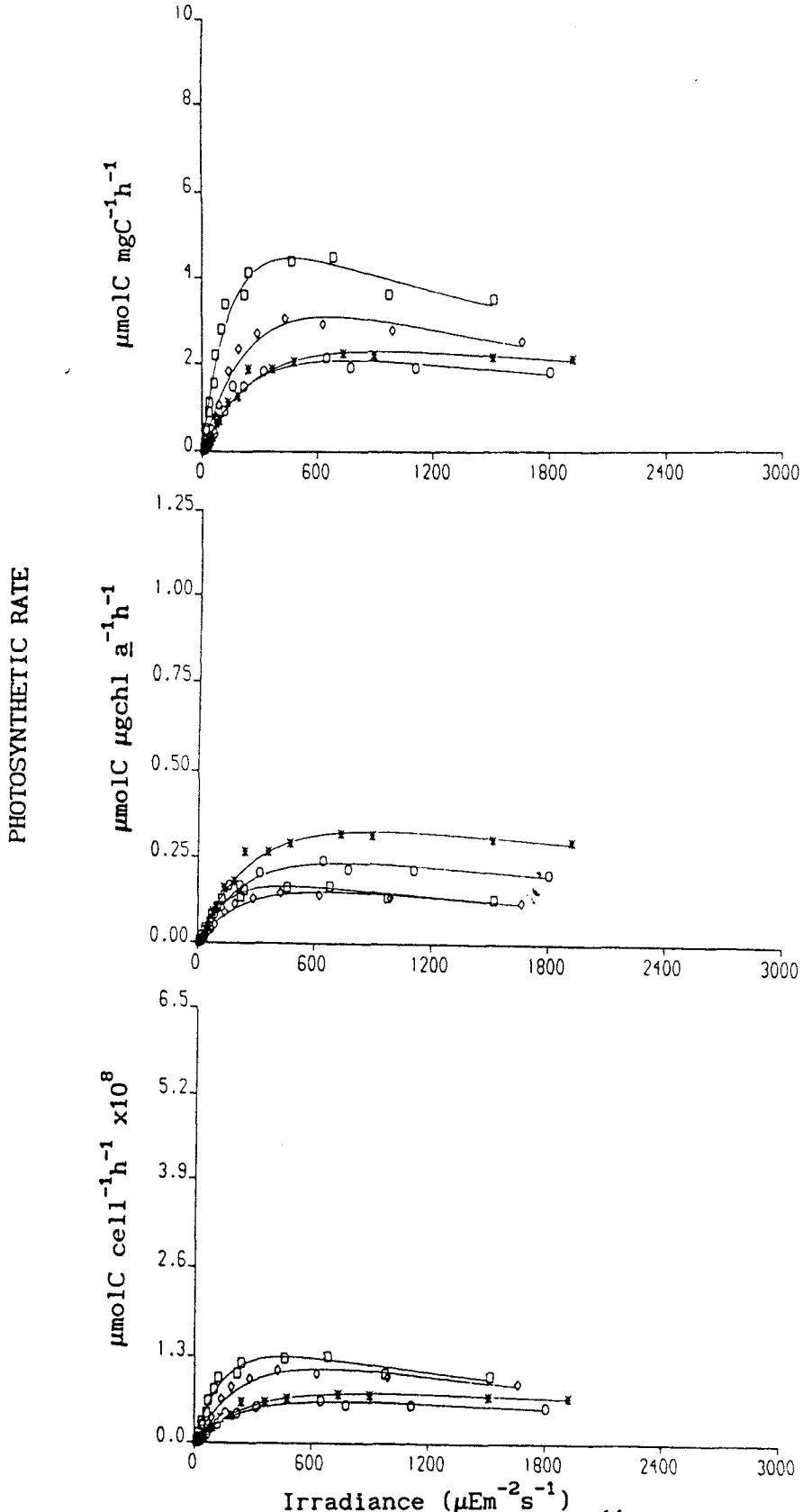

Figure 2.4.7. P *v* I curves derived from ^{14}C measurements, for cells of *Pycnococcus provasolii* grown at 20°C and continuous light at various growth irradiances: (✱) 370, (o) 260, (◇) 140, and (□) 25 µEm^{-2}s^{-1}.

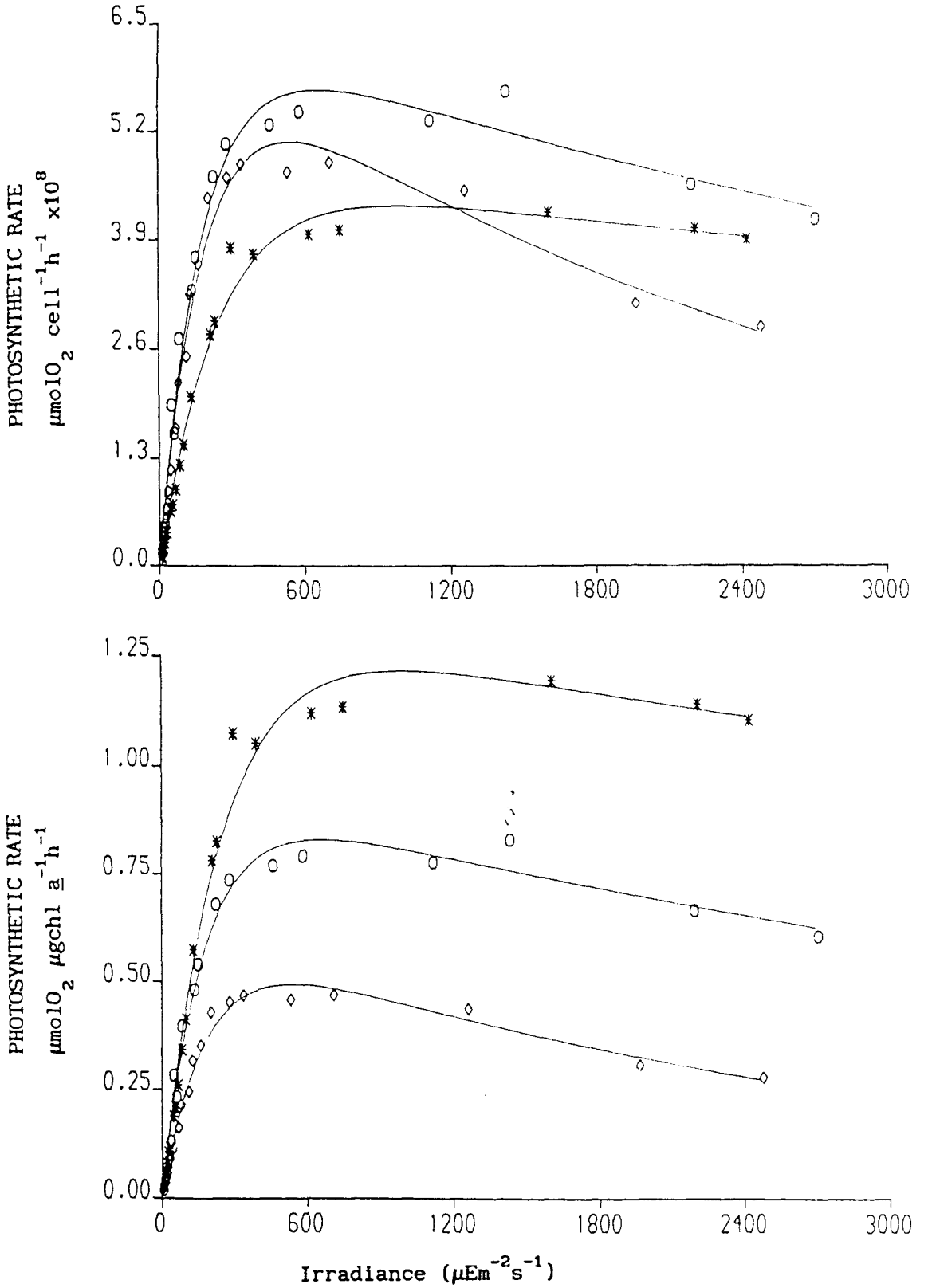

Figure 2.4.8. P *v* I curves derived from gross oxygen production measurements, for cells of *Pycnococcus provasolii* grown at 20°C and 12:12h L:D cycle at various growth irradiances: (*) 360, (o) 110 and (◇) 30 µEm^{-2}s^{-1}.

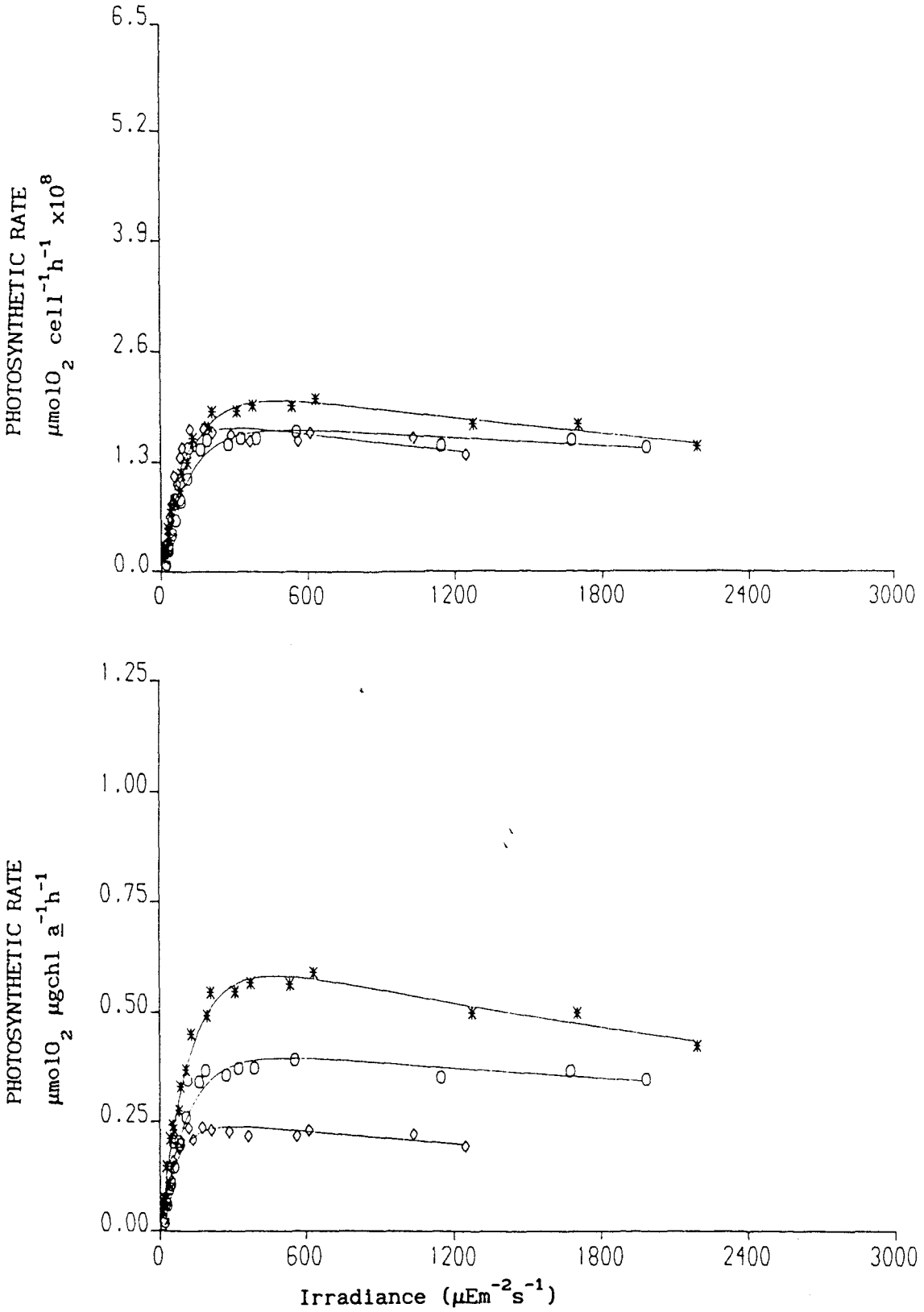

Figure 2.4.9. P *v* I curves derived from gross oxygen production measurements, for cells of *Pycnococcus provasolii* grown at 10°C and 12:12h L:D cycle at various growth irradiances: (✱) 360, (o) 110 and (◊) 20 µEm^{-2}s^{-1}.

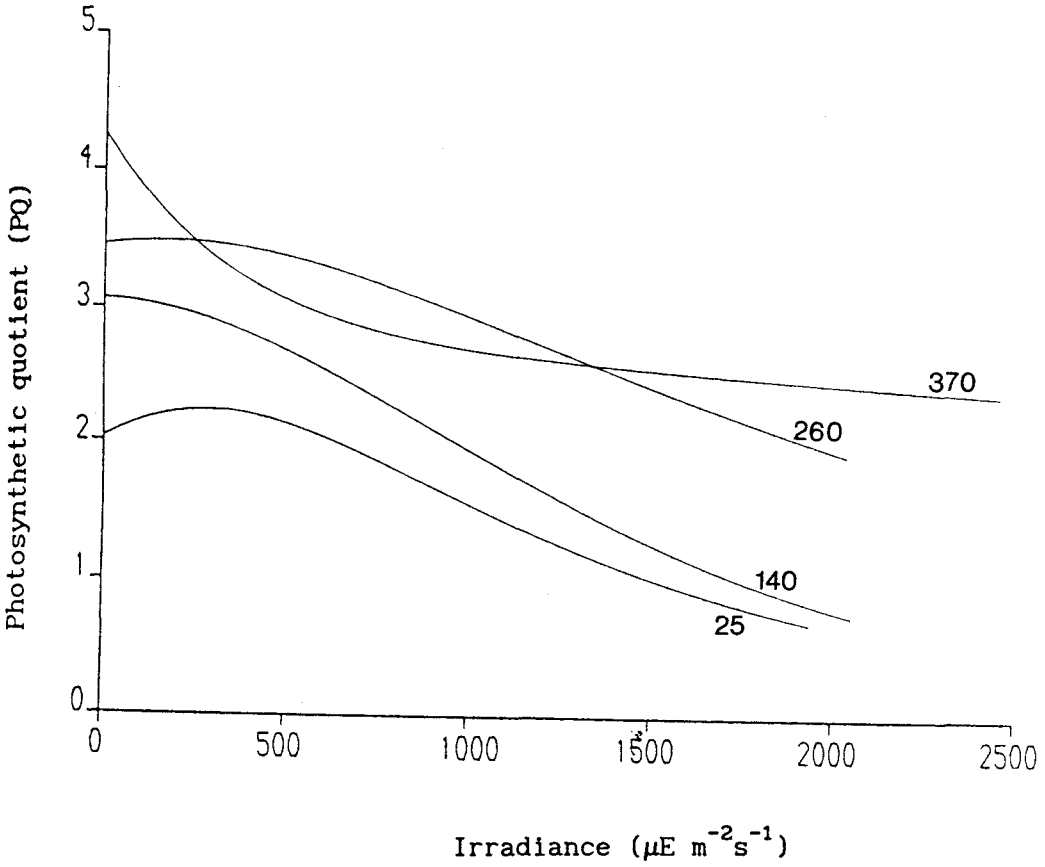

Figure 2.4.10. Photosynthetic quotients estimated over a range of incubation irradiances as derived from parallel measurements of carbon fixation rate and oxygen production rate in the light gradient box incubator, for cells of *Pycnococcus provasolii* grown at various irradiances (indicated in the graph in $\mu Em^{-2}s^{-1}$) under continuous light.

presented in Figure 2.4.10.

PQ's thus obtained ranged between 0.7 and 4.2. Values appeared to decrease with increasing incubation irradiance, i.e. relatively less O_2 production was detected at high photon flux densities (above saturation levels). This decrease was more significant for algae grown at low photon flux density. At all incubation irradiances PQ's were markedly lower for algae grown at lower light levels, decreasing from around 4 for algae grown at 370$\mu Em^{-2}s^{-1}$ to around 2 for algae grown at 25$\mu Em^{-2}s^{-1}$. Thus growth at high irradiance resulted in comparatively less carbon fixation per unit of O_2 produced than growing at low irradiances.

2.4.4. Respiration rates

The presence of bacteria in the algal cultures was considered could introduce a bias in the estimates of algal respiration rate. To evaluate the contribution of bacterial respiration rate to total respiration rate, size fractionated respiration rate measurements were carried out at various algal concentrations. Results are presented in Table 2.4.5. At algal concentrations of $\geq 2.7 \times 10^5$cell ml^{-1}, bacterial contribution was less than 20%, whereas at an algal concentration of 0.5×10^5cell ml^{-1}, bacterial contribution increased to 69.8%. In most experiments in which respiration rate was measured algal concentration was higher than 2×10^5cell ml^{-1} and thus it has been assummed that in the data presented in Table 2.4.6 the primary contributor to the overall rate of respiration was the autotrophic organism.

Rates of respiration were measured at two temperatures (10 and 20°C) in cultures grown over a range of irradiances. At 20°C two photoperiods were investigated: continuous light and 12:12h L:D cycle. Results are presented in Table 2.4.6 as rates of respiration normalized to cell number and as a percentage of the light saturated rate of gross photosynthesis and as a percentage of the photosynthetic performance as measured with the oxygen technique.

Rates of respiration varied one order of magnitude, between 0.014×10^{-8} and $0.486 \times 10^{-8}\mu molO_2$ $cell^{-1}h^{-1}$. Values were markedly influenced by temperature and irradiance, but did not appear to be

Table 2.4.5. Rates of respiration ($\mu molO_2\ l^{-1}h^{-1}$) in unfractionated samples and in the fraction passing through 1μm filters in cultures of *Pycnococcus provasolii,* measured at various algal (Ω) and bacterial cell concentrations (cell $ml^{-1}x10^{-5}$). The percentage contribution by the fraction smaller than 1μm to the overall respiration rate (%) is also shown.

Ω	Bacteria		Respiration rate		
	unfrac	<1μm	unfrac	<1μm	%
0.54	nd	0.82	0.299	0.209	69.8
2.70	nd	2.30	1.433	0.209	14.6
6.32	3.23	1.91	1.918	0.374	19.5

nd= not determined

greatly affected by the photoperiod. At low and intermediate irradiances respiration rates appeared to increase more or less steadly with increasing photon flux density. Above a certain level of irradiance, i.e. around $110\mu Em^{-2}s^{-1}$, however, rates of respiration remained at similar levels for each temperature. A comparison of respiration rate per cell at two irradiances can be made to derive Q_{10} values for respiration at $55\mu Em^{-2}s^{-1}$ (Q_{10}= 2.25) and $360\mu Em^{-2}s^{-1}$ (Q_{10}= 2.12) growth irradiances.

As a percentage of P_{max} respiration varied from 3.2 to 19.5%. Lowest values were estimated for cells grown at low photon flux densities. R/P_{max}(%) was similar for cells grown at 10 and 20°C under 12:12h photoperiod, and was rather higher for cells grown under continuous light than for those grown under 12:12h. As a percentage of the photosynthetic performance, variations with irradiance were not clear. Highest values were recorded at 20°C under continuous light and minima at 10°C under light and dark cycle.

Figure 2.4.11 is a plot of respiration rate as a function of gross photosynthetic performance (P_i). The trend was a linear increase of respiration as photosynthetic performance increased up to a level of P_i, beyond which further increases of P_i did not appear to be paralleled by increases in respiration rate.

Growth rate was also positively correlated (r=0.902, p<0.001) with respiration rates over the entire range of growth rates measured (Figure 2.4.12).

Table 2.4.6. Rates of respiration (R) and photosynthetic performance (P_i) ($\mu molO_2$ $cell^{-1}h^{-1}$) and respiration to photosynthesis ratios at various irradiances (I_g, in $\mu Em^{-2}s^{-1}$), temperatures and photoperiods for cells of *Pycnococcus provasolii*. Values of growth rate (μ) and algal cell concentration are also given.

I_g	R ($x10^{-8}$)	R/P_{max} %	R/P_i %	P_i ($x10^{-8}$)	μ h^{-1}	cell ($x10^5$ ml^{-1})
			A: 10°C, 12:12h			
3	0.014	nd	8.3	0.168	nd	3.79
10	0.051	nd	17.1	0.298	0.005	2.02
20	0.078	4.5	15.0	0.520	0.009	3.14
55	0.079	nd	7.9	1.002	0.011	3.53
110	0.161	11.8	11.6	1.387	0.012	3.96
360	0.193	9.5	14.8	1.304	nd	2.78
			B: 20°C, 12:12h			
22	0.220	nd	18.8	1.170	0.022	2.57
30	0.162	3.2	nd	nd	nd	1.40
55	0.178	nd	9.8	1.816	0.020	11.32
110	0.393	5.8	13.2	2.977	0.026	2.09
360	0.410	9.5	9.4	4.361	0.031	4.35
			C: 20°C, 24h			
25	0.183	7.8	36.7	0.498	0.016	2.81
65	0.377	12.3	28.7	1.313	0.027	2.39
140	0.486	16.6	26.8	1.813	0.029	4.50
260	0.321	15.7	19.6	1.637	0.033	6.00
370	0.422	19.5	20.7	2.038	0.041	2.95

nd= not determined

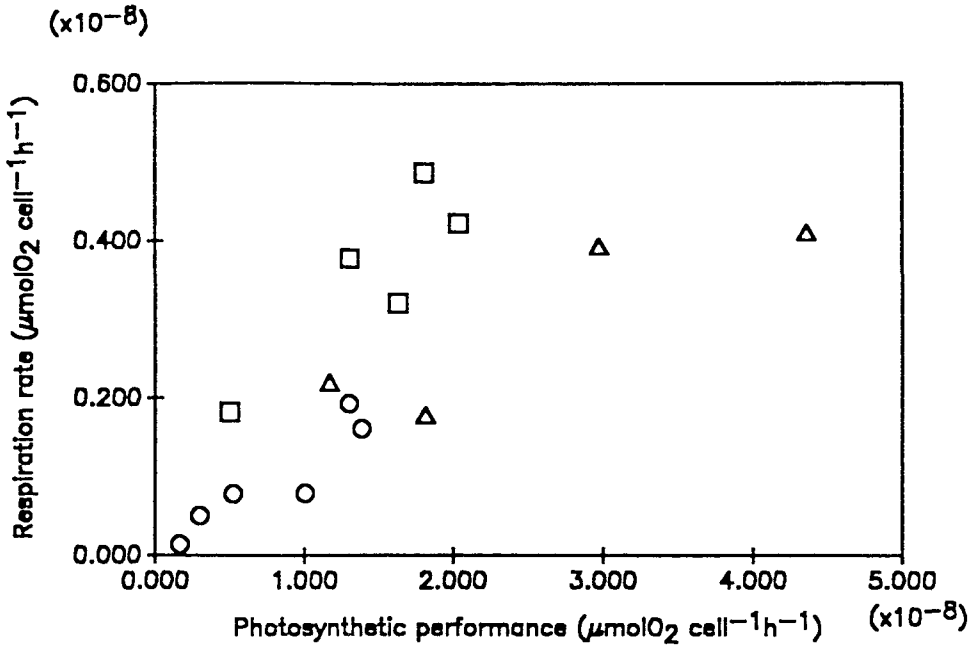

Figure 2.4.11. Relationship between respiration rate and gross photosynthetic performance in *Pycnococcus provasolii*. Data have been combined for various growth conditions: (o) 12:12h L:D cycle and 10°C; (Δ) 12:12h L:D cycle and 20°C and (□) continuous light and 20°C.

