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Effects of Light and Nutrient Gradients on the Taxonomic Composition, Size Structure and Physiological Status of the Phytoplankton Community within A Temperate Eutrophic Estuary.

By

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Under the supervision of Professor Patrick Holligan

Submitted for the degree of Doctor of Philosophy

September-2006

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Abstract:

Estuaries are important ecosystems which are affected by a large range of environmental factors. It is important to measure, characterise and monitor the ecological status of estuaries, and this is the contribution of this thesis for phytoplankton communities in Southampton Water. The size structure and species composition were investigated during 2002 and 2003, with special attention to nanophytoplankton (cells < 5µm in diameter). The main sampling stations were Empress Dock and NW Netley in both years, and Calshot and Horse Elbow in 2002 only. To aid the interpretation of phytoplankton data, information about prevailing meteorological (air temperature, rainfall, irradiance) and hydrographic (tides, water temperature, salinity, and levels of inorganic nutrients) conditions were also collected. In 2002, Empress Dock was sampled weekly between February and October, and other stations were sampled monthly between May and October. In 2003, Dock and NW Netley were sampled weekly between April and September.

Measurements made on the phytoplankton included size fractionated (<2, 2-5, 5-20, >20µm) chlorophyll, photosynthetic pigments by HPLC, cell counts by inverted microscopy, and in year 2003 flow cytometry counts and primary productivity (by oxygen) and Fast Repetition Rate Fluorometer (FRRF). Analyses of the major nanophytoplankton taxa involved oligonucleotide probes using fluorescent *in situ* hybridization (FISH) techniques. Plymouth Routines in Multivariate Ecological Research (PRIMER) statistical analyses were used to calibrate the environmental parameters and phytoplankton carbon biomass.

The phytoplankton populations at the four stations were relatively similar. Maximum values of chlorophyll (chl) and carbon biomass were observed between May and August in both years, with a late diatom bloom in 2002, and a mid summer bloom for various organisms. Phytoplankton (as chl or carbon biomass) distributions for 2002 and 2003 could be divided into four phases associated with irradiance and nutrient level. However, there were rapid chl fluctuations during summer due to different factors, such as water column irradiance, tidal range and flushing rate.

PRIMER analyses showed that phytoplankton carbon biomass distribution was associated with seasonal patterns, related to light irradiance water column light attenuation, tidal range and nutrients (N and P). In addition, Si is related to phytoplankton succession, and P to size fraction.

The nanophytoplankton generally contributed 35-40% of total chlorophyll and up to 60% in winter at the outer stations. Chlorophyll size fraction measurements at Empress Dock (2002) and NW Netley (2003) showed that increased chlorophyll values are generally associated with fractions greater than 5µm (>5µm) and vice versa, although nanophytoplankton (carbon biomass) size structure was dominated by size 2-5µm followed by picophytoplankton, and flagellates >5µm became important in some samples. Comparison of total and fractionated pigment indicated that chlb and $chlc_{2+3}$ were highly related to the nanophytoplankton fraction and probably inductive of the importance of Chlorophyta and Chrysophyta.

Acknowledgement

This is the time to thank every one who gave me help during my study and I ask for forgiveness to any that I forget.

First of all, I acknowledge my parents for their support and encouragement during my life and education, and my family members. Special thanks go to my wife for her support and patience during my study.

For science and academic work, I give greatest thanks to my supervisor, Professor Patrick Holligan, for his guidance, encouragement and patience throughout this work. Without his support, the thesis could not have achieved this level.

I acknowledge that support of PhD panel, Dr Duncan A. Purdie, who trained me in sampling and helped me in analytical techniques, and Dr Lawrence Hawkins.

I gratefully acknowledge the funding agency, Saline Water Conversion Corporation (SWCC), and especially the Desalination Research Institute (Riyadh and Al-Jubaill, Kingdom of Saudi Arabia). The acknowledgment also includes Saleh Al-Jarah and Dr. Mohammad O. Saeed for their support and encouragement.

I am grateful to all the people who have helped me with sample collection and analysis, or given advice during the writing: Dr Elham Ali, Dr. Sinhhue Torres, Mohammed Qurban, Dr. Arantza Iriate, Dr. Alex Poulton, Young-Nam Kim, Dr. Judith Homewood, Dr. Sue Hartman, Russell Davidson, Graeme Leggatt, Matt O'Shaughnessy, Glenn Tarran and Shir Akberi.

Thanks are also due to all technical staff members of the National Oceanography Centre, without whose help the completion of this work would have been impossible.