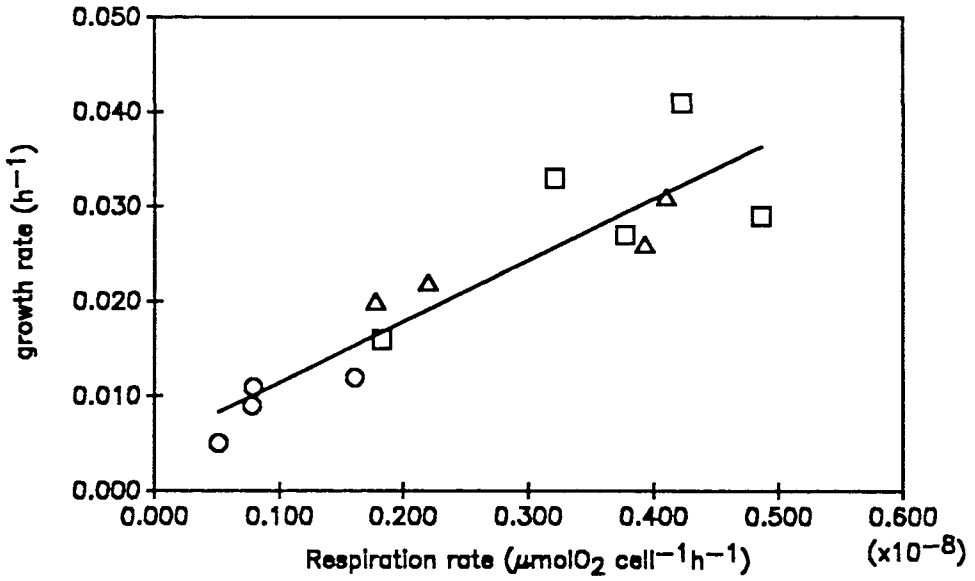

Figure 2.4.12. Relationship between growth rate and respiration rate in *Pycnococcus provasolii*. Data have been combined for various growth conditions: (o) 12:12h L:D cycle and 10°C; (Δ) 12:12h L:D cycle and 20°C and (□) continuous light and 20°C.

2.4.5. Growth rates

From the batch growth experiments growth rates were calculated as rates of increase in cell number, chlorophyll *a* and carbon biomass. Data are presented in Table 2.4.7. A good agreement between growth rates in terms of cell number and chlorophyll *a* concentration was found. Growth rates in terms of increases in carbon concentration often appeared to be lower than cell number and chlorophyll *a* concentration based ones, particularly at intermediate and high photon flux densities.

The relationship between growth rate and photon flux density can be described as a saturation type and a curve expressed by a hyperbolic tangent function (described in section 2.3.2.4) was fitted to the data. μ *v* I curves are presented in (Figures 2.4.13 and 2.4.14). Values of light saturated growth rate (μ_{max}), growth efficiency (α_g), specific respiration rate (μ_o) and irradiance for saturation of growth ($I_{\mu k}$) thus obtained are presented in Table 2.4.8. Estimates of α_g denoted steep slopes in the initial linear portion of the curve at the three temperatures and the two photoperiods investigated. The linearity, however, was only maintained in a very narrow range of photon flux densities, and the curve showed signs of bending towards the horizontal (probably a saturation indication) at relatively low photon flux densities (10-25$\mu Em^{-2}s^{-1}$), although μ_{max} was not achieved until photon flux densities reached values higher than around 100$\mu Em^{-2}s^{-1}$. In the majority of cases α_g was calculated from the first two points of the curve and the availability of and/or differences in growth rates at the very low photon flux densities (lower than 10$\mu Em^{-2}s^{-1}$) appeared critical for the estimation of α_g. Given the standard errors involved in the estimation of the growth efficiencies, α_g was not significantly different between 10 and 20°C under L:D cycle, nor between 10 and 15°C under L:D cycle, nor between L:D and continuous light at 20°C. α_g was significantly higher at 20°C and 15°C under 12:12h photoperiod, but this may have been influenced by the unavailability of growth rate data at 3$\mu Em^{-2}s^{-1}$ at 15°C.

The highest standard error for μ_{max}, however, was only 10% of the estimated value of μ_{max}. For algae grown under 12:12h photoperiod the difference in μ_{max} between 15 and 20°C was statistically insignificant, values ranging between 0.0246 and 0.0284h^{-1} (i.e.

minimum generation times of 28.1 and 24.4 hours respectively) in terms of cell number and chlorophyll *a* biomass. At 10°C, however, μ_{max} was markedly reduced, reaching only half the magnitude at the two highest temperatures ($0.0129h^{-1}$, minimum generation time of 53.7 hours). In terms of increases in carbon concentration μ_{max} was lower than in terms of cell number and chlorophyll *a* concentration increase, reaching values of 0.0164 and $0.0196h^{-1}$ at 15 and 20°C respectively. No growth rate data are available in terms of carbon increase at 10°C. Algae grown at 20°C and continuous light showed enhanced (1.3 fold) μ_{max} in relation to algae grown at the same temperature and 12:12h photoperiod. However the enhancement was very moderate considering the 2 fold increase in the total daily irradiance.

Irradiances for saturation of growth ($\mu_{max}/\alpha_g = I_{\mu k}$) were remarkably low, generally below $25\mu Em^{-2}s^{-1}$. Estimates of the intercept on the y axis (μ_o) yielded positive values at 20°C, but the standard errors involved in the estimation were large enough to make values of μ_o statistically not different from very small negative values. Compensation irradiances derived as μ_o/α_g showed variability upon biomass indicators, but were generally lower than $5\mu Em^{-2}s^{-1}$.

Growth rates obtained at 15 and 20°C and 12:12h L:D cycle were plotted as a function of carbon to chlorophyll *a* ratios (Figure 2.4.15). The pattern of variation was a linear positive relationship between the two parameters up to a saturation point, beyond which further increases in the carbon to chlorophyll *a* ratio were not acompanied by increases in growth rate.

Growth rate (μ_{cell}) data for algae grown under light:dark cycle at 10 and 20°C were found to be well correlated with the net photosynthetic performance ($r=0.893$, $p<0.01$) (Figure 2.4.16).

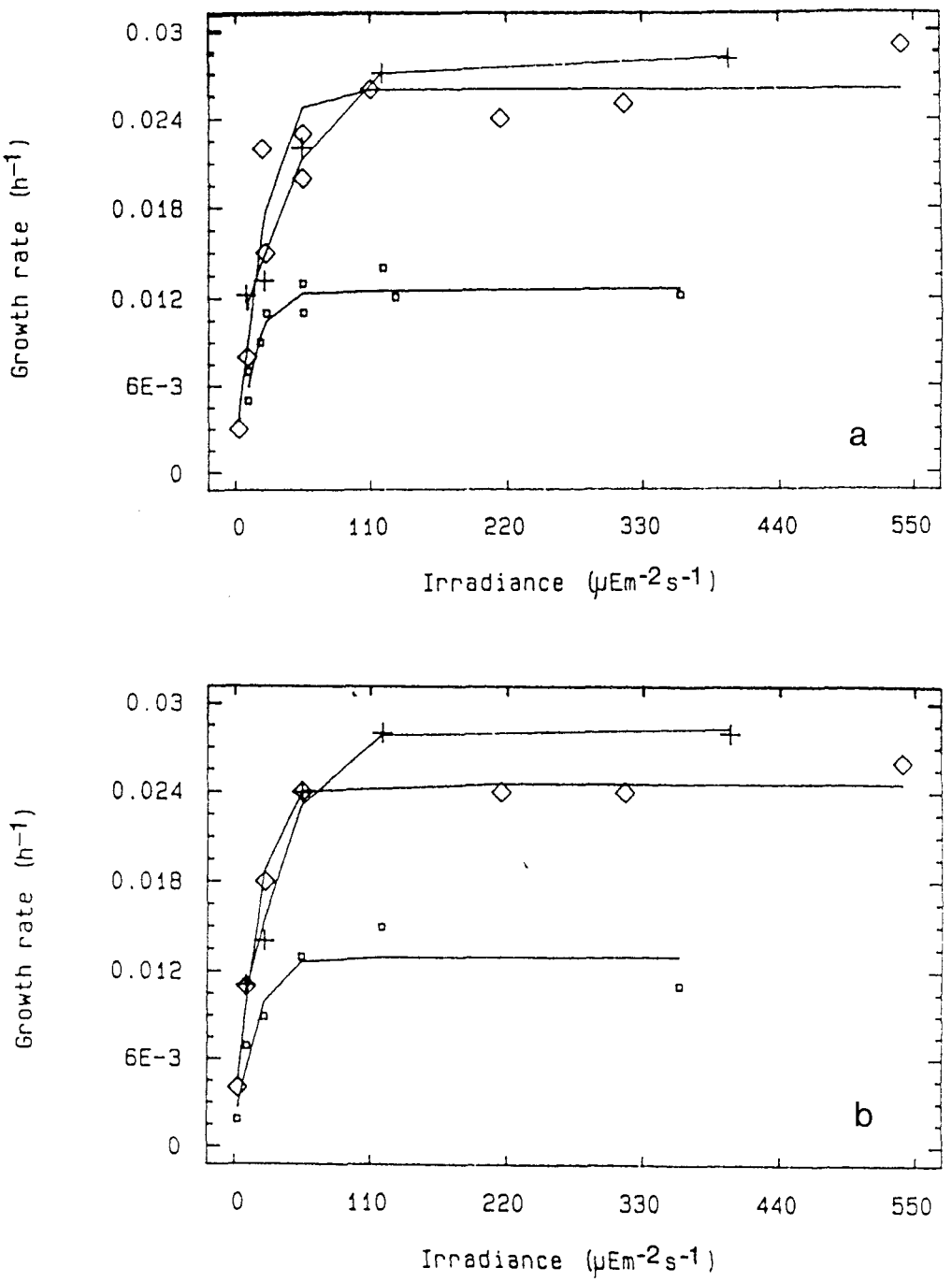

Figure 2.4.13.A comparison of μ *v* I curves for cells of *Pycnococcus provasolii* grown under 12:12h L:D cycle at various temperatures: (□) 10°C, (+) 15°C and (◊) 20°C, determined from (A) increases in cell number and (B) increases in chlorophyll *a* concentration.

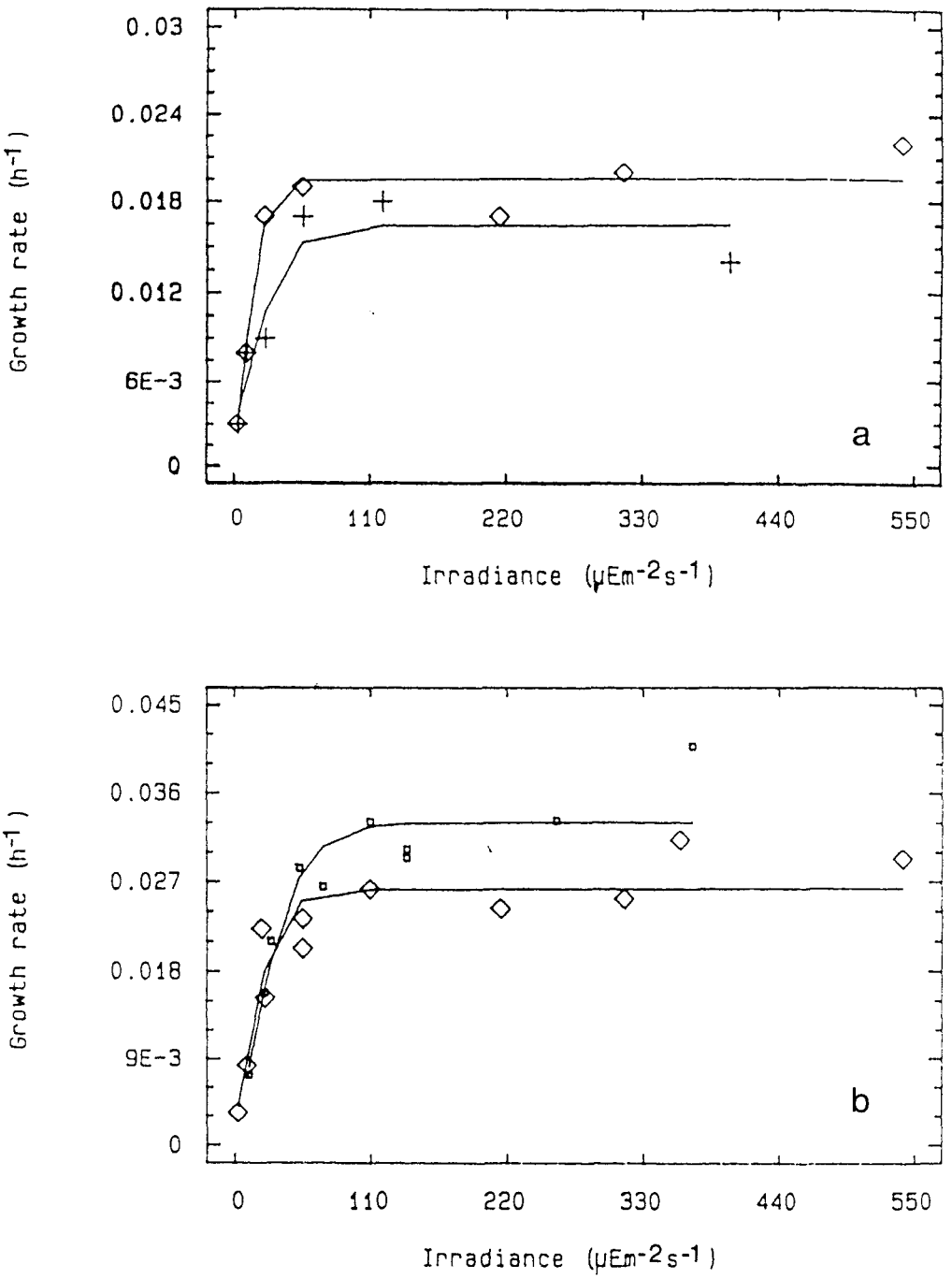

Figure 2.4.14. (A) A comparison of μ *v* I curves for cells of *Pycnococcus provasolii* grown under 12:12h L:D cycle at two temperatures: (+) 15°C and (◇) 20°C, determined from increases in POC concentration. (B) A comparison of μ *v* I curves for cells of *Pycnococcus provasolii* grown at 20°C under two photoperiods: (□) continuous light and (▽) 12:12H L:D cycle, determined from increases in cell number.

Table 2.4.7. Growth rates (h^{-1}) calculated as rates of increase of different biomass indicators for *Pycnococcus provasolii* grown at various combinations of photon flux density (I_g in $\mu Em^{-2}s^{-1}$), photoperiod (PhP, 24h light or 12:12h light:dark cycle) and temperature (T^a in °C) conditions.

I_g	PhP	T^a	μ_{cell}	$\mu_{chl\ a}$	μ_C
3	12	10	-	0.002	-
	12	15	-	-	0.003
	12	20	0.003	0.004	0.003
10	12	10	0.007	0.007	-
	12	15	0.012	0.011	0.008
	12	20	0.008	0.011	0.008
	24	20	0.007	-	-
25	12	10	0.011	0.009	-
	12	15	0.013	0.014	0.009
	12	20	0.015	0.018	0.017
	24	20	0.016	-	-
55	12	10	0.013	0.013	-
	12	15	0.022	0.024	0.017
	12	20	0.023	0.024	0.019
	24	20	0.029	-	-
120	12	10	0.014	0.015	-
	12	15	0.027	0.028	0.018
	24	20	0.033	-	-
215	12	20	0.024	0.024	0.017
260	24	20	0.033	-	-
360	12	10	0.012	0.011	-
360	12	15	0.028	0.028	0.014
315	12	20	0.025	0.024	0.020
370	24	20	0.041	-	-
540	12	20	0.029	0.026	0.022

- no value

Table 2.4.8. Parameters of the μ *v* I curves derived for cells of *Pycnococcus provasolii* grown at various temperatures (T^a) and photoperiods, using different biomass indicators: cell number (cell), chlorophyll *a* (chl *a*) and carbon (C). Values in parenthesis are standard errors.

T^a	biomass	μ_{max}	μ_o	α_g	$I_{\mu k}$
10	cell	0.0125(0.0005)	-0.0003(0.0030)	0.0011(0.0006)	13
	chl a	0.0129(0.0012)	-0.0002(0.0026)	0.0010(0.0010)	13
15	cell	0.0281(0.0012)	-0.0060(0.0019)	0.0007(0.0002)	42
	chl a	0.0284(0.0010)	-0.0040(0.0019)	0.0009(0.0002)	31
	C	0.0164(0.0017)	-0.0010(0.0028)	0.0009(0.0005)	18
20	cell	0.0260(0.0015)	0.0018(0.0043)	0.0019(0.0007)	17
	chl a	0.0246(0.0006)	0.0005(0.0014)	0.0020(0.0003)	12
	C	0.0196(0.0009)	0.0022(0.0026)	0.0022(0.0007)	9
*20	cell	0.0361(0.0017)	0.0034(0.0071)	0.0017(0.0007)	21

T^a (°C); μ_{max} and μ_o (h^{-1}); α_g ($h^{-1}(\mu Em^{-2}s^{-1})^{-1}$); $I_{\mu k}$ ($\mu Em^{-2}s^{-1}$)

* 24h continuous light; the rest of values obtained under 12:12h L:D cycle

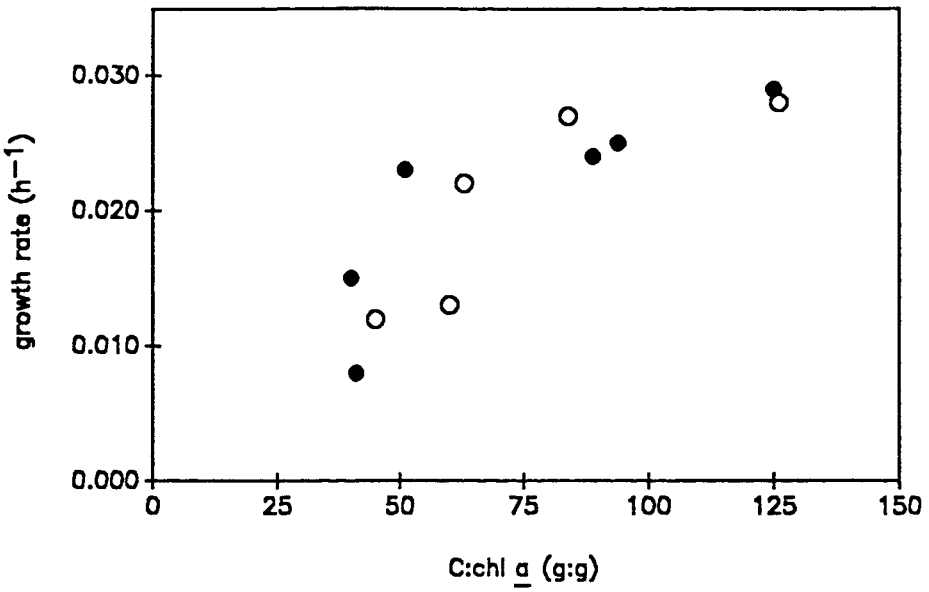

Figure 2.4.15. Relationship between growth rate and carbon to chlorophyll *a* ratio in cells of *Pycnococcus provasolii* grown under 12:12h L:D cycle at (o) 15°C and (●) 20°C.

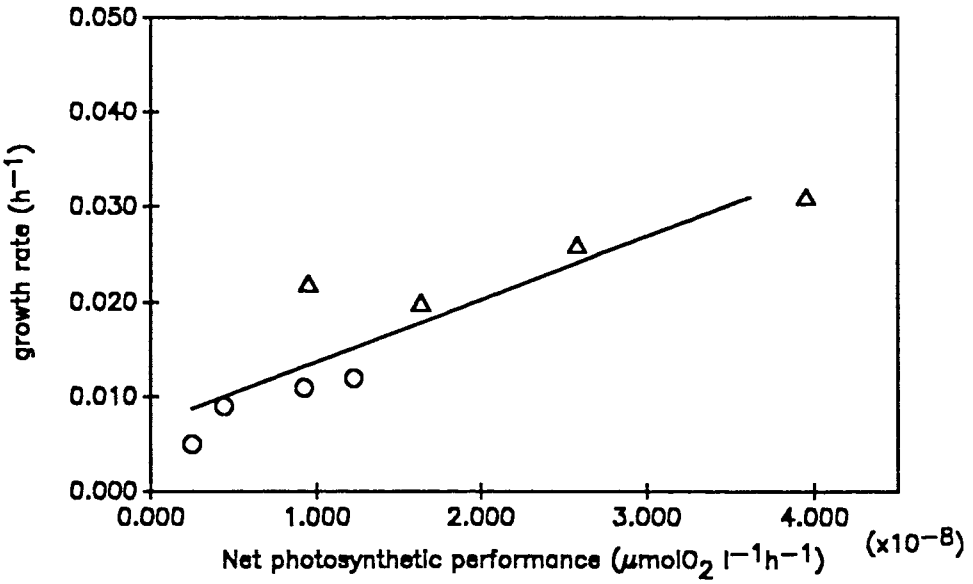

Figure 2.4.16. Relationship between net photosynthetic rate and growth rate in *Pycnococcus provasolii* grown under 12:12h L:D cycle at (o) 10°C and (Δ) 20°C.

2.5. DISCUSSION

2.5.1. Cellular composition and photosynthesis

Phytoplankton have been shown to respond to qualitative and quantitative variations in light with rapid morphological, biochemical and physiological adjustments that tend to offset the disadvantageous conditions and optimize growth rates (Richardson *et al.*, 1983).

A very generalized response to variations in irradiance involves changes in the concentration and relative composition of pigments. Typical variations in cellular chlorophyll *a* concentration range between 2 and 4 fold in an irradiance range between 10 to 500$\mu Em^{-2}s^{-1}$ (Kirk, 1983). In *Pycnococcus provasolii* cellular chlorophyll *a* and chlorophyll *b* decreased exponentially with photon flux density from 3 to 400$\mu Em^{-2}s^{-1}$, chlorophyll *a* decreasing about 4 fold and chlorophyll *b* decreasing about 6 fold, indicating an enhanced light harvesting capability at low irradiances.

An exponential decrease in cellular chlorophyll *a* content with increasing irradiance is a common trend in phytoplankton (e.g. Steeman Nielsen and Jørgensen, 1968; Falkowski and Owens, 1980). In some microalgae at very low irradiances a slight bleaching of chlorophyll *a* has been noted, e.g. *Dunaliella euchlora* and *Skeletonema costatum* (Falkowski and Owens, 1980) at irradiances below 8$\mu Em^{-2}s^{-1}$. In *Gonyaulax polyedra* a marked reduction in chlorophyll *a* and peridinin was observed at irradiances below around 62$\mu Em^{-2}s^{-1}$ (Prézelin and Sweeney, 1978), which was interpreted as an indication of photostress. In some species chlorophyll *a* per cell does not show great variations with irradiance, as for example in *Scenedesmus obliquus* (Senger and Fleischhacker, 1978), *Olisthodiscus lutheus* (Langdon, 1987) and *Dytilum brightwellii* (Perry *et al.*, 1981) or even decreases with decreasing photon flux density, as in the diatom *Thalassiosira fluviatilis* (Perry *et al.*, 1981). But in all these species in which cellular chlorophyll *a* content did not increase at low irradiance, cells showed a reduction in packed volume with decreasing irradiance, and chlorophyll *a*, when expressed on a volume basis, augmented with decreasing irradiance.

Some species reduce their cell size at low irradiances, i.e.

reduce the cell components other than the photosynthetic machinery, mainly reserve compounds (Cook, 1963; Morris *et al.*, 1974), so that with the same or slightly increased light harvesting capacity, there is less carbon to be turned over. Reducing the cell size provides the advantage of arranging the photosynthetic pigments more efficiently, i.e. by diminishing the package effect (Kirk, 1983). Other phytoplankton species, including *Pycnococcus provasolii*, however, do not show appreciable cell carbon content changes with growth irradiance (Verity, 1981; Raps *et al.*, 1983) and compensate for the low incident luminous energy by increasing substantially the size of photosynthetic apparatus. There is no clear evidence of a distinction of pattern with the species cell size or taxonomic position. The abundant and ubiquitous picoplankter *Synechococcus* spp. did not show variations of cell carbon with irradiance, although a slight increase in cell volume was observed under low light conditions (Kana and Glibert, 1987a). It may not be feasible for picophytoplankton to undergo significant reductions in cell size or carbon content because of minimum requirements of incompletely scalable cell components.

The C:chl *a* ratio, i.e. a measure of the amount of photosynthetic machinery employed to achieve a certain level of cell energy content, is thus a better indicator of photoadaptational response than the cellular chlorophyll content. Geider (1987), on reviewing data available in the literature, found that the C:chl *a* ratio increases linearly with increasing irradiance at constant temperature and decreases exponentially with temperature at constant photon flux density. The intercept of the linear regression of C:chl *a* (g:g) on photon flux density ($\mu Em^{-2}s^{-1}$) was found to increase from 6 to 40 gC gchl a^{-1} between 30 and 0°C, and the slope to augment from 0.04 to 1.9 gC gchl a^{-1} $(\mu Em^{-2}s^{-1})^{-1}$ over the same temperature range. The present observations for *Pycnococcus provasolii* at 15 and 20°C showed an intercept of 45.7, somewhat higher than would be expected for the temperature tested, and a slope of 0.17, which is in reasonable agreement with findings for other algae grown at these temperatures. The large value for the intercept may be due to the large amounts of chlorophyll *b* that this algae accumulates, and if the ratio was expressed as C:(chl *a* + chl *b*), the value would be closer to those found for other algae not containing chlorophyll *b*. Geider

(1987) did not report clear taxonomic differences in the slope of the linear regression of C:chl *a* ratio with irradiance.

Elevated ratios of accessory pigments to chlorophyll *a* with decreasing levels of irradiance, indicative of an enhanced light harvesting capacity in relation to reaction centers, have been reported for phytoplankton from a variety of taxa: dinoflagellates (Prézelin, 1976; Coats and Harding, 1988), cyanobacteria (Vierling and Alberte, 1980; Kana and Glibert, 1987a), diatoms (Falkowski and Owens, 1980) and chlorophytes (Falkowski and Owens, 1980). The magnitude of the values for chl *b*/chl *a* ratios determined by Hooks *et al.* (1988) for *Pycnococcus provasolii* grown under white light, of around 0.72 (g:g) (extracted from figure 5) at 140$\mu Em^{-2}s^{-1}$, increasing to around 0.88 (g:g) at 5.2$\mu Em^{-2}s^{-1}$ (extracted from figure 5) are in accordance with the values determined in this work. Although the variation is not very marked, the consistency of pattern observed at the three different temperatures tested in the present study shows the changes to be clearly light dependent (Figure 2.4.6).

Data of cellular PON content made during this study are only available for cells of *P. provasolii* grown at 15°C. Values of PON did not show significant changes with growth photon flux density and neither did the C/N ratio. The increase in chlorophyll *a* and *b* with decreasing irradiance is suggestive of an increase in nitrogen content resulting from the concomitant increase in the proteins bound to the chlorophylls. It is not clear whether this increase in nitrogen would be large enough to result in a detectable change in PON concentration. If this were the case, however, a reduction in other nitrogen-containing compounds, most likely enzymes, must have taken place in the cells at low irradiances to maintain the overall nitrogen content invariant. In some phytoplankters the carboxylating activity of the enzyme RUBISCO has been shown to decrease in low light grown phytoplankton (Beardall and Morris, 1976; Senger and Fleischhacker, 1978; Rivkin *et al.*, 1982a). However, in *Pycnococcus provasolii*, since low light grown cells showed increased rates of light saturated photosynthesis in relation to high light grown cells, the nitrogen offset was not likely balanced by a reduction in carboxylating enzymes of the dark reactions. However, a reduction in enzyme levels of the respiratory metabolism may have occurred, since low light grown cells

exhibited lower rates of respiration per cell. The atomic C:N ratio in phytoplankton has been claimed to approximate the Redfield ratio at maximal growth rates (Goldman *et al.*, 1979). In agreement with this suggestion, Sakshaug *et al.* (1984) found C:N ratios of 6.6 in several dinoflagellates growing under non-limiting conditions. Other workers, however, have shown the ratio to vary among species and low ratios of 3-4 are not uncommon (Falkowski and Owens, 1980; Shifrin and Chisholm, 1981; Raps *et al.*, 1983; Moal *et al.*, 1987) and values higher than 10 have also been reported (Shifrin and Chisholm, 1981; Moal *et al.*, 1987). The C:N ratio has been suggested not to be related to light limitation (Moal *et al.*, 1987) and light limited cells of *Pavlova* sp. have been shown to exhibit C:N ratios approximating the Redfield ratio (Tett *et al.*, 1985), which is in agreement with results obtained in this work for *Pycnococcus provasolii*.

The variations in the concentrations of pigments can be related to changes in the size and/or number of photosynthetic units (PSU). Whether cells change the size or number of PSU's has been suggested can be inferred from the comparison of the light saturated rates of photosynthesis (P_{max}) and initial slope (α) of the P *v* I curves normalized to cell number and to chlorophyll *a* concentration from cells grown at low and high light intensities (Prézelin and Sweeney, 1979; Prézelin, 1981; Ramus, 1981).

If results obtained at 20°C (both 12:12h and 24h photoperiods), i.e. non temperature limiting conditions, are considered, cells of *Pycnococcus provasolii* exhibited reduced P_{max} and α values normalized to cell number under high photon flux density. An increase in the number of PSU's has been suggested to be associated with an enhancement of the cells photosynthetic capacity (Prézelin, 1976; Armond and Mooney, 1978), if the amount of oxygen produced per unit of PSU remains constant. Falkowski (1980), however, has argued that O_2 per PSU may vary as a result of shade adaptation, suggesting that intersystem electron carriers may limit electron flow and light saturated rate of photosynthesis. Vierling and Alberte (1980) suggested that high light grown cells of *Anacystis nidulans* are capable of producing more O_2 per reaction center I than low light grown cells. Kirk (1983) has further pointed out that an increase in the number of PSU's does not have to be necessarily associated with an

enhancement of P_{max} per cell, since low levels of enzymes of the dark reactions of photosynthesis can be responsible for lowering the rate of light saturated photosynthesis. Conversely, higher amounts of carboxylating enzymes could be responsible for increasing the rates of light saturated rates of photosynthesis. There are no reports, however, of enhanced levels of ribulose bisphosphate carboxylase at low irradiance, and in the present case the reduced P_{max} at high light may be indicative of a slight decrease in the number of PSU's.

When P_{max} and α are expressed per unit of chlorophyll *a* biomass, clearly high light grown cells (i.e. cells with lower chlorophyll content) displayed elevated rates at all light levels. The loss of efficiency of the chlorophyll *a* in producing photosynthetic output as the cells accumulate more chlorophyll *a* per unit volume, has been ascribed to a reduction in the absorption cross section normalized to chlorophyll a, i.e. increased package effect (Geider and Osborne, 1986). Interpretation in terms of PSU suggests an increase in the size of the PSU's, i.e. larger increases in the light harvesting pigments in relation to those of reaction center chlorophylls, and the subsequent loss of energy in the transference of energy from the former to the latter. The trend of increase in the chlorophyll *b/a* ratio observed in *Pycnococcus provasolii* with decreasing photon flux density supports the contention of an increased light harvesting capability in relation to reaction center chlorophylls (Falkowski, 1980), and thus of some increase in the size of the PSU.

The validity of this procedure of interpretation of P *v* I curve parameters to predict changes in PSU number and size has been questioned (Gallagher *et al.*, 1984; Barlow and Alberte, 1985) and hence some caution should be exercised. If the interpretations are correct, *Pycnococcus provasolii* appears to compensate for low irradiances mainly by increasing the size of PSU as well as possibly the number of them when grown at low photon flux density. In the various models predicting theoretical responses in the P *v* I curves of algae expressing different strategies of photoadaptation, suggested by Prézelin and Sweeney (1979), Ramus (1981), Prézelin (1981) and Richardson *et al.* (1983), no account has been made for the possibility of algae encompassing both strategies, i.e. variations in the size as well as the number of PSU's. However, there are reports of

phytoplankton species suggested to exhibit both types of changes in PSU, e.g. the cyanobacterium *Synechococcus* clone WH7803 (Kana and Glibert, 1987a) and the dinoflagellate *Prorocentrum mariae-lebouriae* (Coats and Harding, 1988), as determined from methods other than the analysis of the P *v* I curve parameters.

Another indication of positive photoadaptation to low light levels is the lower requirement of light intensity for saturation of photosynthesis, which was brought about despite the increases in P_{max} in low light grown cells of *Pycnococcus provasolii*.

Oxygen derived P *v* I curves showed significant reduction of photosynthesis at irradiances over saturation for cells grown at low photon flux densities, a characteristic typical of shade adapted algae. ^{14}C derived P *v* I curves, however, did not evidence photoinhibitory responses, which suggests larger rates of photorespiration in low light grown cells as responsible for the low I_b values (see section 2.5.2). It is not clear what is the physiological role of photorespiration, if indeed there is one, but it has been suggested that photorespiratory energy consumption may protect plants from photo-oxidative damage (Osmond and Björman, 1972; Heber and Krause, 1980). If this concept is correct, it seems sensible to suggest that low light grown cells, which accumulate larger amounts of photosynthetic pigments, should show higher rates of photorespiration as a means of energy dissipation when incubated at high irradiances.

The initial slope and light saturated rate of photosynthesis of cultures of *Pycnococcus provasolii* were sensitive to changes in photoperiod. Both parameters were enhanced under 12:12h relative to 24h light regime when normalized to cell number as well as to chlorophyll *a* concentration. Cells showed higher chlorophyll *a* concentrations under 12:12h and this explains at least partially the higher rates of photosynthesis expressed on a cell number basis. Increased P_{max} even when normalized to chlorophyll *a* under a photoperiod in which cells accumulated more chlorophyll *a* can be indicative of increases in the enzymes of dark reactions of photosynthesis. The elevated initial slopes of the P *v* I curve normalized to chlorophyll *a* concentration are suggestive of smaller

PSU's and increased number of PSU's under 12:12h in comparison to growth under continuous light. Increasing the number of PSU's may be more advantageous than increasing the size of PSU's when changing to growth conditions of shorter photoperiod, since the instantaneous light dose does not become more limiting, and thus increasing the light harvesting capability may not be so essential.

There is little information in the literature on the effect of daylength on the photosynthetic parameters and on the variations in the size and number of PSU's in marine phytoplankton. Verity (1981) observed the opposite effect to that described for *Pycnococcus provasolii* with the diatom *Leptocylindricus danicus*, which, when grown under 9:15h (L:D) photoperiod had lower P_{max} and α values normalized to chlorophyll *a* concentration, relative to cells grown under 12:12h (L:D). Cells grown under 12:12h and 15:9h, however, did not seem to show appreciable differences in α and P_{max} in this diatom.

A comparison of growth rates of *Pycnococcus provasolii* at three different temperatures showed that growth at 10°C resulted in reduced ability to photosynthsize at all growth irradiances. The most pronounced effect was a reduction in P_{max} (by more than 2 fold), not only on a cell number basis, but also when normalized to chlorophyll *a* concentration. This is likely to be due primarily to the lower thermal energy supply for the catalysts of dark reactions (Davison, 1991). α was also reduced on a cell number basis, though to a lesser extent (1.48 fold). This may be explainable to some extent by the slight chlorosis exhibited by cells grown at 10°C. Reduced cellular chlorophyll *a* content with decreased temperature has also been observed in other microalgae, e.g. the diatoms *Leptocylindricus danicus* (Verity, 1981), *Phaeodactylum tricornutum* and *Nitzschia closterium* (Morris and Glover, 1974), *Skeletonema costatum* (Yoder, 1979) and may be the result of a reallocation of cell resources to the catalysts of dark reactions at the expense of light harvesting pigments (Geider, 1987).

The similarity of α normalized to chlorophyll *a* concentration at different temperatures, for cells grown at similar growth photon flux densities, suggests the specific reaction rates for the light reactions of photosynthesis are independent of temperature and thus

have no enzymatic control. This contrasts with results obtained in other studies in which a positive relationship of α with temperature was found and an enzymatic control suggested (Verity, 1981; Palmisano *et al.*, 1987).

As a consequence of the higher Q_{10} for P_{max} than for α, photosynthesis saturated at a lower irradiance at low temperature for *Pycnococcus provasolii*. This pattern was also observed with the cryptophyceae *Cryptomonas erosa* (Morgan and Kalff, 1979). This lowering of I_k was clearly not indicative of an enhanced performance at low light when grown at the lowest temperature and shows the need for knowledge on both P_{max} and α when interpreting the parameter I_k.

In contrast to findings for other algae (Davison, 1991), *Pycnococcus provasolii* did not appear to be more susceptible to photoinhibition at low temperature.

2.5.2. Photosynthetic quotients

The molar ratio of oxygen produced to carbon dioxide assimilated (PQ) estimated in the present study for cultures of *Pycnococcus provasolii* was in the range 0.7 to 4.2 and two trends of variation with irradiance were apparent: a) PQ's decreased with incubation irradiance above saturation levels; b) PQ's in the entire range of incubation irradiance were reduced for algae grown at lower photon flux densities.

The algae are assumed to have been growing on nitrate, given that the culture medium was prepared with nitrate (900μM) and PQ's in the range of *ca.* 2 would have been expected on grounds of the nitrogen PQ (Williams *et al.*, 1979; Davies and Williams, 1984; see section 1.5.2 in chapter one). Incubation time was short, i.e. 3 hours and respiration was at the most around 36% of photosynthesis (see Table 2.4.6), so the ^{14}C uptake is likely to have been closer to gross photosynthesis than to net photosynthesis.

It is not clear if any of the previous hypothesis put forward to explain higher PQ's at low photon flux densities, i.e. a higher affinity of enzymes involved in reducing nitrate to ammonium than the carboxylating enzymes for photoreductants (Megard *et al.*, 1985) or a preferential uptake of $^{12}CO_2$ in relation to ^{14}C-bicarbonate (Andersen

and Sand Jensen, 1980) at low irradiances, could explain the lower PQ's observed at subsaturating irradiance levels in the present study with *P. provasolii*, since PQ's as high as 3 to 4 were estimated at irradiances up to $500\mu Em^{-2}s^{-1}$. Fahnenstiel and Carrick (1988) have reported PQ's as high as 2.78 at surface irradiance, but their incubation periods were of 9 to 16 hours and the underestimation of gross ^{14}C uptake was suggested could account for the high PQ in their study.

In the present study the marked depression of photosynthetic rate observed at high incubation irradiances in the oxygen *P v I* curves was not apparent in the carbon based *P v I* curves. This would suggest that the decrease in the photosynthetic rates was not essentially due to photochemical damage to the photosynthetic apparatus, unless these damages had affected more severely the oxygenic photosynthesis, i.e. the light reactions, and had a minor effect on the dark reactions, which could be the case if there is an uncoupling between the two sets of reactions. The decrease of the PQ at irradiances above saturation can thus be explained in terms of an underestimation of the gross photosynthetic rate by the oxygen method resulting from the failure to account for the losses due to photorespiration. This was also apparent in measurements undertaken with laboratory cultures of *Gyrodinium aureolum* (Garcia, 1989).

A similar trend of variation of reduced PQ's for cells grown at lower growth irradiances to that observed for *Pycnococcus provasolii* has also been observed for *Gyrodinium aureolum* (Garcia, 1989), but the opposite trend was noted for *Braciomonas* sp. (Garcia, 1989). This contrasts with the pattern of increased PQ with depth observed in natural plankton assemblages (Holligan *et al.*, 1984; Megard *et al.*, 1985). C/N ratios did not vary with growth irradiance for *P. provasolii* at 15°C and if these results are applicable to 20°C, the variation in PQ cannot be accounted for by variations in the carbon/nitrogen metabolism. Another light induced biochemical effect on the photosynthetic apparatus is likely to be responsible, which is presumably species-specific and therefore probably reflects the different strategies of photoadaptation such as variations in the size and or number of PSU's and possible variations in the rate of oxygen production per PSU and its coupling with the dark reactions of

photosynthesis.

2.5.3. Respiration rates

Rates of respiration derived from this study should be taken with some caution due to the presence of bacteria in the cultures. However, the pattern of variation of respiration with growth irradiance and the results from size-fractionated respiration rate measurements suggest that at the algal cell concentrations at which the measurements were undertaken the largest proportion of the rates of respiration are attributable to the phototrophs, the bacteria probably being responsible for some of the noise observed in these patterns.

Rates of respiration in the dark by *Pycnococcus provasolii* were influenced by growth irradiance and temperature. Cells grown at higher irradiances and at higher temperatures showed elevated rates of oxygen consumption. The respiratory metabolism results in a net loss of carbon, at the same time providing energy in the form of ATP and reductant power, and carbon skeletons. Respiration rate is thus expected to be controlled primarily by the energetic demands and these can be divided into maintenance requirements, swimming demands and biosynthetic requirements.

Maintenance respiration rate shows a marked interespecific variability (Langdon, 1988; Geider and Osborne, 1989), varying from from 0.01 to $0.4d^{-1}$. The energetic cost of flagellar motility in dinoflagellates has been estimated to account for only 10^{-3} of the energy required for overall cell synthesis (Raven and Richardson, 1984).

It is generally assumed that under conditions of non-temperature limitation respiration rate is largely regulated by the rate of biosynthesis (Geider and Osborne, 1989). Under conditions of high biosynthetic activity there will be a demand for carbon skeletons and maybe also for ATP and NADPH, if the supply through photophosphorylation is not enough, thus increasing the rate of respiration. Under reduced rates of biosynthetic activity respiration rate will be limited through a reduced supply of substrates for oxidation. This is in agreement with the present findings that growth

at low irradiances was accompanied by lowered rates of respiration in *Pycnococcus provasolii*. This pattern has already been reported for a variety of phytoplankters (Verity, 1982; Falkowski *et al.*, 1985; Grobelaar and Soeder, 1985; Geider *et al.*, 1985; Langdon, 1987) and has been interpreted as a means of reducing carbon losses as the photon flux density for growth decreases (Raven and Glidewell, 1975). Other studies, however, have not shown variations in respiration rate with growth irradiance (Geider and Osborne, 1986).

The positive correlation between growth rate and respiration rate observed for *Pycnococcus provasolii* has also been noted for other phytoplankton species (Myers and Graham, 1971; Laws and Caperon, 1976; Laws and Bannister, 1980; Verity, 1982a; Langdon, 1987). This has been taken as an indication of the dependence of respiration rate on the biosynthetic requirements. However, since growth can be viewed as the outcome of the difference between the synthetic and the loss processes of carbon, the emphasis should probably be placed in the relationship between respiration and photosynthesis. A positive correlation between respiration rate and photosynthesis rate was observed in the present study and has also been reported for other phytoplankton species (Myers and Graham, 1971; Verity, 1982a). Similarly to the present observations Verity (1982a) found the relationship to be independent of temperature and daylength and to deviate from linearity at high photosynthetic rates, being best described by a power curve. This pattern suggests that photosynthetic rate undergoes larger variations than respiration rate upon changes in environmental parameters (i.e. daylength, irradiance and temperature).

At 20°C low light grown cells respired more in proportion to their rates of photosynthesis (25-35%) than cells grown at saturating irradiance (10%). The same trend becomes apparent in the data reported by Falkowski *et al.* (1985) for *Isochrysis galbana* and by Langdon (1987) for *Skeletonema costatum and Olisthodiscus lutheus.* Geider and Osborne (1989) expressed respiration as a proportion of growth rate and for a variety of microalgae they found respiration to be about 20-30% of growth rate under optimal conditions of light and nutrients and the ratio of respiration rate to growth rate to increase under suboptimal conditions.

Expressed as a percentage of the light saturated rates of

photosynthesis (R/P_{max} %), however, respiration clearly increased with growth photon flux density. This was the result of both, the increase of respiration with growth irradiance and the decrease of P_{max} with growth irradiance. A similar trend was reported by Prézelin and Sweeney (1978) for *Gonyaulax tamarensis*. Falkowski and Owens (1980), however, found no variation in the R/P_{max} ratio with growth irradiance for *Dunaliella tertiolecta* and *Skeletonema costatum*, since respiration and P_{max} increased proportionately with growth photon flux density. Thus R/P_{max} is not always good at identifying specifically respiratory responses, since the ratio will be affected not only by the influence of growth irradiance on respiration but also on P_{max} (Verity, 1982a).

Respiration rate has classically been considered to be only 10% of the light saturated rate of photosynthesis (Steeman Nielsen and Hansen, 1959; McAllister *et* al., 1964; Parsons *et al.*, 1984b) and is often adopted in such constant proportion in phytoplankton productivity models (Tett, 1987; Murray, 1991). There is growing evidence, however, that respiration is a variable proportion of the light saturated rate of photosynthesis, depending on environmental conditions such as irradiance, temperature and pH, reaching values as high as 50% (Falkowski and Owens, 1978; Prézelin and Sweeney, 1978).

A decrease of respiration rate with decreasing temperature observed for *Pycnococcus provasolii* (Q_{10} 2.1-2.2) can best be understood in the context of the thermal dependence of enzyme mediated reactions. Reduced rates of respiration will also be influenced by the lower photosynthetic activity imposed by the lower temperature. Reynolds (1984) recorded a similar value of Q_{10} for respiration for natural freshwater plankton samples dominated by *Asterionella.* Verity (1982a) found a stronger temperature dependence of respiration rate in the diatom *Leptocylindricus danicus*, with an estimated Q_{10} of 4.0 at μ_{max}. Morgan and Kalff (1979) also found respiration rate to increase with temperature in the cryptophyte *Cryptomonas erosa.* Ryther and Guillard (1962), however, were unable to show a consistent trend in the respiratory response to temperature in six marine diatoms.

2.5.4. Growth rates

The minimum generation time (i.e. $\log_e 2/\mu_{max}$) recorded of 19.2

hours in continuous light and 24.4 hours in 12:12h photoperiod at 20°C compare well with the 19 hours estimated for this isolate by Glover *et al.* (1987) in 14/10 hours L:D cycle at saturating irradiance and under the same temperature. It is well established for metazoans and heterotrophic unicells that metabolic rate is an inverse power function of organism size and an exponent of 0.75 is commonly found (i.e. 3/4 rule). Similarly maximum growth rate has been suggested to be inversely related to cell size in phytoplankton (Banse, 1976; Schlesinger *et al.*, 1981; Geider *et al.*, 1986; Langdon, 1988). Various authors have suggested different coefficients for the inverse power expression. Blasco *et al.*, (1982) formulated the relationship as:

$$\mu_{max} = 0.1084W^{-0.1414}$$

where

μ_{max} is the growth rate at 320μEm^{-2}s^{-1} (h^{-1})

W is the cell carbon content (pg C cell^{-1})

Langdon (1988), on analysing another set of data found the following expression:

$$\mu_{max} = 4.261W^{-0.28}$$

where

μ_{max} is the maximum growth rate (div d^{-1})

W is the cell carbon content (pg C cell^{-1})

However, the maximum growth rate of *Pycnococcus provasolii* appears to be rather low for an organism in this size range (2-3μm in diameter, around 3pgC cell^{-1}) according to the relationship between size and μ_{max} found by both, Blasco *et al.* (1982) and Langdon (1988). Langdon's equation predicts a μ_{max} of 3.12div d^{-1} (minimum generation time of 7.7 hours) for cells of 3pgC cell^{-1}. The equation derived by Blasco *et al.* (1982) predicts values of 3.2div d^{-1} (7.4 hours minimum generation time). Similarly, for a picoplankter in the size range of *Synechococcus* spp., 0.25pgC cell^{-1} (Kana and Glibert, 1987a), the model of Langdon (1988) predicts a μ_{max} of 6.28div d^{-1} (minimum generation time of 3.8 hours) and the equation of Blasco *et al.* (1982)

predicts a light saturated growth rate of 4.56div d^{-1} (5.2 hours minimum generation). Kana and Glibert (1987a), however, empirically recorded a μ_{max} of only 1.87d^{-1} (minimum generation time of 9 hours), and Morris and Glover (1981) and Iriarte (1986), for the same strain, determined a minimum generation time of 11 and 12 hours respectively. In the derivation of the equation by Langdon (1988) no data for organisms below 5pgC $cell^{-1}$ (as observed from the graph) were included and in the data set analyzed by Blasco *et al.* (1982) the smallest species was of 11pgC $cell^{-1}$. Hence neither of the two models was tested at the very low end of the size spectrum.

A comparison of the two different light regimes: 24h and 12:12h photoperiod, shows that α_g is similar and μ_{max} is slightly higher under continuous light. The algae attain similar growth rates even with half the amount of light on a daily basis. It could be anticipated that cells under 12:12 L:D cycle should show either enhanced rates of photosynthesis, decreased rates of respiration or reduced packed cellular volume, or a combination of any of these characteristics. Cellular carbon content and respiration rates appeared to be similar under both photoperiods. Growth rates were maintained by greatly enhacing the rates of photosynthesis, which was achieved at least partially by increased pigmentation under L:D cycle, possibly involving an incresae in the number of PSU's.

Brand and Guillard (1981) investigated the effect of continuous light and light and dark cycle on the growth rates of a taxonomically wide array of species and concluded that there was not a unique trend: some species reproduced faster in continuous, others under L:D cycle and some grew at roughly the same rate under both conditions. No phylogenetical trend was observed. Data suggested that species from coastal waters tend to reproduce at the same rate or even more rapidly in continuous light than in L:D cycle, whilst many oceanic species are harmed by continuous irradiance. *Pycnococcus provasolii* is an oceanic isolate, but does not seem to be affected negatively by the 24 hour light regime, although light energy was less efficiently utilized under the continuous light conditions. Increases in μ_{max} under continuous light less than proportional to the increase in daily light dose are not uncommon (Foy and Gibson, 1976; Yoder, 1979).

A comparison between temperatures (10 and 20°C) showed a clear decrease in μ_{max} at low temperature, a feature well documented in the literature for phytoplankton (Cloern, 1977; Yoder, 1979; Meeson and Sweeney, 1982; Verity, 1982b; Post *et al.*, 1985). Data in the literature suggests growth rates of *Pycnococcus provasolii* continue to increase at temperatures higher than 20°C (e.g less than 12 hours of generation at 24°C (Guillard *et al.*, 1991)). The reduction in μ_{max} with decreasing temperature observed in the present study was mainly ascribed to the reduced rates of photosynthesis at high irradiances of cells grown at 10°C in comparison to those grown at 20°C, a feature likely to be related to a reduced activity of the enzymes of the dark reactions of photosynthesis. There was no clear indication of changes in other parameters of the μ *v* I curve, i.e. the growth efficiency, the saturation light intensity and the maintenance respiration rate, with temperature.

For cultures of *Pycnococcus provasolii* grown at 15 and 20°C under L:D cycle, growth rates were shown to be maintained at near maximal rates only in a range of C:chl *a* ratios. C:chl *a* ratios below this range were accompanied by decreased growth rates. This pattern has been observed for other phytoplankton species (Geider, 1987) and can be interpreted as decreases in C:chl *a* ratio not being able to fully compensate for the light limitation below a certain level of luminous energy supply.

Despite the difficulties encountered to derive reliable values of α_g, given the rapid deviation from linearity at low photon flux densities observed for the isolate in this study, it appears to be higher than average, following Langdon's (1988) review. This can be interpreted as an efficient management of resources at the very low photon flux densities, these algae being adapted to low light better than the majority of phytoplankton species. Saturation of growth occurred at very low photon flux densities, 13-42$\mu Em^{-2}s^{-1}$, owing to both steep initial slopes and low μ_{max}. I_k for photosynthesis, however, was around 66-239$\mu Em^{-2}s^{-1}$. Saturation of growth rate at lower irradiance than photosynthesis as derived from short term incubation measurements is a common feature in phytoplankton (Beardall and

Morris, 1976; Prézelin and Sweeney, 1978; Morris and Glover, 1981; Verity, 1982; Garcia, 1989), although the reasons behind are as yet poorly understood. It was also difficult to obtain reliable values of compensation irradiance (i.e. large standard errors of estimates). This can be accounted for by the difficulties associated with accurately measuring low irradiances, and those related with obtaining a range of irradiances in the very low end of the irradiance spectrum, with the technique used for varying irradiance in this study (i.e. combination of layers of mosquito netting) and also, with the difficulties associated with the measurement of slow growth rates. These pitfalls have also been experienced in other studies (Verity, 1982b).

In summary, *Pycnococcus provasolii* appears as a well suited organism to growth at low irradiance, a capacity achieved mainly by increasing the light harvesting potential, aided to some extent by reducing carbon losses through reduced rates of respiration. This capacity to grow with low fluxes of light, together with previous findings of efficient growth under blue-violet and blue light (i.e. the wavelength bands with deepest penetration in the water column) (Glover *et al.*, 1987), are a good reflection of the general habitat of this species, the depth of the pycnocline in open ocean waters (Guillard *et al.*, 1991). The increased chlorophyll *b* (Jeffrey, 1976; Gieskes *et al.*, 1978; Vernet and Lorenzen, 1987) and prasinoxanthin concentrations (Hooks *et al.*, 1988) associated with the pycnocline areas of oceanic waters, suggest species such as *Pycnococcus provasolii* can prove typical significant members of the chlorophyll maximum layer of stratified oceanic waters. These are also the areas where picophytoplankton have been found to make the most significant contribution to the overall oceanic phytoplankton community primary production (Li *et al.*, 1983; Platt *et al.*, 1985) and *Pycnococcus provasolii* is thus likely to be a prominent primary producer in the worlds oceans.

CHAPTER THREE

CONCLUSIONS AND FUTURE WORK

3.1. Studies with natural plankton assemblages

3.1.1. Studies in Southampton Water

The temporal and spatial distribution of total and size fractionated chlorophyll *a* biomass and primary production were investigated in Southampton Water, with particular reference to the picoplankton size fraction.

In the estuary proper, the temporal cycle of chlorophyll *a* biomass was characterized by a double, spring and summer, peak. In agreement with previous studies (Bryan, 1979; Rees and Williams, 1982), the timing of the spring phytoplankton bloom is suggested to be primarily dominated by the flushing rate. In 1990 the colonial prymnesiophyte algae *Phaeocystis* sp. was shown to dominate the spring bloom (i.e. first ever reported in this estuary), as opposed to diatoms in previous studies (Savage, 1965; Burkill, 1978; Kifle, 1989). Recovery in nutrient levels during summer months, likely to result mainly by effluent discharges and the respiratory activity in the water column, provided conditions for a bloom of major proportions in mid summer, which was dominated by the red-water forming phototrophic ciliate *Mesodinium rubrum*, as has been reported in previous studies (Rees and Williams, 1982; Soulsby *et al.*, 1984; Whitfield, 1985; Wilde, 1988; Kifle, 1988; Antai, 1990; Crawford, 1991). At the mouth of the estuary, the maximum chlorophyll *a* concentration was recorded in spring. The greater dilution effect by the offshore waters causes nutrient levels in this area to be lower than in the inner estuary. It is hypothesized that, in summer, phosphate concentration becomes limiting for phytoplankton growth in the estuary, resulting in low overall chlorophyll *a* biomass.

Along the longitudinal axis of the estuary (as determined from a survey carried on one day in September 1990) chlorophyll *a* concentration peaked in the mid estuary region, diminishing both towards the seaward and landward ends.

Estimates of the annual rate of total primary production (POC+DOC) indicated Southampton Water to be a moderately productive estuary: 207 and 162gC $m^{-2}yr^{-1}$ at NW Netley and Calshot Spit respectively. The only other estimate of annual production for Southampton Water is based on a survey carried out more than a decade ago (1973/1974) by Bryan (1979). Although estimates from the present study are between 30 and 90% higher than those reported by Bryan for similar positions in the estuary, the low temporal resolution of the sampling programmes of both studies (i.e. maximum twice per month) and year to year climatological variations preclude conclusions on possible trends of progressive eutrophication in the estuary.

Concentrations of PE-containing picocyanobacteria in the estuary ranged between $2x10^4$ and $1.3x10^7$ cells ml^{-1} and values are in good agreement with numbers recorded in other estuarine waters. PE-containing picocyanobacteria numbers were positively correlated with temperature, and the seasonal peak was recorded in summer at both stations sampled. True (i.e. excluding *Phaeocystis* cells) picoplanktonic eukaryotic algae also peaked during the summer, just after the peak of picocyanobacteria ($1.2x10^7$ cells ml^{-1}). This appears to be a generalized pattern in temperate coastal and estuarine waters. It is argued that numbers of picophytoplankton are likely to be readily controlled by grazing throughout most of the year. In summer, temperature enhanced growth rates and increased bacterial concentrations possibly allow the picophytoplankton biomass accumulation rate to increase above the grazing rate by protozoans.

The surface distribution of PE-containing picocyanobacteria along the estuary showed the concentration of cells to increase exponentially with increasing salinity, a pattern likely to reflect the requirement for large amounts of ions reported for cultures of PE-containing marine *Synechococcus* spp. (Waterbury *et al.*, 1986). Eukaryotic algae passing through a 5μm filter also showed an increase with salinity, but the relationship was not so clear.

From the vertical distribution pattern of PE-containing picocyanobacteria there was some evidence to suggest that they were more abundant deeper in the water column under stratified conditions, but not in mixed water columns.

Size fractionated measurements showed chlorophyll *a* levels in

the <1μm size fraction in Southampton Water to be similar to values reported for offshore waters. As a percentage of total phytoplankton standing stock and primary production rate, however, the <1μm fraction was a relatively minor component (*ca.* 6% of integrated annual plankton community primary production). The smaller than 3(5)μm fraction represented 20% of the total primary production. A comparison with data in the literature for other marine environments shows the impact of the picophytoplankton on phytoplankton production diminishes considerably from offshore (>50%) to coastal and estuarine waters (<20%).

The <1μm size fraction made a greater contribution to the plankton community primary production rate than to the chlorophyll *a* biomass, whereas the reverse was the case for the >3(5)μm size fraction, and values of α^B and P_m^B were significantly larger for the <1μm size fraction than for the >3(5)μm size fraction. There was no clear trend for the 1-3(5)μm size fraction.

The percentage contribution of the <1μm and the 1-3(5)μm was shown to generally decrease with increasing chlorophyll *a* biomass. It is argued that the size structure of the phytoplankton community is primarily influenced by the concentration of larger phytoplankton cells and thus factors limiting the accumulation of these larger forms are the primary determinants of the switch from a large cell dominated system to a small cell dominated system. Thus in Southampton Water, the <1μm and the 1-3(5)μm size fractions, although in absolute concentrations were more numerous in summer, they made up for a larger proportion of the total phytoplankton community in winter and autumn, when larger phytoplankton were likely to be more severely light limited. At the mouth of the estuary, similarly to the pattern described for coastal waters (e.g. Joint *et al.*, 1986), the <1μm size fraction had the greatest impact in summer, when larger phytoplankton were more likely to be nutrient limited.

The importance of these investigations on picophytoplankton distribution and productivity carried out in Southampton Water relies on their originality, i.e. there are virtually no other data of chlorophyll *a* concentration and primary production for the <1μm size fraction in temperate estuarine waters. This data set therefore provides indications that enable us to fill the gap in the global view

of the significance of this size fraction in the marine environment. Additionally, the investigation of spatio-temporal trends of variation in picophytoplankton abundance and productivity has provided useful information for a comprehensive analysis of factors determining the size structure of phytoplankton communities.

3.1.2. Studies in the North Sea

The distribution and abundance of PE-containing picocyanobacteria and size fractionated chlorophyll *a* biomass and primary production rate were investigated in central and southern North Sea waters during March and June/July 1989.

Numbers of PE-containing picocyanobacteria were in agreement with previous reports for the North Sea; they were found to be least numerous in areas near the east coast of England. Their vertical distribution pattern showed no evidence for a preferential accumulation deep in the euphotic zone, even under conditions of stratified water column and thus the genotypic nature of their possible low light adaptation characteristic can be further questioned.

In agreement with results obtained in Southampton Water, the <3μm size fraction contributed a mean of 20% to the plankton community chlorophyll *a* concentration in June/July. In contrast, the contribution to the rate of carbon fixation was only of the order of 10%. It is argued that the use of filter types of same nominal pore size, but different retention capacity, could be partly responsible for this large difference between the chlorophyll *a* and primary production percentage.

The <3μm size fraction was shown to be the major contributor to the plankton community respiration rate, thus suggesting that bacteria and microflagellates are primary consumers of photosynthetically fixed carbon.

Photosynthetic quotients typically ranged between 2 and 3. Values could not be explained in terms of the oxidation state of the inorganic nitrogen assumed to be assimilated. It is argued that up to 35% of the discrepancy between the oxygen technique and the ^{14}C technique can be explained in terms of the net/gross problem, but the

explanation for higher discrepancies is at present unclear.

3.2. Studies with laboratory cultures of *Pycnococcus provasolii*

The photosynthesis, respiration and growth response to varying conditions of temperature, photoperiod and irradiance in the oceanic micromonadophyceae *Pycnococcus provasolii* (clone Ω48-23) were investigated.

Values of Q_{10} for respiration rate, P_{max} and μ_{max} of approximately 2 were estimated, and this is consistent with a typical value for the Q_{10} of phytoplankton growth rate (Raven and Geider, 1988).

A comparison between continuous light and 12:12h L:D cycle showed a more efficient photosynthetic response (i.e. higher α and P_{max} values) under the reduced irradiance dose regime.

P. provasolii responded to growth at low irradiance with an increase in the light harvesting potential (increased chlorophyll *a* and *b* concentrations and increased chlorophyll *b* to *a* ratio). Investigation of P *v* I responses at various growth irradiances showed that at 20°C cells had lower P_{max} and α values normalized to cell number when grown at high irradiance, in comparison to growth at low and intermediate irradiances. The pattern suggested primarily an increase in the PSU size, although there were also indications of some simultaneous increase in the number of PSU's. Respiration rates were also shown to decrease with decreasing growth irradiance and a significant positive correlation was found between growth rate and respiration rate.

P. provasolii appeared to have very low requirements for white light to optimize growth rate ($I_{\mu k}$ values were in general lower than $20\mu Em^{-2}s^{-1}$) and it is concluded that it is a well suited organism to growth at low irradiance. This capacity, together with previous reports of its suitability to grow under blue/violet light (Glover *et al.*, 1987) shows its potential capacity to grow deep in the euphotic zone of stratified oceanic waters, where it has been found to be most abundant (Guillard *et* al., 1991). This is also the area where picophytoplankton have been shown to make the most significant contribution to the oceanic microflora standing stock and primary

production (Li *et al.*, 1983; Murphy and Haugen, 1985). If, as chemotaxonomic indications suggest, the prasinophyte (micromonadophyte)-like organisms are important components of the oceanic picophytoplankton (Hooks *et al.*, 1988), this species may prove a significant member of the chlorophyll maximum layer of stratified oceanic waters.

3.3. Future work

The eco-physiological investigations on picophytoplankton carried out in the present study have prompted a number of questions that need to be addressed in the near future for a better understanding of the factors controlling the spatio-temporal distribution of picophytoplankton and the size structure of phytoplankton assemblages in the marine environment.

A fundamental question arising from the studies of temporal distribution pattern of picophytoplankton in temperate coastal and estuarine waters is how influential is water temperature. Are Q_{10} values for growth of picocyanobacteria and other picophytoplankton higher than the Q_{10} values for growth of their grazers?. Is the increase in bacterial abundance in summer responsible for some reduction in grazing pressure upon picophytoplankton?. A simultaneous comparison of growth rates of picocyanobacteria and bacteria and grazing rates by microflagellates and ciliates throughout the year in temperate estuarine and coastal waters could provide useful information. Comparisons of Q_{10}'s for growth in laboratory grown cultures of picophytoplankton, bacteria and protozoan isolates together with measurements of ingestion rates by microflagellates and ciliates with varying concentrations of picophytoplankton and bacteria would provide complementary data.

The abundance of PE-containing *versus* salinity relationship suggested a growth requirement for high salinity, yet, PE-containing picocyanobacteria have also been reported in abundant numbers in low salinity waters (e.g. Baltic Sea, Kuosa, 1988; Jochem, 1988). Investigation of strain-specific variations in the salinity tolerance of picocyanobacteria could prove very useful.

In general, more extensive work on the significance of

picophytoplankton (both prokaryotic and eukaryotic) in temperate coastal and particularly estuarine areas is needed to compare and contrast the present results from Southampton Water and the North Sea and allow a more global assesment of the impact of small phytoplankton on the marine biota.

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APPENDIX

Table A.1. Seasonal variation of the vertical distribution of temperature (°C) at Calshot Spit and NW Netley.

Calshot Spit

Depth	Date								
(m)	24.01	21.02	07.03	05.04	23.04	08.05	22.05	04.06	20.06
1	8.3	nd	8.6	10.1	10.5	14.7	15.0	15.7	15.9
2	8.4	nd	nd	10.1	10.5	14.6	15.0	15.7	nd
3	8.4	nd	nd	10.2	10.5	14.5	15.0	15.7	15.9
4	8.4	nd	8.6	10.2	10.5	14.4	14.9	15.7	nd
5	8.4	nd	nd	10.2	10.5	14.4	14.9	15.7	nd
6	8.4	nd	nd	10.2	10.5	14.4	15.0	15.7	nd
7	8.4	nd	nd	10.2	10.5	14.3	15.0	15.7	nd
8	8.4	nd	8.6	10.2	10.4	14.3	15.0	15.7	15.9
9	8.4	nd	nd	10.2	10.4	14.3	15.0	15.7	nd
10	8.4	nd	nd	10.2	10.4	14.3	15.0	15.7	nd

Depth	Date				
(m)	03.07	02.08	31.08	18.09	01.10
1	16.3	20.4	18.9	17.5	15.9
2	16.3	20.1	19.0	17.5	15.9
3	16.3	19.8	19.1	17.5	15.9
4	16.3	19.9	18.8	17.5	15.9
5	16.3	19.9	18.8	17.5	15.9
6	16.3	19.8	18.9	17.5	15.9
7	16.3	19.9	19.2	17.5	15.9
8	16.3	nd	19.2	17.5	15.9
9	16.3	nd	nd	17.5	15.9
10	16.3	nd	nd	17.5	15.9

(Table A.1 cont')

NW Netley

Depth (m)	Date 24.01	21.02	07.03	05.04	23.04	08.05	22.05	04.06	20.06
1	nd	9.3	8.7	10.2	10.9	15.5	15.9	16.0	16.5
2	nd	9.3	8.7	10.5	10.8	15.4	15.9	16.0	16.5
3	nd	9.0	8.7	10.5	10.8	15.2	15.6	16.0	16.5
4	nd	9.2	8.7	10.5	10.8	15.1	15.6	15.9	16.5
5	nd	9.3	8.6	10.4	10.8	14.7	15.5	15.9	16.5
6	nd	9.3	8.6	10.4	10.8	14.6	15.5	15.9	16.4
7	nd	9.3	8.6	nd	10.7	14.6	15.3	16.0	16.4
8	nd	9.3	8.6	nd	10.7	14.6	15.2	nd	16.2

Depth (m)	Date 03.07	02.08	31.08	18.09	01.10
1	16.7	21.4	19.4	17.3	15.6
2	16.7	20.9	19.4	17.3	15.7
3	16.7	21.0	19.6	17.3	15.9
4	16.4	21.0	19.6	17.3	16.0
5	16.3	20.2	19.8	17.4	16.0
6	16.3	20.0	19.8	17.3	16.0
7	16.3	nd	19.8	17.4	16.0
8	nd	nd	19.5	17.4	16.0

nd= not determined

Table A.2. Seasonal variation of the vertical distribution of salinity (ppt) at Calshot Spit and NW Netley.

Calshot Spit

Depth	Date								
(m)	24.01	21.02	07.03	05.04	23.04	08.05	22.05	04.06	20.06
1	33.0	nd	32.0	32.4	32.8	33.3	33.4	33.4	33.7
2	33.0	nd	nd	32.4	32.8	33.3	33.4	33.4	nd
3	33.0	nd	nd	32.5	32.8	33.2	33.4	33.4	33.7
4	33.1	nd	32.2	32.6	32.8	33.2	33.4	33.4	nd
5	33.1	nd	nd	32.6	32.8	33.2	33.4	33.4	nd
6	33.1	nd	nd	32.6	32.8	33.2	33.4	33.4	nd
7	33.1	nd	nd	32.6	32.8	33.2	33.4	33.4	nd
8	33.3	nd	32.3	32.6	32.8	33.2	33.4	33.4	33.7
9	33.3	nd	nd	32.6	32.8	33.2	33.4	33.4	nd
10	33.3	nd	nd	32.6	32.8	33.3	33.4	33.4	nd

Depth	Date				
(m)	03.07	02.08	31.08	18.09	01.10
1	33.8	34.8	34.6	34.0	34.2
2	33.6	34.8	34.4	34.0	34.2
3	33.6	34.8	35.0	34.1	34.2
4	33.6	34.8	34.9	34.0	34.2
5	33.6	34.8	34.9	34.1	34.2
6	33.6	34.8	34.8	34.1	34.1
7	33.6	34.8	34.8	34.0	34.1
8	33.6	nd	34.8	34.0	34.1
9	33.6	nd	nd	34.1	34.1
10	33.6	nd	nd	34.1	34.1

(Table A.2 cont')

NW Netley

Depth	Date								
(m)	24.01	21.02	07.03	05.04	23.04	08.05	22.05	04.06	20.06
1	nd	27.1	29.3	30.0	31.1	31.7	31.4	32.1	32.3
2	nd	26.3	29.8	31.2	31.3	31.9	31.4	32.2	32.3
3	nd	30.6	31.2	31.5	31.4	31.9	31.8	32.3	32.3
4	nd	32.4	31.8	32.1	31.9	32.2	31.9	32.7	32.3
5	nd	32.6	32.2	32.2	31.8	32.4	32.0	32.9	32.3
6	nd	32.7	32.2	32.4	31.9	32.4	32.5	33.2	32.3
7	nd	32.6	32.3	nd	32.0	32.4	32.5	33.2	32.4
8	nd	32.6	32.3	nd	32.0	32.5	32.7	nd	32.6

Depth	Date				
(m)	03.07	02.08	31.08	18.09	01.10
1	31.4	33.3	33.4	33.1	31.5
2	31.9	33.2	33.6	33.1	32.0
3	32.8	33.0	33.8	33.1	33.0
4	32.9	33.4	34.3	33.2	33.6
5	33.5	33.8	34.3	33.3	33.8
6	33.5	33.8	34.5	33.4	33.8
7	33.5	nd	34.8	33.6	33.8
8	nd	nd	34.8	33.6	33.8

nd= not determined

Table A.3. Phosphate (P) and nitrate plus nitrite (N) concentration (μM) as measured in the three depth composite samples collected during the seasonal survey at Calshot Spit and NW Netley.

Date	Calshot Spit		NW Netley	
	N	P	N	P
24.01	21.0	1.2	39.5	3.94
21.02	29.7	5.1	38.3	11.98
07.03	5.6	0.6	10.2	0.90
05.04	11.5	1.0	18.4	1.02
23.04	6.3	1.5	29.2	3.33
08.05	4.2	0.2	1.8	0.13
22.05	4.9	0.1	-	0.13
04.06	2.9	0.3	34.7	0.75
20.06	4.4	0.3	10.2	1.10
03.07	7.3	0.2	10.5	0.44
02.08	3.5	0.1	2.0	0.29
31.08	17.9	2.0	7.2	0.71
18.09	7.2	0.7	16.1	0.95
01.10	6.2	0.9	65.2	2.40

-: sample lost

Table A.4. Mean size fractionated and total chlorophyll *a* concentration (mg m^{-3}) as measured in the depth composite samples from the seasonal survey at Calshot Spit and NW Netley.

Date	Size fraction			Sum of frac.	Tot (unfrac.)
	>3(5)μm	1-3(5)μm	<1μm		
Calshot Spit					
24.01	nd	nd	nd	nd	0.60
21.02	nd	nd	nd	nd	0.58
07.03	nd	nd	nd	nd	0.47
05.04	0.66	0.09	0.05	0.80	0.59
23.04	9.72	0.13	0.08	9.93	15.68
08.05	2.37	0.79	0.08	3.24	3.44
22.05	(1.19)	(1.18)	0.08	2.45	3.81
04.06	1.83	0.10	0.09	2.02	2.55
20.06	0.99	0.13	0.10	1.22	1.42
03.07	3.98	0.16	0.14	4.28	4.78
02.08	(2.58)	(0.46)	0.16	3.20	4.22
31.08	(1.90)	(0.51)	0.13	2.54	2.49
18.09	1.07	0.05	0.05	1.17	1.27
01.10	0.88	0.09	0.07	1.04	0.82
NW Netley					
24.01	nd	nd	nd	nd	0.76
21.02	nd	nd	nd	nd	0.52
07.03	nd	nd	nd	nd	0.52
05.04	0.35	0.05	0.04	0.44	0.46
23.04	3.72	0.12	0.04	3.88	4.95
08.05	8.69	2.13	0.08	10.89	10.99
22.05	(2.30)	(0.39)	0.03	2.71	3.32
04.06	1.15	0.11	0.14	1.40	1.69
20.06	1.34	0.12	0.13	1.59	1.71
03.07	8.79	0.20	0.13	9.11	11.55
02.08	(7.04)	(2.14)	0.56	9.74	18.84
31.08	(7.50)	(0.38)	0.20	8.07	8.92
18.09	1.56	0.05	0.07	1.69	1.83
01.10	0.90	0.08	0.10	1.08	1.11

nd: not determined

Table A.5. Mean size fractionated and total phaeopigment concentration (mg m^{-3}) as measured in the depth composite samples during the seasonal survey at Calshot Spit and NW Netley.

Date	Size fraction			Sum of frac.	Tot (unfrac.)
	>3(5)μm	1-3(5)μm	<1μm		
Calshot Spit					
24.01	nd	nd	nd	nd	0.82
21.02	nd	nd	nd	nd	1.09
07.03	nd	nd	nd	nd	0.43
05.04	0.30	0.03	0.03	0.36	0.57
23.04	1.18	0.09	0.04	1.31	0.84
08.05	0.80	0.14	0.01	0.95	1.24
22.05	(0.52)	(0.33)	0.06	0.91	0.66
04.06	0.64	0.07	0.05	0.76	1.20
20.06	0.27	0.07	0.08	0.42	0.65
03.07	1.50	0.12	0.13	1.75	1.14
02.08	(1.16)	(0.27)	0.16	1.59	1.97
31.08	(0.48)	(0.24)	0.11	0.83	0.95
18.09	0.82	0.03	0.05	0.90	1.12
01.10	0.35	0.06	0.04	0.45	0.62
NW Netley					
24.01	nd	nd	nd	nd	0.68
21.02	nd	nd	nd	nd	0.22
07.03	nd	nd	nd	nd	0.28
05.04	0.24	0.03	0.01	0.28	0.41
23.04	0.66	0.08	0.02	0.76	0.74
08.05	1.22	0.30	0.03	1.55	1.75
22.05	(0.35)	(0.21)	0.03	0.59	0.91
04.06	0.32	0.07	0.07	0.46	1.39
20.06	0.36	0.07	0.07	0.50	0.51
03.07	0.82	0.10	0.14	1.06	1.66
02.08	(4.83)	(0.60)	0.34	5.77	3.82
31.08	(0.83)	(0.22)	0.16	1.21	1.49
18.09	0.83	0.04	0.07	0.93	0.96
01.10	0.43	0.07	0.09	0.59	0.62

nd= not determined

Table A.6. Percentage contribution by the various size fractions to total phytoplankton community chlorophyll *a* concentration (sum of fractions) as determined in the three depth composite samples collected during the seasonal survey at Calshot Spit and NW Netley.

Date	Calshot Spit			NW Netley		
	>3(5)μm	1-3(5)μm	<1μm	>3(5)μm	1-3(5)μm	<1μm
05.04	82.9	11.3	5.8	79.4	12.2	8.4
23.04	97.9	1.3	0.8	95.9	3.1	1.0
08.05	73.2	24.4	2.4	79.8	19.5	0.7
22.05	(48.6)	(48.3)	3.1	(84.6)	(14.4)	1.0
04.06	90.1	5.2	4.7	82.4	7.8	9.8
20.06	81.5	10.4	8.1	84.1	7.8	8.1
03.07	92.9	3.8	3.3	96.4	2.2	1.4
02.08	(80.6)	(14.3)	5.1	(72.3)	(21.9)	5.8
31.08	(75.0)	(20.0)	5.0	(92.8)	(4.7)	2.5
18.09	91.0	4.5	4.5	92.5	3.1	4.4
01.10	84.6	8.7	6.7	82.9	7.8	9.3

Table A.7. Cell concentration of PE-containing picocyanobacteria (PEP) and red-fluorescing picophytoplankton (RFP) passing through 3(5)μm filters (cell ml^{-1}) as measured in the three depth composite samples from Calshot Spit and NW Netley.

	Calshot Spit		NW Netley	
Date	PEP	RFP	PEP	RFP
24.01	113	229	20	95
21.02	36	-	143	154
07.03	57	84	63	88
05.04	182	2077	323	2306
08.05	45	-	271	295922
22.05	(78)	(360)	(82)	(735)
04.06	187	1663	274	1550
20.06	687	3422	393	746
03.07	10080	2649	1532	167
19.07	(12570)	(158)	(13442)	(545)
02.08	(12883)	(7465)	(3684)	(13387)
31.08	(4142)	(9068)	(1889)	(5922)
18.09	1130	5000	539	6135
01.10	942	5580	1136	10561

-: not available

Table A.8. Carbon fixation rate (μmolC $l^{-1}h^{-1}$) by the various plankton size fractions (as measured by serial filtration) and by the total plankton community, as measured both, in the particulate phase i.e. retained on a 0.2μm filter (Total POC) and in the dissolved phase (Total DOC) in samples collected at Calshot Spit. Total DOC is also given as a % of Total POC+Total DOC. I_o is the mean irradiance (PAR) during the incubation period.

	% I_o					
	100	58	28	19	10	5.2
Date : 05.04	**I_o = 818 $\mu Em^{-2}s^{-1}$**					
>3μm	0.173	0.286	0.289	0.249	0.136	0.060
3-1μm	0.016	0.037	0.041	0.032	0.021	0.011
1-0.4μm	0.006	0.007	0.009	0.008	-	0.005
Sum of frac	0.195	0.330	0.339	0.289	-	0.076
Total POC	0.150	0.319	0.347	0.231	0.209	0.069
Total DOC	ud	0.016	0.026	0.005	0.001	ud
%DOC	-	4.8	6.9	2.2	0.6	-
Date : 23.04	**I_o = not determined**					
>3μm	3.350	3.110	2.674	2.034	0.977	0.484
3-1μm	0.059	0.058	0.072	0.044	0.036	0.027
1-0.4μm	0.040	0.040	0.040	0.030	0.017	0.014
Sum of frac	3.449	3.208	2.786	2.108	1.030	0.525
Total POC	2.929	3.413	2.958	2.188	1.520	0.626
Total DOC	0.321	0.355	1.189	0.830	0.724	0.222
%DOC	9.9	9.4	28.7	27.5	37.6	26.2
Date : 08.05	**I_o = 1193 $\mu Em^{-2}s^{-1}$**					
>3μm	0.642	1.522	0.969	0.548	0.260	0.174
3-1μm	0.069	0.146	0.126	0.063	0.022	0.031
1-0.4μm	0.040	0.042	0.039	0.034	0.022	0.010
Sum of frac	0.751	1.710	1.134	0.645	0.304	0.215
Total POC	0.813	1.004	0.842	0.767	0.452	0.224
Total DOC	0.737	0.166	0.775	0.323	0.213	0.439
%DOC	47.5	14.2	47.9	29.6	32.0	27.1
Date : 22.05	**I_o = 1389 $\mu Em^{-2}s^{-1}$**					
>5μm	0.829	1.786	1.580	0.845	0.578	0.414
5-1μm	0.367	0.469	0.605	0.481	0.336	0.197
1-0.4μm	0.082	0.114	0.149	0.090	0.051	-
Sum of frac	1.278	2.369	2.334	1.416	0.965	-
Total POC	1.453	1.586	1.412	1.043	0.846	0.420
Total DOC	0.242	1.954	2.116	0.902	0.399	0.156
%DOC	14.3	55.2	59.9	46.4	32.0	27.1

(Table A.8 cont')

Date : 04.06	**I_o = 706 $\mu Em^{-2}s^{-1}$**					
>3μm	0.962	0.978	0.723	0.591	0.388	-
3-1μm	0.080	0.074	0.074	0.041	0.023	-
1-0.2μm	0.084	0.051	0.038	0.037	0.028	-
Sum of frac	1.126	1.103	0.835	0.669	0.439	-
Total POC	1.002	1.015	0.889	0.721	0.383	-
Total DOC	0.008	0.020	-	0.048	0.050	-
%DOC	0.8	1.9	-	6.3	11.7	-
Date : 20.06	**I_o = 664 $\mu Em^{-2}s^{-1}$**					
>3μm	0.507	0.509	0.330	0.217	0.188	0.083
3-1μm	0.136	0.097	0.054	0.039	0.022	0.009
1-0.2μm	0.064	0.036	0.099	0.040	0.027	0.009
Sum of frac	0.707	0.642	0.483	0.296	0.237	0.101
Total POC	0.860	0.777	0.514	0.499	0.153	0.111
Total DOC	0.080	0.075	0.048	0.048	ud	-
%DOC	8.5	8.8	8.5	8.8	-	-
Date : 03.07	**I_o = 991 $\mu Em^{-2}s^{-1}$**					
>3μm	1.362	1.605	1.436	1.067	0.533	0.269
3-1μm	0.067	0.112	0.094	0.056	0.033	0.016
1-0.2μm	0.144	0.130	0.098	0.082	0.053	0.032
Sum of frac	1.573	1.847	1.628	1.205	0.619	0.317
Total POC	1.318	1.175	1.263	0.830	0.682	0.339
Total DOC	0.013	0.182	0.120	0.064	0.097	0.028
%DOC	1.0	13.4	8.7	7.1	12.5	7.6
Date : 02.08	**I_o = 1217 $\mu Em^{-2}s^{-1}$**					
>5μm	1.155	1.172	1.282	0.926	0.595	0.273
5-1μm	0.300	0.363	0.358	0.215	0.122	0.086
1-0.2μm	0.176	0.182	0.189	0.187	0.092	0.077
Sum of frac	1.631	1.717	1.829	1.328	0.809	0.436
Total POC	1.978	2.200	2.184	1.711	1.019	0.490
Total DOC	0.088	0.289	0.181	0.270	0.133	-
%DOC	4.2	11.6	7.6	13.6	11.5	-
Date : 31.08	**I_o = 335 $\mu Em^{-2}s^{-1}$**					
>5μm	0.853	0.481	0.375	0.171	0.080	0.042
5-1μm	0.202	0.131	0.089	0.046	0.032	0.014
1-0.2μm	0.085	0.080	0.047	0.030	0.028	0.016
Sum of frac	1.140	0.692	0.511	0.247	0.140	0.072
Total POC	0.841	0.676	0.517	0.240	0.232	0.091
Total DOC	0.032	0.116	0.060	0.033	0.123	-
%DOC	3.6	14.7	10.4	12.1	34.6	-

(Table A.8 cont')

Date : 18.09	**I_o = 305 $\mu Em^{-2}s^{-1}$**					
>3μm	0.268	0.199	0.171	0.084	0.055	0.037
3-1μm	0.021	0.032	0.031	0.022	0.009	0.004
1-0.2μm	0.007	0.101	0.100	0.008	0.007	0.006
Sum of frac	0.296	0.242	0.212	0.114	0.071	0.047
Total POC	0.644	0.200	0.269	0.107	0.097	0.047
Total DOC	0.004	0.005	0.070	ud	0.073	ud
%DOC	-	2.7	20.7	-	42.8	-
Date : 01.10	**I_o = 668 $\mu Em^{-2}s^{-1}$**					
>3μm	0.223	0.311	0.219	0.206	0.088	0.032
3-1μm	0.045	0.084	0.053	0.035	0.027	0.009
1-0.2μm	0.010	0.013	0.014	0.016	0.012	0.004
Sum of frac	0.278	0.408	0.286	0.257	0.127	0.045
Total POC	0.314	0.345	0.376	0.299	0.186	0.064
Total DOC	0.091	0.041	0.068	0.063	0.045	0.017
%DOC	22.4	10.6	15.3	17.3	19.4	21.4

ud= undetected

-= unreliable value or lost sample

Table A.9. Carbon fixation rate (μmolC $l^{-1}h^{-1}$) by the various plankton size fractions (as measured by serial filtration) and by the total plankton community, as messured both, in the particulate phase i.e. retained on a 0.2μm filter (Total POC) and in the dissolved phase (Total DOC) in samples collected at NW Netley. Total DOC is also given as a % of the Total POC+Total DOC. I_o is the mean irradiance (PAR) during the incubation period.

	% I_o					
	100	58	28	19	10	5.2
Date : 05.04	**I_o = 818 μEm^{-2}s^{-1}**					
>3μm	0.035	0.084	0.086	0.061	0.040	0.034
3-1μm	0.008	0.021	0.026	0.016	0.011	0.008
1-0.4μm	0.005	0.014	0.012	0.018	0.009	0.008
Sum of frac	0.048	0.119	0.124	0.095	0.060	0.050
Total POC	-	0.132	0.137	0.082	-	-
Total DOC	-	0.053	0.053	0.014	-	-
%DOC	-	28.8	27.8	15.1	-	-
Date : 23.04	**I_o = not determined**					
>3μm	1.099	1.378	1.109	0.672	0.374	0.185
3-1μm	0.051	0.059	0.058	0.046	0.017	0.012
1-0.4μm	0.044	0.032	0.030	0.026	0.013	0.011
Sum of frac	1.194	1.469	1.197	0.744	0.405	0.208
Total POC	1.251	1.187	1.535	0.748	0.339	0.174
Total DOC	0.022	0.194	0.183	0.013	-	0.027
%DOC	1.7	14.0	10.6	1.8	-	13.4
Date : 08.05	**I_o = 1193 μEm^{-2}s^{-1}**					
>3μm	1.737	2.526	1.594	1.179	0.804	0.438
3-1μm	0.075	0.187	0.199	0.142	0.115	0.084
1-0.4μm	0.131	0.142	0.123	0.091	0.077	0.032
Sum of frac	1.943	2.855	1.916	0.412	0.996	0.554
Total POC	1.609	2.489	1.828	1.127	1.173	0.419
Total DOC	1.276	1.197	0.506	2.978	0.748	0.134
%DOC	44.2	32.5	21.7	72.5	38.9	24.2
Date : 22.05	**I_o = 1389 μEm^{-2}s^{-1}**					
>5μm	1.152	-	1.711	0.945	0.584	0.258
5-1μm	0.155	0.196	0.217	0.190	0.139	0.079
1-0.4μm	0.046	0.061	0.065	0.046	0.039	0.039
Sum of frac	1.353	-	1.993	1.181	0.762	0.360
Total POC	1.244	1.535	1.264	1.337	0.749	0.281
Total DOC	0.030	0.236	0.315	0.270	0.130	0.002
%DOC	2.4	13.3	19.9	16.8	14.8	0.7

(Table A.9 cont')

Date : 04.06	**I_o = 706 $\mu Em^{-2}s^{-1}$**					
>3μm	0.525	0.545	0.426	0.393	0.303	-
3-1μm	0.047	0.061	0.039	0.027	0.026	-
1-0.2μm	0.127	0.047	0.052	0.462	0.373	-
Sum of frac	0.699	0.653	0.517	0.462	0.372	-
Total POC	-	0.722	0.563	0.463	0.495	-
Total DOC	-	ud	ud	0.010	0.015	-
%DOC	-	0.0	0.0	2.1	3.0	-
Date : 20.06	**I_o = 664 $\mu Em^{-2}s^{-1}$**					
>3μm	0.464	0.512	0.365	0.327	0.122	0.075
3-1μm	0.044	0.054	0.038	0.023	0.013	0.005
1-0.2μm	0.040	0.046	0.044	0.039	0.038	0.017
Sum of frac	0.549	0.611	0.447	0.389	0.174	0.097
Total POC	0.616	0.668	0.542	0.394	0.238	0.091
Total DOC	-	-	-	-	-	-
%DOC	-	-	-	-	-	-
Date : 03.07	**I= 991 $\mu Em^{-2}s^{-1}$**					
>3μm	3.097°	3.539	2.890	1.774	1.264	0.632
3-1μm	0.069	0.100	0.079	0.061	0.042	0.025
1-0.2μm	0.088	0.132	0.128	0.098	0.069	0.041
Sum of frac	3.254	3.772	3.097	1.933	1.375	0.698
Total POC	2.590	3.229	2.607	1.567	1.104	0.604
Total DOC	0.138	1.026	0.714	0.339	-	0.166
%DOC	5.1	24.1	21.5	17.8	-	21.5
Date : 02.08	**I_o = 1217 $\mu Em^{-2}s^{-1}$**					
>5μm	4.375	5.792	5.480	4.118	2.002	1.018
5-1μm	2.274	3.568	3.205	1.791	0.652	0.304
1-0.2μm	0.404	0.522	0.559	0.492	0.421	0.210
Sum of frac	7.053	9.882	9.244	6.401	3.075	1.532
Total POC	7.842	10.879	10.412	7.498	4.069	1.707
Total DOC	0.397	0.599	0.967	0.679	0.829	0.410
%DOC	4.8	5.2	8.5	8.3	16.9	19.4
Date : 31.08	**I_o = 335 $\mu Em^{-2}s^{-1}$**					
>5μm	3.228	2.462	1.437	0.780	0.555	0.269
5-1μm	0.267	0.194	0.126	0.058	0.049	0.030
1-0.2μm	0.130	0.103	0.083	0.042	0.036	0.026
Sum of frac	3.625	2.759	1.646	0.880	0.640	0.325
Total POC	3.311	3.391	1.771	1.216	0.575	0.366
Total DOC	ud	ud	0.143	0.033	ud	0.226
%DOC	0.0	0.0	7.5	2.7	0.0	38.1

(Table A.9 cont')

Date : 18.09	**I_o = 305 $\mu Em^{-2}s^{-1}$**					
>3μm	0.402	0.289	0.215	0.120	0.070	0.044
3-1μm	0.060	0.043	0.025	0.013	0.008	0.003
1-0.2μm	0.043	0.040	0.030	0.017	0.012	0.008
Sum of frac	0.505	0.372	0.270	0.150	0.080	0.055
Total POC	0.591	0.392	0.280	0.162	0.091	0.063
Total DOC	ud	ud	ud	0.019	ud	ud
%DOC	0.0	0.0	0.0	10.5	0.0	0.0
Date : 01.10	**I_o = 668 $\mu Em^{-2}s^{-1}$**					
>3μm	0.204	0.267	0.246	0.178	0.105	0.059
3-1μm	0.054	0.078	0.071	0.047	0.027	0.015
1-0.2μm	0.047	0.047	0.048	0.045	0.023	0.016
Sum of frac	0.305	0.392	0.365	0.270	0.155	0.091
Total POC	0.440	0.519	0.413	0.302	0.168	0.097
Total DOC	0.014	0.081	0.025	0.040	0.007	0.027
%DOC	3.0	13.5	5.8	11.8	4.2	21.7

ud= undetected

-= unreliable value or sample lost

Table A.10. Depth integrated daily rates of carbon fixation into particulate material (gC $m^{-2}d^{-1}$) by the various plankton size fractions, as determined from samples collected during the seasonal survey at Calshot Spit and NW Netley.

Date	Calshot Spit			NW Netley		
	>3(5)μm	1-3(5)μm	<1μm	>3(5)μm	1-3(5)μm	<1μm
05.04	0.171	0.024	0.007	0.066	0.017	0.013
23.04	2.131	0.046	0.030	0.837	0.042	0.026
08.05	0.526	0.061	0.023	0.841	0.095	0.060
22.05	(0.745)	(0.307)	0.066	(0.553)	(0.099)	0.034
04.06	0.640	0.046	0.041	0.350	0.030	0.042
20.06	0.317	0.058	0.051	0.237	0.021	0.033
03.07	0.693	0.043	0.061	1.172	0.036	0.054
02.08	(0.664)	(0.181)	0.119	(2.145)	(1.062)	0.273
31.08	(0.386)	(0.104)	0.068	(1.712)	(0.146)	0.092
18.09	0.068	0.010	0.006	0.084	0.010	0.012
01.10	nd	nd	nd	nd	nd	nd

nd= not determined due to unavailability of reliable daily irradiance data

Table A.11. Percentage contribution by the various size fractions to the daily depth integrated total plankton community rate of carbon fixation (sum of fractions; DOC not included) as measured from samples collected during the seasonal survey at Calshot Spit and NW Netley.

Date	Calshot Spit			NW Netley		
	>3(5)μm	1-3(5)μm	<1μm	>3(5)μm	1-3(5)μm	<1μm
05.04	84.6	11.9	3.5	68.8	17.7	13.5
23.04	96.5	2.1	1.4	92.5	4.6	2.9
08.05	86.2	10.0	3.8	84.4	9.6	6.0
22.05	(66.6)	(27.5)	5.9	(80.6)	(14.4)	5.0
04.06	88.0	6.3	5.7	82.9	7.1	10.0
20.06	75.9	12.8	11.3	81.4	7.2	11.4
03.07	87.0	5.4	7.6	92.9	2.8	4.3
02.08	(68.9)	(18.8)	12.3	(61.7)	(30.5)	7.8
31.08	(69.2)	(18.6)	12.2	(87.8)	(7.5)	4.7
18.09	81.0	11.9	7.1	79.2	9.5	11.3
01.10	75.6	18.7	5.7	67.0	18.4	14.6

Table A.12. Temperature (°C), salinity (ppt), and numbers of PE-containing picocyanobacteria (PEP) and red-fluorescing picophytoplankton (RFP) (cell ml^{-1}) counted on 5µm filter filtrates, along a longitudinal axis of the estuary from Redbridge to the vicinity of Calshot Spit buoy on September 3rd 1990.

Sample n°	salinity	T[a]	PEP	RFP
1	15.6	19.1	277	3138
2	16.5	18.8	75	2756
3	13.4	18.9	469	4966
4	12.0	18.9	272	4598
5	10.5	18.8	363	4941
6	14.5	19.1	557	5716
7	23.8	19.6	422	3422
8	27.8	19.9	749	4541
9	18.3	19.4	337	4931
10	11.8	18.9	124	6380
11	14.3	18.9	248	6805
12	15.0	19.0	304	7035
13	17.8	19.1	449	6103
14	14.9	19.0	215	4459
15	12.0	18.9	234	4032
16	8.9	18.7	113	3646
17	8.1	18.7	212	4480
18	7.5	18.7	91	4723
19	22.9	19.6	-	-
20	28.3	20.0	330	2477
21	26.3	19.9	523	5605
22	25.3	19.9	945	9939
23	24.0	19.7	541	-
24	29.1	19.9	374	10293
25	32.0	20.0	1090	9853
26	32.7	19.8	1196	7842
27	33.0	19.9	1397	5341
28	33.2	19.8	1460	8574
29	33.4	19.8	1515	4883
30	33.2	19.8	1246	7848
31	33.6	20.3	1715	13079
32	34.2	20.0	3208	13283
33	34.5	20.1	2738	10144
34	34.5	19.8	3609	12895
35	34.5	19.8	2779	10790

-: sample lost

Table A.13. Phosphate and nitrite plus nitrate concentration (μM) in samples collected along a longitudinal axis of the estuary from Redbridge to the vicinity of Calshot Spit buoy on September 3rd 1990.

Sample n°	Nitrate+nitrite	Phosphate
1	168.4	3.2
2	252.8	4.2
3	209.3	3.3
4	231.1	4.4
5	239.2	4.5
6	196.2	4.2
7	124.4	3.0
8	42.4	1.8
9	231.1	5.0
10	227.5	4.3
11	201.4	4.1
12	185.8	4.1
13	170.5	3.9
14	195.3	-
15	190.1	4.4
16	251.9	5.1
17	256.5	4.9
18	-	4.6
19	65.0	-
20	45.8	2.0
21	41.9	1.9
22	74.2	2.1
23	71.3	2.6
24	53.0	4.1
25	22.3	3.0
26	25.1	3.7
27	17.4	1.1
28	17.5	0.8
29	22.0	0.8
30	15.1	0.1
31	11.9	1.2
32	11.2	0.8
33	11.0	0.9
34	9.5	2.2
35	11.0	0.7

-: sample lost

Table A.14. Size fractionated chlorophyll *a* and phaeopigment concentration (mg m^{-3}) determined in samples collected along a longitudinal axis of the estuary from Redbrigde to the vicinity of Calshot Spit buoy on September 3rd 1990.

Sample	Chlorophyll *a*				Phaeopigment			
n°	>5μm	1-5μm	0.2-1μm	Sum	>5μm	1-5μm	0.2-1μm	Sum
1	*	*	*	*	*	*	*	*
2	1.59	0.39	0.10	2.08	0.44	0.47	0.18	1.09
3	1.47	0.40	0.11	1.98	0.36	0.45	0.18	0.99
4	1.40	0.37	0.10	1.87	0.29	0.44	0.20	1.03
5	1.10	0.47	0.07	1.64	0.38	0.62	0.17	1.17
6	2.05	0.36	0.13	2.54	0.42	0.47	0.21	1.10
7	4.45	0.33	0.18	4.96	0.64	0.31	0.18	1.13
8	4.83	0.34	0.16	5.33	0.59	0.29	0.15	1.03
9	1.28	0.49	0.06	1.83	0.24	0.48	0.14	0.86
10	0.96	0.24	0.03	1.23	0.29	0.38	0.12	0.79
11	2.21	0.23	0.08	2.52	0.51	0.37	0.17	1.05
12	2.08	0.40	0.10	2.58	0.66	0.52	0.18	1.36
13	2.50	0.32	0.10	2.92	0.38	0.36	0.10	0.84
14	3.40	0.51	0.17	4.08	1.35	0.74	0.34	2.43
15	1.81	0.41	0.15	2.37	0.99	0.73	0.36	2.08
16	0.97	0.37	0.13	1.47	0.66	0.65	0.35	1.66
17	1.28	0.49	0.09	1.86	0.91	0.97	0.27	2.15
18	1.06	0.48	0.08	1.62	0.70	0.91	0.32	1.93
19	4.43	0.27	0.17	4.87	1.46	0.48	0.29	2.23
20	0.50	0.35	0.22	5.57	1.69	0.45	0.28	2.42
21	4.64	0.22	0.17	5.03	1.40	0.28	0.27	1.95
22	4.18	0.27	0.17	4.62	1.24	0.35	0.28	1.87
23	4.40	0.34	0.21	4.95	1.37	0.40	0.26	2.03
24	6.75	0.39	0.22	7.36	1.37	0.30*	0.10	2.27*
25	10.71	0.49	0.19	11.39	2.76	0.00*	0.13	2.89*
26	9.60	0.61	0.23	10.44	2.61	0.15	0.14	2.90
27	11.83	0.58	0.26	12.67	3.50	0.13	0.18	3.81
28	10.00	0.63	0.18	10.81	1.91	0.20	0.05	2.16
29	8.92	0.62	0.18	9.72	2.16	0.11	0.11	2.38
30	11.28	0.83	0.28	12.39	0.65	0.29	0.23	1.17
31	9.34	0.74	0.18	10.26	0.71	0.31	0.19	1.21
32	6.68	0.88	0.23	7.79	0.99	0.36	0.22	1.57
33	3.54	0.58	0.37	4.49	0.50	0.28	0.27	1.05
34	3.80	0.84	0.29	4.93	0.77	0.31	0.25	1.33
35	2.58	0.52	0.21	3.31	0.57	0.22	0.10	0.89

* unreliable value

Table A.15. Size distribution of total phytoplankton community chlorophyll *a* and phaeopigment, expressed as a percentage of the sum of fractions in samples collected along a longitudinal axis of the estuary from Redbridge to the vicinity of Calshot Spit buoy on September 3rd 1990.

Sample	Chlorophyll a			Phaeopigment		
n°	>5μm	1-5μm	0.2-1μm	>5μm	1-5μm	0.2-1μm
1	*	*	*	*	*	*
2	76.7	18.7	4.6	40.2	43.2	16.6
3	74.4	20.1	5.5	36.6	45.0	18.4
4	74.9	19.7	5.4	39.0	42.0	19.0
5	66.9	28.7	4.4	32.2	53.2	14.6
6	80.6	14.1	5.3	38.5	42.8	18.7
7	89.8	6.6	3.6	56.7	27.3	16.0
8	90.7	6.3	3.0	57.2	28.0	14.8
9	70.2	26.6	3.2	28.2	55.7	16.1
10	77.9	19.9	2.2	36.3	48.0	15.7
11	87.8	9.0	3.2	48.9	35.3	15.8
12	80.6	15.5	3.9	48.7	38.3	13.0
13	85.7	10.9	3.4	45.1	42.6	12.3
14	83.3	12.5	4.2	55.4	30.6	14.0
15	76.2	17.4	6.4	47.4	35.2	17.3
16	66.1	25.3	8.6	39.9	39.2	20.9
17	68.9	26.2	4.9	42.3	45.1	12.6
18	65.5	29.7	4.7	36.2	47.2	16.6
19	91.0	5.6	3.4	65.5	21.6	12.9
20	89.8	6.3	3.9	69.8	18.8	11.4
21	92.3	4.4	3.3	71.5	14.6	13.9
22	90.4	5.9	3.7	66.3	18.7	15.0
23	88.8	7.0	4.2	32.3	19.6	12.7
24	91.6	5.4	3.0	82.4	13.3	4.3
25	94.0	4.3	1.7	95.6	0.0	4.4
26	92.0	5.8	2.2	89.7	5.3	5.0
27	93.3	4.6	2.1	91.8	3.4	4.8
28	92.5	5.8	1.7	88.4	9.1	2.5
29	91.8	6.4	1.8	90.8	4.5	4.7
30	91.0	6.7	2.3	56.0	24.6	19.4
31	91.0	7.2	1.8	59.0	25.5	15.5
32	85.7	11.4	2.9	63.4	22.8	13.8
33	78.9	12.9	8.2	47.5	26.6	25.9
34	77.0	17.1	5.8	58.0	23.2	18.8
35	78.0	15.8	6.2	64.2	24.9	10.9

* unreliable value

Table A.16. Percentage phaeopigment (phaeopigment/(chlorophyll a + phaeopigment)x100) in the various size fractions as determined in samples collected along a longitudinal axis of the stuary from Redbridge to the vicinity of Calshot Spit on September 3rd 1990.

Sample n°	% phaeopigment			
	>5μm	1-5μm	0.2-1μm	sum of frac
1	*	*	*	*
2	21.6	54.9	65.2	34.5
3	19.8	52.9	62.9	33.4
4	22.4	54.3	66.1	35.7
5	25.5	56.9	70.0	41.5
6	17.1	56.7	60.6	30.2
7	12.5	48.4	50.1	18.5
8	10.8	45.9	49.0	16.1
9	15.9	49.5	70.4	32.0
10	23.2	60.9	82.0	39.3
11	18.8	62.0	67.3	29.4
12	24.1	56.6	63.4	34.5
13	13.1	52.9	51.4	22.3
14	28.4	52.9	66.5	37.3
15	35.3	63.9	70.5	46.7
16	40.5	63.6	73.3	53.0
17	41.6	66.6	74.8	53.7
18	39.6	65.3	80.5	54.3
19	24.7	63.9	63.3	31.4
20	25.2	56.4	56.1	30.3
21	23.1	56.3	62.1	28.0
22	22.8	56.2	62.3	28.7
23	23.8	53.5	55.8	29.1
24	16.9	43.4*	30.7	23.6*
25	20.4	0.00*	39.5	20.2
26	21.3	20.2	38.8	21.7
27	22.8	18.0	41.1	23.1
28	16.0	23.9	22.5	16.6
29	19.4	14.7	38.4	19.6
30	5.4	25.7	44.3	8.6
31	7.1	29.3	50.9	10.5
32	12.9	28.7	48.5	16.7
33	12.4	32.7	42.8	19.1
34	16.8	26.7	46.6	21.2
35	18.2	30.0	30.0	21.3

* unreliable value

Table A.17. Depth distribution of nutrients (μM) in North Sea waters during June/July 1989. The temperature (°C) distribution is also given. Data have been accessed through NERC's North Sea Project data base.

Exp no	Depth	PO_4	NH_4	NO_3	NO_2	Si	T[a]
1	1.7	0.09	0.80	-	0.09	0.20	15.4
	7.6	0.08	0.77	-	0.08	0.10	15.4
	22.7	0.09	0.67	-	0.08	0.20	15.6
2	3.1	0.36	1.00	0.50	0.13	0.70	16.0
	7.1	0.33	1.07	0.50	0.12	0.70	15.4
	29.7	0.31	1.00	0.50	0.13	0.70	15.6
3	0.5	0.04	0.50	0.41	0.06	0.20	-
	5.3	0.04	0.30	0.50	0.05	0.20	14.3
	19.2	0.05	0.33	0.41	0.06	0.20	14.3
4	0.9	0.12	1.10	0.66	0.09	0.27	15.2
	15.0	0.10	0.60	0.59	0.08	1.40	14.2
	28.4	0.11	0.80	0.54	0.09	2.10	9.6
5	0.4	0.02	2.37	0.88	0.17	1.20	14.7
	13.9	0.05	2.40	0.88	0.17	1.37	14.2
	23.6	0.13	5.37	1.54	0.30	3.33	10.0
6	0.7	0.08	1.03	0.66	0.14	0.40	13.5
	13.0	0.09	1.73	0.66	0.16	0.40	13.5
	31.5	0.14	1.13	0.66	0.16	0.40	12.0
7	1.5	0.10	0.87	0.38	0.09	0.40	12.5
	22.5	0.29	1.60	0.50	0.12	0.60	11.9
	59.5	0.48	2.10	1.00	0.23	1.10	8.3

(Table A.17 cont')

7A	1.6	0.05	0.60	0.38	0.11	0.50	
	13.9	0.07	0.70	0.38	0.10	0.60	
	27.8	0.29	1.57	0.59	0.13	1.60	
8	1.6	0.09	0.47	0.25	0.04	0.60	
	17.6	0.12	0.50	0.38	0.03	0.70	
	70.7	0.42	1.53	1.13	0.21	1.90	
9	0.7	0.11	0.50	0.63	0.07	0.20	14.4
	13.9	0.11	0.50	0.63	0.06	0.30	13.6
	29.1	0.11	0.30	0.63	0.06	0.30	13.4
10	0.8	0.14	1.07	0.38	0.10	0.30	13.9
	29.1	0.25	0.90	0.41	0.10	1.80	10.4
	59.8	0.31	0.97	0.46	0.08	2.03	10.2
10AB	2.5	0.11	0.87	0.54	0.10	0.37	
	23.7	0.17	0.93	0.50	0.09	0.50	
	58.3	0.18	0.83	0.46	0.08	0.57	

Table A.18. Size-fractionated rate of carbon fixation (μmolC $l^{-1}h^{-1}$) determined from on deck incubations of samples from North Sea waters during June/July. (I_o is the mean irradiance (PAR) during incubation period).

Size fraction	%I_o					
	100	58	28	19	10	5.2
Experiment number= 1		I_o = 1098$\mu Em^{-2}s^{-1}$				
>3μm	0.062	0.244	0.265	0.198	0.180	0.110
3-0.2μm	0.016	0.020	0.028	0.027	0.024	0.017
Sum of frac	0.078	0.264	0.287	0.226	0.196	0.127
Experiment number= 2		I_o = 994$\mu Em^{-2}s^{-1}$				
>3μm	0.239	0.205	0.183	0.125	0.097	0.065
3-0.2μm	0.051	0.077	0.057	0.051	0.046	0.022
Sum of frac	0.291	0.282	0.240	0.176	0.144	0.087
Experiment number= 3		I_o = 480$\mu Em^{-2}s^{-1}$				
>3μm	0.077	-	0.091	0.069	0.032	0.013
3-0.2μm	0.010	-	0.008	0.006	0.004	0.003
Sum of frac	0.087	-	0.099	0.075	0.036	0.016
Experiment number= 4		I_o = 862$\mu Em^{-2}s^{-1}$				
>3μm	0.091	0.154	0.154	0.120	0.092	0.058
3-0.2μm	0.004	0.004	0.005	-	0.005	0.003
Sum of frac	0.095	0.158	0.159	-	0.097	0.061
Experiment number= 5		I_o = 1011$\mu Em^{-2}s^{-1}$				
>3μm	0.307	0.417	0.444	0.355	0.251	0.127
3-0.2μm	0.008	0.013	0.020	0.011	0.006	0.002
Sum of frac	0.315	0.430	0.464	0.366	0.257	0.129
Experiment number= 6		I_o = 199$\mu Em^{-2}s^{-1}$				
>3μm	0.112	0.097	0.073	0.046	0.019	0.008
3-0.2μm	0.007	0.006	0.004	0.003	0.001	0.0005
Sum of frac	0.119	0.103	0.077	0.049	0.020	0.009
Experiment number= 9		I_o = 1199$\mu Em^{-2}s^{-1}$				
>3μm	0.065	0.098	0.095	0.069	0.053	0.024
3-0.2μm	0.005	0.009	0.008	0.005	0.004	0.003
Sum of frac	0.070	0.107	0.103	0.074	0.057	0.027

(Table A.18 cont')

Experiment number= 10 $I_o = 1131 \mu Em^{-2}s^{-1}$

>3μm	0.061	0.073	0.080	0.060	0.044	0.025
3-0.2μm	0.005	0.006	0.004	0.004	0.003	0.003
Sum of frac	0.066	0.079	0.084	0.064	0.047	0.028

-: unreliable value or lost sample

Table A.19. Standard error (expressed as a percentage) of the estimates of the parameters P_s, α and β derived from the P v I fitted curves and the percentage variance accounted for by the fitting procedure in measurements in cultures of *Pycnococcus provasolii*.

Ig	P_s	α	β	%var
20°C continuous light (oxygen)				
25	35.0	9.6	60.0	92.1
65	19.5	7.5	3.6	95.7
140	63.7	8.6	93.7	94.6
260	20.6	7.7	4.1	97.3
370	2.5	2.6	10.3	99.6
20°C continuous light (carbon)				
25	9.3	7.2	39.4	97.5
140	14.9	7.9	51.0	97.6
200	6.9	6.4	20.9	97.9
260	14.7	9.2	68.0	96.8
370	13.1	8.2	73.4	97.6
20°C 12:12h L:D cycle				
30	6.2	5.1	19.0	98.1
110	5.6	6.0	27.7	98.2
360	6.6	5.5	54.0	98.6
10°C 12:12h L:D cycle				
20	6.3	7.6	49.4	94.3
110	7.6	8.8	68.8	93.9
360	3.9	4.7	22.1	98.1

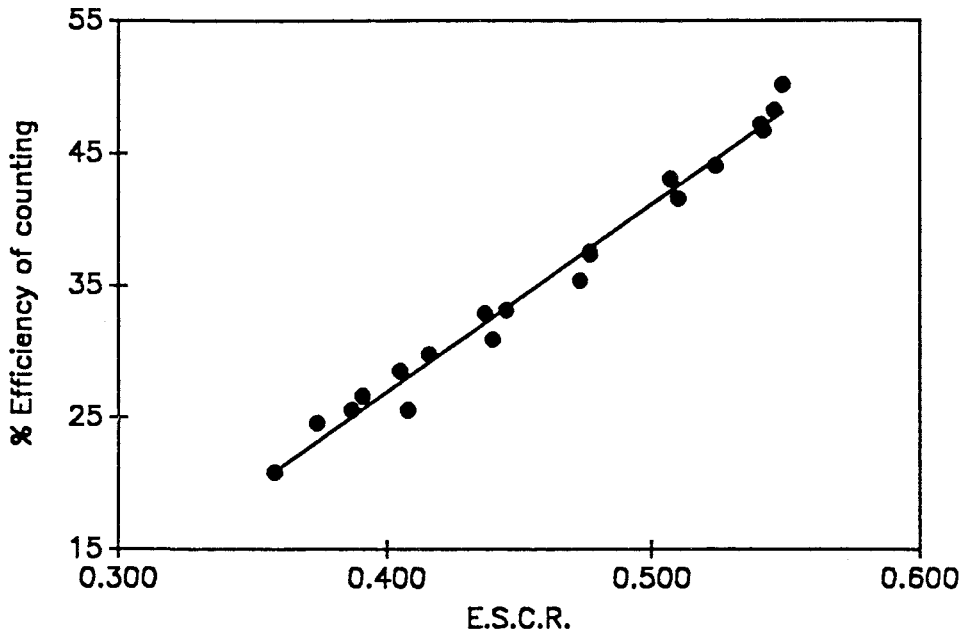

Figure A.1. ^{14}C Quench curve, relating the external standard channels ratio (E.S.C.R.) and the percentage efficiency of counting of radioactivity, determined for Optiphase Hisafe 3 liquid scintillation cocktail using standard ^{14}C-sucrose.

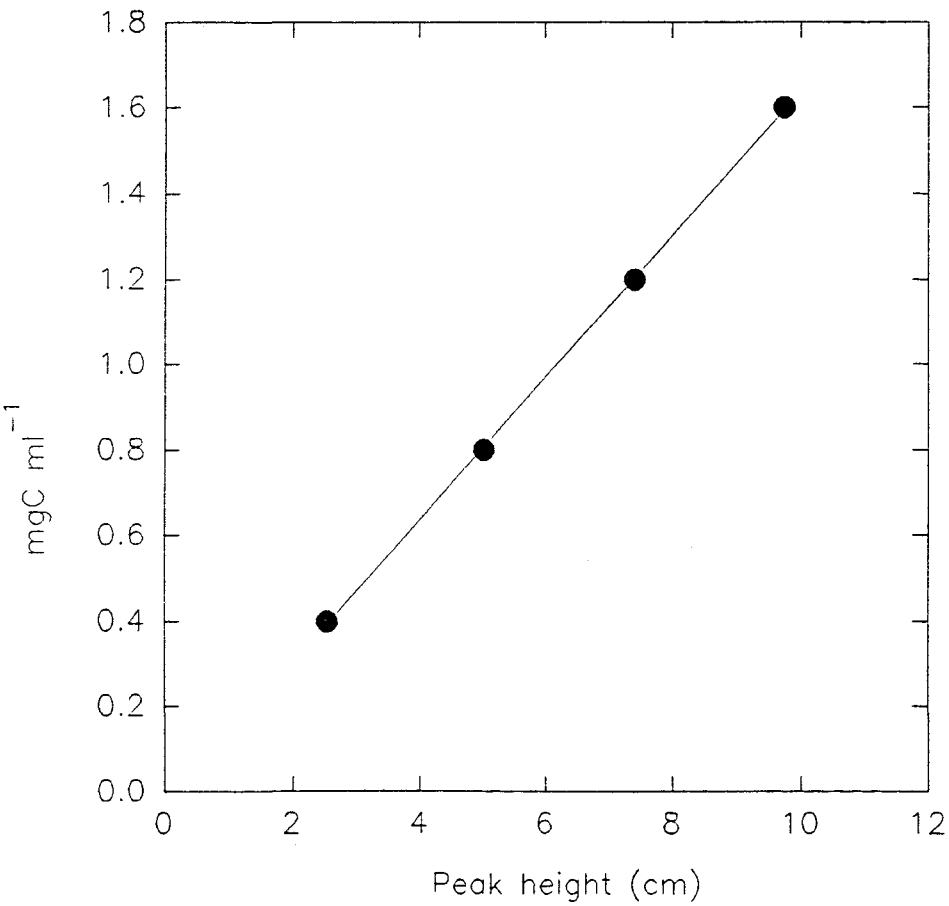

Figure A.2. Typical standard calibration curve for POC measurements, as measured in an infra red gas analyzer, using a standard sucrose solution.

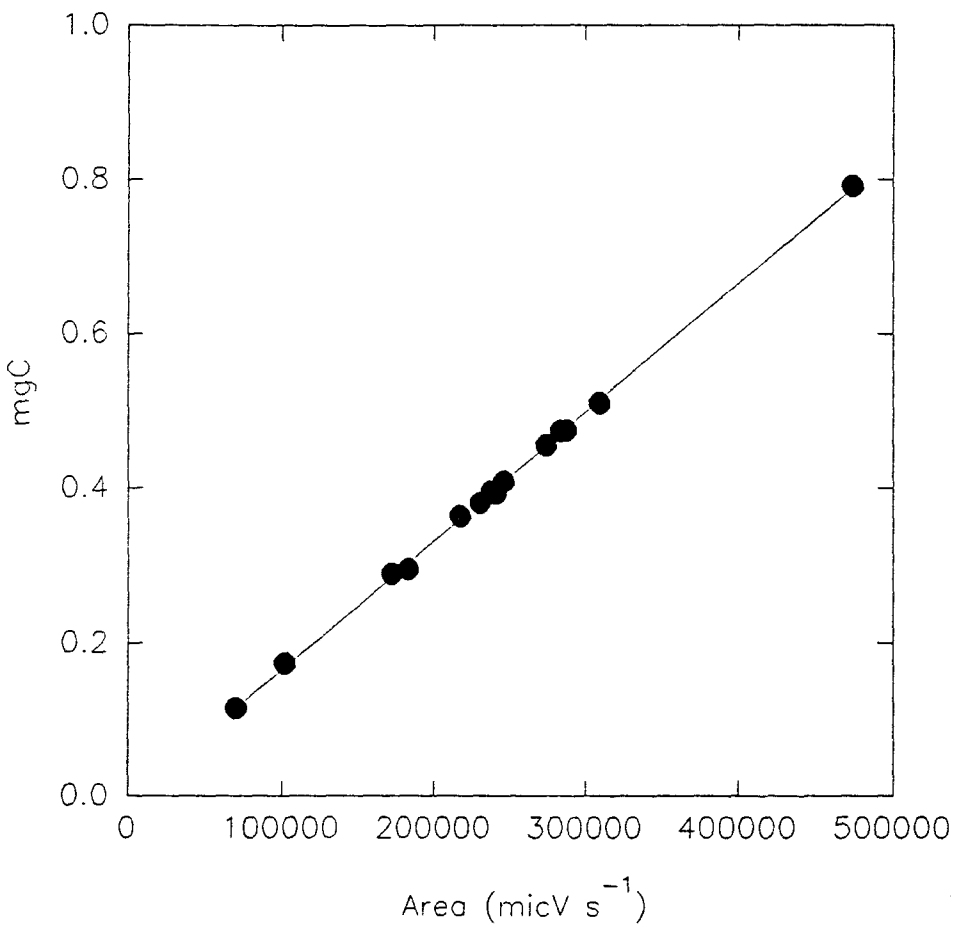

Figure A.3. Typical standard calibration curve for POC measurements, as measured in the Elemental Analyzer, using Acetanilide (Analar).

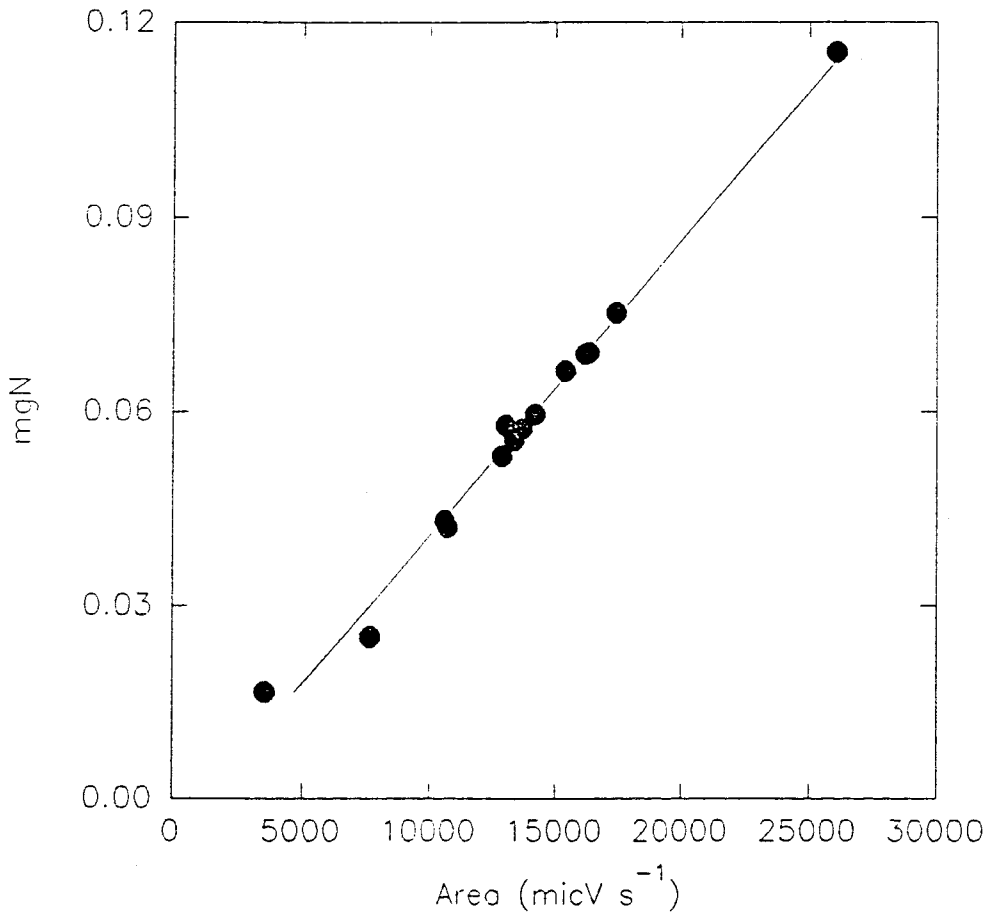

Figure A.4. Typical standard calibration curve for PON measurements, as measured in the Elemental Analyzer, using Acetanilide (Analar).

Plankton community respiration and its relationship to chlorophyll *a* concentration in marine coastal waters

Plankton
Respiration
Chlorophyll *a*
Coastal waters
Microheterotrophs

Plancton
Respiration
Chlorophylle *a*
Eaux littorales
Microhétérotrophes

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ABSTRACT

Rates of plankton community respiration and chlorophyll *a* levels were measured in water samples collected at several times of the year in the North Sea, and during July in the English Channel. Planktonic community respiration rates ranged from 0.025 to 0.830 µmol O_2 l^{-1} h^{-1}. At low and moderate chlorophyll *a* concentrations (0-5 µg l^{-1}) the correlation between algal biomass and community respiration was not significant. However, respiration and chlorophyll *a* did correlate significantly and positively at chlorophyll *a* levels in excess of 5 µg l^{-1}. This is discussed in terms of the relative contribution to the total community respiration of autotrophs and microheterotrophs. It is argued that at low and moderate chlorophyll *a* concentrations, the microheterotrophs make the most substantial contribution to community respiration rates whilst at exceptionally high concentrations of chlorophyll *a* autotrophic dark respiration can be the dominant component of the plankton community respiration in the water column. The ratio of respiration to maximum photosynthesis (R/P_{max}%) is considered to be a useful indicator of the trophic balance of the plankton community. The range of this ratio varied from 2.2 to 75.9, lowest values being recorded in water masses with high rates of net algal growth.

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RÉSUMÉ

Relation entre chlorophylle *a* et taux de respiration du plancton dans les eaux littorales

Nous avons mesuré la chlorophylle *a* et le taux de respiration provenant de communautés planctoniques prélevées dans la Manche en juillet et dans la Mer du Nord à différentes époques de l'année. Les valeurs du taux de respiration sont comprises entre 0.025 et 0.830 µmol O_2 l^{-1} h^{-1}. Lorsque la concentration en chlorophylle *a* est inférieure à 5 µg l^{-1}, aucune corrélation significative n'existe

entre la biomasse phytoplanctonique et le taux de respiration de l'ensemble du plancton. Par contre, les deux paramètres sont étroitement liés dès que la teneur en chlorophylle *a* excède 5 µg l^{-1}. L'explication proposée repose sur la dominance d'espèces autotrophes ou d'espèces hetérotrophes quant à leurs contributions au taux de respiration global. Il est montré qu'aux faibles concentrations en chlorophylle *a*, les espèces microhétérotrophes contrôlent le taux de respiration global, tandis qu'aux concentrations élevées le mode de respiration autotrophe domine.

De ce fait, le rapport entre le taux de respiration et le taux maximum de photosynthèse (R/P_{max}%) peut servir à exprimer l'équilibre trophique de la communauté planctonique. Ce rapport évolue entre 2,2 et 75,9, et les valeurs inférieures sont enregistrées dans des masses d'eau connaissant un taux élevé de croissance nette des espèces phytoplanctoniques.

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INTRODUCTION

Respiration, defined as a biologically controlled oxidation of organic compounds (Burris, 1980), is the major consumption process of photosynthetically fixed carbon in the marine water column. Knowledge of respiration rates is essential for the quantification and modelling of energy and carbon flux within marine food webs. However, the relative contribution of the various metabolic groups to total planktonic respiration has yet to be established.

Data on plankton community respiration rates are remarkably sparse in the literature, particularly when compared to the number of photosynthesis measurements that have been reported (Peterson, 1980). This scarcity can mainly be attributed to the low routine precision of the Winkler assay, the established chemical method for measuring dissolved oxygen in sea water, and the low sensitivity of oxygen electrodes for measuring small changes in dissolved oxygen concentration in discrete samples. Both these approaches have been unsuitable for measuring changes in oxygen concentration in low to moderately productive waters *e.g.* oceanic oligotrophic waters. The alternative approach to measuring total plankton respiration has been to determine the biochemical activity of the electron transport system (ETS) using an enzyme assay procedure (Packard *et al.*, 1971; Packard, 1985). Although the principle behind this technique is reasonably straightforward, acceptance of the method has suffered from problems of interpretation of results (Williams, 1984).

Direct measurements of respiration from changes in dissolved oxygen concentration in the dark are now reliable due to substantial improvements in the precision of the Winkler titration technique (Bryan *et al.*, 1976; Williams and Jenkinson, 1982) and new approaches to using oxygen electrochemical sensors (Langdon, 1984; Smith and Horner, 1982). Thus potential artifacts associated with prolonged incubations (Steemann Nielsen, 1952) or preconcentration of natural plankton samples (Pomeroy and Johannes, 1968; Holm-Hansen *et al.*, 1970; Souza Lima, 1971) have been eliminated for respiration measurements.

Until comparatively recently (Williams, 1981 *a*) the notion that microheterotrophs (< 200 µm), including bacteria and protozoa (*e.g.* microflagellates, loricate and aloricate ciliates), played only a minor role in the structure and function of marine plankton communities had led to the suggestion that most of the oxidation of photoassimilated carbon is carried out by the larger members of the zooplankton or the photoautotrophs themselves (Steele, 1974). In the last decade, due to considerable improvements in techniques available for measuring bacterial biomass (Hobbie *et al.*, 1977; Porter and Feig, 1980) and production (Hagstrom *et al.*, 1979; Fuhrman and Azam, 1982), the role of planktonic microorganisms in the sea has undergone a thorough re-examination and evidence has been put forward to suggest that bacteria can be one of the major direct consumers of photosynthetically fixed carbon (Azam *et al.*, 1983 ; Linley *et al.*, 1983). In agreement with these results there is evidence suggesting that respiratory activity by the small fraction of the plankton (< 3 µm) can be significant (Williams, 1981 *b*; Kuparinen, 1984; Harrison, 1986). However, as yet few studies have attempted to determine the relative contribution of photoautotrophic and microheterotrophic oxygen consumption to overall plankton community respiration. Also, there has been little attention focused on the difference between the relative algal oxygen demand of low phytoplankton biomass communities and phytoplankton bloom populations.

We present here data on rates of plankton community respiration measured at various times of the year in several regions of the southern and central North Sea and during a summer bloom of the dinoflagellate *Gyrodinium aureolum* in the western English Channel. Results are discussed in terms of the relative contribution of the photosynthetic and non-photosynthetic organisms to the planktonic community respiration rates under different concentrations of phytoplankton biomass as indicated from measurements of chlorophyll *a*.

Data on the ratio of respiration to maximum rate of photosynthesis expressed as a percentage (R/P_{max}%) are also included in this paper. There is little information in the literature regarding respiration to photosynthesis ratios for

natural plankton communities. Most of the data available refers to unialgal culture studies (Humphrey, 1975; Falkowski and Owens, 1978; Geider and Osborne, 1989; Grande *et al.*, 1989). The R/P_{max} ratio of phytoplankton in culture has been shown to be an indicator of the physiological state of the algae (Ryther, 1954) and respiration rate has been suggested to be a small proportion of the photosynthetic rate in rapidly growing phytoplankton (Peterson, 1980; Smith, 1982). Also, plankton community respiration rates have been found to be lower at the beginning of a bloom period than when the bloom is scenescent (Keller and Riebesell, 1989). In this paper R/P_{max} ratios estimated from waters containing algal populations in different stages of development are presented. This ratio is proposed as a useful index of the trophic balance of the plankton community.

MATERIALS AND METHODS

Study areas

Water samples were collected from the southern and central North Sea during the months of March, April, May, June, July and October 1989, while on board the RV *Challenger* and in the English Channel during July 1987 on board the RV *Frederick Russell*. Measurements in North Sea waters were made opportunistically on several research cruises undertaken as part of the British Natural Environmental Research Council North Sea Project. The majority of the sampling sites were located in the shallow (less than 50 m) well mixed waters of the southern bight between latitudes 51°18'N and 53°00'N and between longitudes 1°50'E and 4°00'E, with most samples being taken in Dutch coastal waters, between latitudes 52°10'N and 52°50'N and longitudes 3°00'E and 3°40'E. Exceptions were during May, June and July when sampling was extended to areas in the stratified Central North Sea, between latitudes 54°10'N and 55°50'N and between longitudes 1°10'W and 7°00'E. In the English Channel measurements were made at station F87 at about 49°14'N and 4°30'W, in a bloom of the dinoflagellate *Gyrodinium aureolum* (Garcia and Purdie, 1991).

Photosynthesis and respiration measurements

Natural samples were collected from surface waters using 10 or 30 l Niskin bottles and dispensed through silicon rubber tubing into 125 or 60 ml borosilicate glass bottles. Incubation time varied between 3 and 17 hours and samples were held within 2°C of ambient seawater temperature. Dissolved oxygen concentration was measured using a precise, automated microprocessor controlled Winkler titration technique similar to that described by Williams and Jenkinson (1982). Respiration rates were determined from the difference between zero time and dark incubated measurements of oxygen. A series of six screened deck incubators was used to measure photosynthesis from oxygen production under various natural light levels (100, 58, 28, 19, 10, 5.2 % of surface incident irradiance) as described by Garcia (1989).

Since respiration is a process common to both heterotrophic and autotrophic organisms, rates measured in untreated natural samples (*i.e.* without prefiltration) are regarded as plankton community respiration rates (Williams, 1984). In practice, however, there is an upper limit to the size of planktonic organism studied, which is constrained by the volume of incubation containers used (usually less than 200 ml) in relation to the low density of larger organisms, *i.e.* mainly zooplankton.

Size-fractionation procedure

Natural water samples were fractionated by reverse filtration by gravity through large diameter (142 mm) 3 µm pore sized Nuclepore filters cemented to perpex tubing (Williams, 1981 *b*).

Chlorophyll *a* measurements

Duplicate samples were filtered onto Whatman GF/F filters and stored frozen until analysis on return to the laboratory. Chlorophyll *a* was extracted by homogeneization of filters in 90 % acetone and the concentration determined fluorometrically, correcting for phaeopigments, in an Aminco fluoro-colorimeter as described by Parsons *et al.* (1984). The concentration of a commercial standard solution of chlorophyll *a* (extracted from *Anacystis nidulans*, Sigma Ltd.) was determined spectrophotometrically in a Pye Unicam SP6-350 using the equations of Lorenzen (1967).

RESULTS AND DISCUSSION

Plankton community respiration rates measured during this study span one order of magnitude, ranging from 0.025 to 0.83 µmol O_2 l^{-1} h^{-1} (Tab. 1). This range is in agreement with other published data for coastal areas, reviewed by Williams (1984).

Regression analysis indicated rates of community respiration, expressed as µmol O_2 l^{-1} h^{-1}, to be significantly correlated ($p < 0.001$) with both, chlorophyll *a* concentration ($r = 0.88$; Fig. 1), and water temperature($r = 0.59$; Fig.2). In laboratory grown cultures an increase in the rate of respiration with increasing temperature has been observed for both phytoplankton (Morgan and Kalff, 1979; Verity,

Table 1

Plankton community respiration and photosynthesis rates measured in the North Sea during March, April, May, June, July and October 1989, in the English Channel during July 1987 and in Southampton water during June 1980.
**Data from Southampton water from June 1980 taken from Shamsudin (1980).*

Date	Temp. (°C)	Chl *a* ($\mu g\ l^{-1}$)	Respiration $\mu molO_2\ l^{-1}h^{-1}$)-	Spec. respiration ($\mu molO_2\ \mu gChl^{-1}h^{-1}$)	Inc. time (hours)	P_{max} ($\mu molO_2\ l^{-1}h^{-1}$)	R/P_{max} (%)
06.80*	19.0	6.7	0.33	0.049	-	-	-
06.80*	19.0	22.0	0.76	0.034	-	-	-
06.80*	19.0	23.5	0.83	0.035	-	-	-
07.87	16.8	12.8	0.68	0.053	4.0	4.01	16.9
07.87	16.4	11.2	0.60	0.053	4.0	1.80	33.4
07.87	16.8	11.7	0.79	0.068	4.0	2.84	27.7
07.87	16.9	10.1	0.70	0.070	4.0	1.96	35.7
07.87	16.9	12.2	0.59	0.048	4.0	2.24	26.3
07.87	16.4	11.2	0.65	0.058	4.0	-	-
07.87	16.9	10.1	0.52	0.052	4.0	-	-
07.87	16.9	14.1	0.63	0.045	4.0	-	-
07.87	16.9	12.2	0.66	0.054	4.0	-	-
03.89	8.1	-	0.05	-	12.0	0.37	14.3
03.89	7.0	-	0.05	-	12.0	0.53	10.0
03.89	-	-	-	0.05	-	3.0	-
03.89	-	-	0.05	-	6.5	-	-
04.89	7.0	1.5	0.05	0.033	14.0	0.49	10.3
04.89	6.9	1.2	0.08	0.062	13.0	0.36	21.0
04.89	7.0	1.6	0.03	0.015	12.0	0.45	5.5
04.89	8.4	1.6	0.18	0.115	9.5	0.97	18.5
04.89	8.7	2.9	0.07	0.025	12.4	3.19	2.2
04.89	8.7	3.5	0.11	0.031	12.2	4.10	2.7
04.89	8.7	5.8	0.10	0.017	5.0	-	-
04.89	8.7	5.8	0.14	0.025	9.0	-	-
04.89	8.7	5.6	0.07	0.022	12.0	3.20	2.3
04.89	7.0	1.5	0.19	0.123	3.0	-	-
04.89	7.0	1.4	0.12	0.086	3.0	-	-
04.89	7.3	1.7	0.26	0.149	3.0	-	-
04.89	7.1	1.6	0.18	0.114	3.0	-	-
04.89	7.0	1.8	0.22	0.123	3.0	-	-
04.89	8.7	3.8	0.44	0.117	3.0	-	-
05.89	10.7	0.6	0.19	0.308	10.8	0.27	71.0
05.89	10.6	0.8	0.14	0.169	12.0	0.21	67.6
05.89	10.7	0.9	0.18	0.208	12.0	0.23	75.9
05.89	11.2	0.7	0.24	0.334	6.5	-	-
05.89	8.3	2.6	0.11	0.041	11.5	1.16	9.3
05.89	8.8	2.8	0.15	0.053	13.0	1.27	11.5
05.89	11.1	1.2	0.15	0.122	9.5	0.35	42.8
05.89	10.2	0.6	0.12	0.204	12.0	0.27	47.9
06.89	15.4	5.1	0.16	0.031	17.0	0.73	21.9
06.89	15.8	1.7	0.14	0.085	16.5	0.72	19.4
06.89	14.3	1.4	0.21	0.150	18.0	0.36	58.3
06.89	14.7	2.4	0.31	0.131	10.5	0.76	40.8
06.89	15.4	5.1	0.12	0.024	6.0	-	-
06.89	15.8	1.7	0.16	0.097	8.5	-	-
06.89	14.3	1.4	0.25	0.178	10.3	-	-
06.89	12.0	2.8	0.32	0.115	10.8	-	-
06.89	14.7	2.4	0.16	0.067	10.5	-	-
07.89	13.0	1.3	0.17	0.129	8.0	-	-
07.89	13.5	0.6	0.10	0.178	16.3	0.25	40.0
07.89	12.5	0.8	0.18	0.220	17.0	0.40	45.0
07.89	14.4	0.5	0.10	0.217	15.5	0.17	58.8
07.89	13.0	0.5	0.09	0.169	16.5	0.21	42.8
10.89	16.8	2.9	0.20	0.068	8.0	-	-
10.89	16.2	2.8	0.15	0.054	12.0	2.41	6.2
10.89	16.1	2.8	0.09	2.032	7.0	-	-
10.89	16.1	2.8	0.09	0.032	7.0	-	-
10.89	15.8	3.2	0.13	0.041	10.0	1.72	7.6
10.89	15.8	3.3	0.13	0.040	5.0	-	-
10.89	15.8	3.5	0.13	0.037	11.0	2.09	6.2
10.89	15.5	2.2	0.11	0.050	9.5	0.72	15.3
10.89	15.2	2.5	0.19	0.076	5.0	1.29	14.7

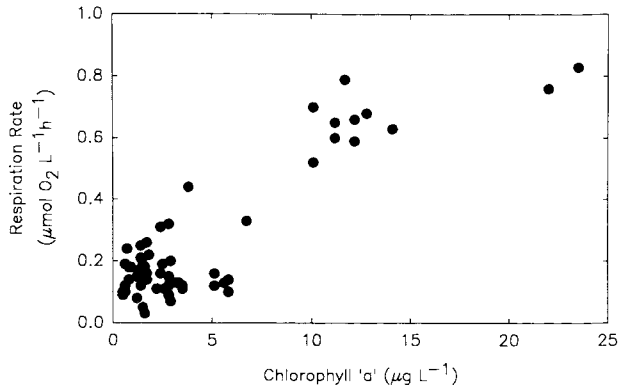

Figure 1

Relationship between chlorophyll a *concentration and plankton community respiration rate.*

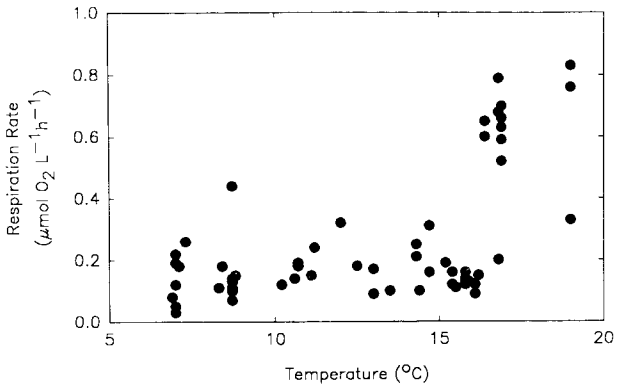

Figure 2

Relationship between water temperature and plankton community respiration rate.

1982) and microheterotrophs (Antai,1990) as might be expected from thermodynamic considerations. In natural waters, however , other factors co-vary with temperature *i.e.* insolation increases and surface mixed layer depth can decrease if a pycnocline develops. In temperate seas an increase in water temperature throughout the surface mixed layer is usually associated with increased organic production by the algae as the incident radiation increases and the water column stabilizes. Thus greater availability of organic carbon to the microbial community (including both autotrophic and heterotrophic populations) is probably more important in determining absolute respiration rates than the direct physiological effect of fluctuations in sea temperature.

Plankton respiration rates have been shown to correlate with chlorophyll *a* concentration by a number of authors *e.g.* Packard (1979), Setchell and Packard (1979) Packard and Williams (1981), Holligan *et al.* (1984) and Laanbroek *et al.* (1985), although some other data have shown little correlation (Packard, 1979; Harrison, 1986). A more detailed analysis of our results suggests that the correlation between chlorophyll *a* and respiration is influenced by the level of chlorophyll *a* present in the samples. If data expressed in Table 1 where respiration rates were determined at low to moderate chlorophyll a concentrations (0.46 to 5 μg l^{-1}) are analyzed separately from those at higher concentrations, the correlation between absolute respiration rate (μmolO_2 l^{-1} h^{-1}) and chlorophyll *a* concentration is statistically insignificant. Respiration rates determined in waters containing low levels of chlorophyll *a* were obtained over a wide range of seawater temperatures and so, correlation of respiration rate with chlorophyll *a* concentration in this low to moderate concentration range was re-determined by separating the data into two groups, a low temperature (< 12°C) and a high temperature (> 12°C). In neither of the two data sets was the correlation significant. This analysis further indicates that seawater temperature was not a dominant factor controlling rates of respiration in the water column. Packard (1979) suggests that at low algal biomass levels, the correlation between chlorophyll *a* concentration and respiration rate will be weak, since under these conditions, and given that zooplankton respiration is often reported as a minor component of total plankton respiration (Andersen and Jacobsen, 1979; Baars and Fransz, 1984; Bell and Kuparinen, 1984; Harrison *et al.*, 1987; Williams, 1981 *b*), microheterotrophs should make up a major proportion of plankton community respiration. Our results support this observation, since chlorophyll *a* specific respiration rate regressed against chlorophyll *a* concentration significantly deviates from a constant relationship at low chlorophyll *a* levels *i.e.* below 5 μg l^{-1} (Fig. 3). Martinez *et al.* (1990) have recently reported similar results from a study in the Mediterranean Sea. In general, good agreement was found between chlorophyll *a* levels and plankton community ETS activity in their study. The enhanced ETS activity at high chlorophyll *a* concentration was mainly attributed to high phytoplankton metabolic activity in frontal boundary regions. However, the authors also noted a high variability in ETS activity, with some relatively high values in low chlorophyll *a* (< 0.2 μg l^{-1}) waters, suggesting an important microheterotrophic contribution to the rates of respiration. Fractionation respiration experiments we conducted in the North Sea in June and July 1989 (chlorophyll *a* levels of 1.4 to 2.8 μg l^{-1}) further substantiate this point. Rates of respiration determined for the fraction passing a 3 μm filter ranged from 70 to 100 % (mean 80 %) of the rates determined for the unfractionated sample, whereas in

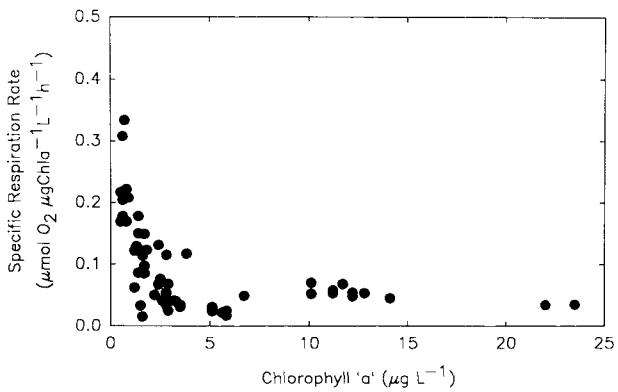

Figure 3

Relationship between chlorophyll a *concentration and chlorophyll* a *specific respiration rate.*

Table 2

Contribution of the fraction passing 3 μm Nuclepore filters to overall community chlorophyll a concentration and respiration rate measured in June/July, 1989 in the North Sea.

Exp	* Chlorophyll *a* TOT	< 3 μm	%	** Respiration rate TOT	< 3 μm	%
2	1.65	0.126	7.6	0.16	0.13	83
3	1.40	0.245	17.5	0.25	0.17	70
4	2.78	0.214	7.7	0.32	0.25	80
5	2.36	0.300	12.7	0.16	0.16	100

* μg Chl *a* l^{-1}.
** μmol O_2 l^{-1} h^{-1}.
% percentage contribution of the smaller than 3 μm fraction to the total sample.

terms of chlorophyll *a* concentration, the small size fraction made up between 7 and 17 % of the unfractionated sample (Tab. 2). Although Salonen and Kononen (1984) have shown that some caution should be taken in interpreting rates of respiraton activity in filtrates, data from size-fractionation measurements reported by Harrison (1986) in Arctic waters also showed microheterotrophs to be responsible for a substantial part of the plankton community respiration at low chlorophyll *a* concentrations, (mean chlorophyll *a* level of 4.12 μg l^{-1}). Similar fractionation results are reported by Williams (1981 *b*), though the chlorophyll *a* levels were not specified in this study.

A close coupling between plankton respiration activity and chlorophyll *a* concentration can be interpreted in two ways: either a) photosynthetic organisms are the major contributors to plankton community respiration rates (Packard, 1979); or b) the growth and/or standing stock of phytoplankton populations significantly enhances the presence and/or activity of microheterotrophs, the latter being the most important contributors to overall respiration even under conditions of high chlorophyll *a* concentration. It has been shown that bacteria numbers are often positively correlated with phytoplankton standing stock (Antai, 1990; Bird and Kalff, 1984; Ferguson and Palumbo, 1979; Fuhrman *et al.*, 1980; Linley *et al.*, 1983) and sometimes also with primary production (Derenbach and Williams, 1974; Larsson and Hagstrom, 1982; Lancelot and Billen, 1984). What proportion of the respiration can be attributed to microheterotrophs and how much to phytoplankton is still a matter of debate. Holligan *et al.* (1984) considered that under conditions of high chlorophyll *a* concentration, during a developing bloom of *Gyrodinium aureolum* in the English Channel, most of the respiratory activity could be attributed to the dominant microalgae. Soulsby *et al.* (1984) reported that in Southampton water dense aggregations of the phototrophic ciliate *Mesodinium rubrum* can lead to a severe depletion of oxygen in the lower part of the water column. This was attributed to night time oxygen demand of the ciliate. Williams (1981 *b*), however, from measurements of respiration in coastal waters using size-fractionation techniques, showed that a substantial proportion of the respiratory activity could be attributed to the < 1 μm fraction, even during diatom blooms, although levels of chlorophyll *a* at the time of the bloom were not specified. Kuparinen (1984) reported that microheterotrophs were the major contributors to overall respiration in surface waters off the coast of Finland on an annual basis, but only 20 % of the respiration during the spring diatom

Table 3

A summary of plankton community respiration rates, maximum rates of photosynthesis and ratios of respiration to maximum photosynthesis measured over a few days in water bodies containing phytoplankton populations in different states of development during 1989 in the North sea and 1987 in the western English Channel (a: μmolO_2 $l^{-1}h^{-1}$, b: μmolO_2 μg Chl $a^{-1}h^{-1}$, n: number of measurements).

n	Chl *a* μg l^{-1}	Respiration a	Respiration b	P_{max} a	P_{max} b	R/P_{max} %	Comments
3	2.9-5.6	0.07-0.11	0.022-0.031	3.19-4.10	0.57-1.17	2.2-2.7	High net production April *Phaeocystis* bloom southern NS
3	2.8-3.5	0.12-0.15	0.037-054	1.72-2.41	0.54-0.86	6.2-7.6	Low net production October *Rhizosolenia* population southern NS
3	0.6-0.9	0.14-0.19	0.169-0.308	0.21-0.27	0.26-0.45	67.6-75.9	Scenescent May *Phaeocystis* bloom southern NS
2	2.6-2.8	0.11-0.15	0.041-053	1.16-1.27	0.45	9.3-11.5	May 14-16 *Nitzschia* population central NS unstratif.
2	0.6-1.2	0.12-0.15	0.122-0.204	0.27-0.35	0.29-0.45	42.8-47.8	May 18-20 low diatom concentration central NS stratif.
5	10.1-12.8	0.59-0.79	0.048-0.070	1.80-4.01	0.16-0.31	16.9-35.7	July *G. aureolum* bloom English Channel

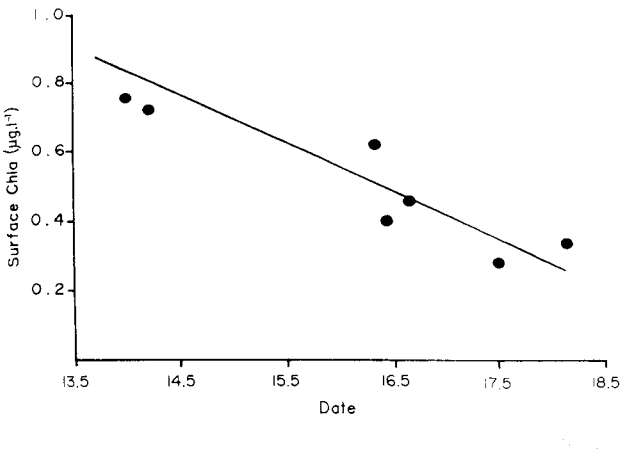

Figure 4

Variation of chlorophyll a *concentration in surface waters during a scenescent bloom of* Phaeocystis *sp in May 1989 in the southern North Sea.*

bloom (chlorophyll *a* levels of 10 to 22 µg l^{-1}) could be attributed to the < 3 µm fraction. Data from freshwater environments show a similar pattern: bacterial respiration accounted for only 30 % of the community respiration during the spring diatom bloom (chlorophyll levels up to 23 µg l^{-1}) in Lake Erken, Sweden (Bell and Kuparinen, 1984). Schwaerter *et al.* (1988) determined from lake studies that bacterial contribution to community respiration could be up to 60 to 70 % at low algal biomass, decreasing to 20 % under conditions of high algal biomass.

Table 3 summarizes rates of respiration, light saturated photosynthesis and ratios of respiration to light saturated photosynthesis (R/P_{max}) from waters in which phytoplankton populations were sampled over a period of a few days. Lowest values of R/P_{max} (2.2 to 2.7 %) were obtained during April 1989 in a bloom patch of *Phaeocystis* sp. in Dutch coastal waters. Values of light saturated photosynthesis normalized to chlorophyll *a* were high (0.57-1.17 µmolO_2 µg chl a^{-1} h^{-1}) and chlorophyll *a* specific respiration rates were low (0.022-0.031 µmolO_2 µg chl a^{-1} h^{-1}), indicating efficient growth of the microalgae. Water samples were collected over a period of four days from a coherent water body sampled in the vicinity of a drogued buoy; high values of net water column production were indicated over the four days from increases in oxygen saturation (110-120 %), chlorophyll *a* concentration (2.9-5.8 µg l^{-1}) and total *Phaeocystis* cell number (3.9-14 x 10^6 cell l^{-1}; Daneri and Purdie, 1990 *b*). Low chlorophyll *a* specific respiration rates were also measured while following the development of a small autumn diatom bloom dominated by *Rhizosolenia stolterfothii* in a coherent water body during October 1989 in the southern bight (Tab. 3; Daneri and Purdie 1990 *a*). The range of community respiration was somewhat higher than that measured during the *Phaeocystis* bloom, 0.12-0.15 µmolO_2 $l^{-1}h^{-1}$ and 0.07-0.11 µmolO_2 $l^{-1}h^{-1}$ respectively, and the range of P_{max} (1.72-2.41 µmolO_2 $l^{-1}h^{-1}$) was lower than during the *Phaeocystis* bloom (3.19-4.1 µmolO_2 $l^{-1}h^{-1}$). As a result, the range of the ratio R/P_{max} measured during the autumn diatom bloom (6.2-7.6 %) was more than double that of

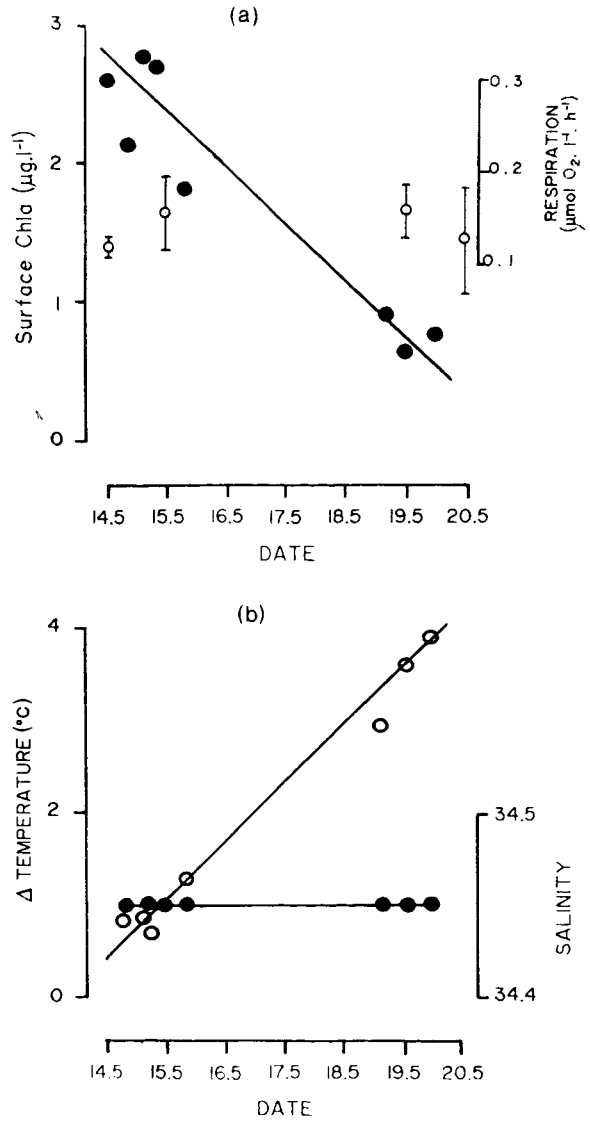

Figure 5

a) variation of chlorophyll a *concentration (•) and respiration rate (± S.E.) (o) in surface water during a bloom of* Nitzschia pseudoseriata *during May 1989 in the central North Sea; b) variation of the temperature difference between the surface and the bottom of the water column (o), showing the development of a thermocline, and variation of salinity (•), indicating that sampling was performed from a coherent water body.*

the spring *Phaeocystis* bloom (2.2-2.7). The values of chlorophyll *a* specific respiration determined during the autumn diatom bloom were higher than during the April *Phaeocystis* bloom and P_{max} normalized to chlorophyll *a* was lower in the autumn than in the April samples, reflecting the lower net rate of increase in the algal population determined from changes in diatom cell numbers (Daneri and Purdie, 1990 *a*). During May 1989 two locations were sampled in the North Sea. The first location was in the southern bight where *Phaeocystis* sp. was the dominant microalgae (over 90 % by cell count). However, the colonies were in a scenescent state, as revealed by microscopic examination, and decreasing levels of chlorophyll *a* were recorded over a period of five days in this region (Fig. 4). Chlorophyll *a* specific rates of respiration were high, P_{max} normalized to chlorophyll *a* was low and R/P_{max} was high

(above 65 %), indicating little potential for positive net community production (Tab. 3). In accordance with these features, levels of oxygen saturation were close to or slightly below 100 % in surface waters, showing an increased biological oxygen demand by the plankton community. The second location visited during May 1989 was in the central area of the North Sea and a population of *Nitzschia pseudoseriata* dominated the phytoplankton when the water column was well mixed on the 14th and 15th of May 1989. The region was sampled again on the 19th and 20th of May when a temperature difference of up to 3°C had established between surface and bottom waters. The phytoplankton were now concentrated below the thermocline and levels of chlorophyll *a* had dropped significantly in surface waters over the six day period (Fig. 5). Absolute respiration rates in surface waters, however, remained at a similar level to that prior to stratification (Fig. 5) and chlorophyll *a* specific respiration rates were consequently increased. This observation is further circumstantial evidence supporting the contention that plankton community respiration is often dominated by non-photosynthetic organisms. Data is also presented in Table 3 of respiration rates determined in samples collected from a *Gyrodinium aureolum* bloom in the English Channel during July 1987. Respiration rates were high but little *in situ* net growth in the population was detected from cell counts determined over a period of 5 days (Garcia and Purdie, 1991). R/P_{max} values were high (16.9 to 35.7) and specific respiration rates were higher than those determined for actively growing blooms in the North Sea. The dinoflagellate population P_{max} values normalized to chlorophyll *a* were relatively low (0.16-0.31 $\mu molO_2$ μg chl $a^{-1}h^{-1}$) and other parameters measured over the five days of observations *i.e.* increase in the percentage of phaeopigments and decrease in the percentage oxygen saturation, suggest the bloom was in an early phase of scenescence due to nutrient limitation (Garcia and Purdie, 1991). Water column gross primary production was approximately in balance with the respiratory oxygen demand (Garcia and Purdie, 1991).

In general, results from Table 3 show that low values of chlorophyll *a* specific respiration rates together with high values of P_{max} % normalized to chlorophyll *a* and lowest values of R/P_{max} % were determined in water masses where the phytoplankton was showing high rates of net growth *i.e.* water masses with high rates of net community production, as indicated by independent methods. We, therefore, suggest R/P_{max} % as being a useful indicator of the trophic balance of the plankton community, lowest values (*i.e.* 2-10 %) being found in photosynthetically active algal dominated plankton populations and values greater than 20 % occurring in plankton populations where heterotrophic oxygen consumption is dominant or the phytoplankton population is in a scenescent state. In agreement with these conclusions, Grande *et al.* (1991) found that light intensity had a less marked effect on respiration rates in plankton communities with higher ratios of dark respiration to P_{max} (%). This result also suggests that heterotrophic organisms make a greater contribution to the overall plankton community respiration rate when the R/P_{max} ratio is high.

CONCLUSIONS

The range of plankton community respiration rates presented is in agreement with data from the literature for coastal waters. Results suggest that at low and moderate chlorophyll levels microheterotrophs play a major role in the overall rates of respiration, however, under conditions of exceptionally high phytoplankton biomass the microalgae can dominate the plankton community respiration rates. The ratio of R/P_{max} is regarded as a good index of the trophic balance of the plankton community. Lowest values of R/P_{max} were measured in water masses where high rates of net community production were occurring *i.e.* water masses with high rates of net algal population growth.

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