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Abbreviations

Abbreviation	Description
Stations	
Dock	Empress Dock
NWN	North West Netley
HE	Horse Elbow
Pigments	
chl and Tchl	Chlorophyll
Fchl	Fractionated chlorophyll
chla	HPLC chlorophyll *a*
chlb	Chlorophyll *b*
$chlc_{1+2}$	Chlorophyll c_{1+2}
$chlc_3$	Chlorophyll c_3
perid	Peridinin
19 but	19-Butanoyloxyfucoxanthin
fuco	Fucoxanthin
19 hex	19-Hexanoyloxyfucoxanthin
brasino	Brasinoxanthin
viol	Violaxnthin
allo	Alloxanthin
zea	Zeaxanthin
Chemicals	
POC	Particulate organic carbon
PON	Particulate organic nitrogen
DOC	Dissolved organic carbon
POM	Particulate organic matter
N	Nitrate
Si	Silicate
P	Phosphate
Statistics	
PRIMER	Plymouth Routines In Multivariate Ecological Research
MDS	Multidimensional scaling
SIMPER	Similarity percentage of species contribution
BIOENV	The relationship between environmental and biological data analyses
Light	
PAR	Photosynthetically available radiation
K_d	Attenuation coefficient
P *vs* I	photosynthesis *vs* light irradiance
Ps	Maximum potential photosynthetic rate
ά	Initial light-limited slope of the curve
HPLC	High Performance Liquid Chromatography
FRRF	Fast Repetition Rate Fluorometer

Chapter 1 - INTRODUCTION

1-1 Overview of Estuarine pelagic biology

1-1-1 Estuarine environments

Estuaries are variable and important environments often with large human populations living along or adjacent to them. The interaction between man and estuaries is therefore very intimate, and they are used for a variety of activities including transportation, recreation, industrial production and fishing. These and other human activities, such as dredging and dumping have profound effects on estuarine ecosystems.

The vital role of estuaries requires that habitats are continuously studied to measure the impact of artificial and natural changes. Such changes are bound to affect estuarine biological productivity, which is characteristically high. This productivity begins with the primary photosynthetic organisms. The basis of primary production in aquatic habitats is phytoplankton but also includes benthic algae and seagrasses as well as various types of intertidal vegetation. Estuarine phytoplankton has been extensively studied in terms of a number of parameters, e.g. chlorophyll biomass, community structure and succession, and the effect of environmental parameters on primary production. Chlorophyll is the traditional indicator of phytoplankton biomass and is used as an index of seasonal and regional variations in abundance and bloom dynamics (Li and Smayda, 1988).

The above elements are all important to ecology, and the main (general) purpose of this thesis is to contribute to knowledge about these environments through study of a particular location (Southampton Water) over the slice of time available for data collection (2002 and 2003).

Estuaries are variously described, each definition conditioned by reference to a certain perspective. Pritchard's definition (1967) is the most widely used. It states that “an estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which the sea water is measurably diluted with fresh water derived from land drainage” (Cameron and Pritchard, 1963; Pritchard, 1967). Hopkinson and Hoffman (1984) refined this definition as follows: "An estuary is a narrow, semi-enclosed coastal body of water which has a free connection with the open sea at least intermittently and within which the salinity of the water is measurably different from the salinity in the open ocean."

Estuaries are characterised by high biological productivity. They trap nutrients, sediments and other materials that enter from rivers, and play a fundamental role in the biogeochemical recycling of elements such as carbon and nitrogen. Estuaries also support a wide range of human activities, e.g. transportation, industrialisation, aquaculture and recreational activities (Kennish, 2000).

Three major forces affect estuaries: input from the land (rivers and other drainage), sea tides and wind (turbulence). Accordingly, Mann (2000) and Klee (1999) classify estuary water as stratified, partially-mixed and fully-mixed, based on salinity/density gradients.

Estuarine resources are influenced by the human disturbance of topographical, chemical, physical and biological aspects, all of which are interrelated (Tett, 1987). Large densely populated cities, major industrial activities and busy ports are located on estuaries (Kennish, 1997; 2000). Civil, industrial and navigational activities discharge effluent into estuaries in the form of land run-off, sewage and industrial waste. These discharges alter the physico-chemical composition and balance of estuarine water. The most important result of external input is nutrient enrichment. Estuarine eutrophication is a major factor influencing estuarine ecological systems (Cloern, 2001; Pinckney *et al.*, 2001; Smith, 2003; Beman *et al.*, 2005).

Discharge of toxic materials, such as heavy metals and chlorinated hydrocarbons, could harm the food chain, and of cooling systems at high temperatures could affect

species diversity and composition, as well as disrupting primary production (Kennish, 1997).

Estuarine ecology is influenced by the quantity and quality of riverine water input. Decreased riverine flow has many effects, including increased salinity, and reduced nutrient concentration, which limits algal photosynthesis and diminishes faunal biomass (Edgar *et al.*, 1999). Increased water flow raises organic and inorganic material input and the turbidity of the water column and may affect the amount of photosynthetically available solar irradiance, due to increased light absorption material and scattering coefficients through the water column (Gallegos *et al.*, 2005). Inversely low flow rate may reduce flushing time and the rate at which material leaves the estuary. Finally, riverine flow also changes the biotic community structure and composition.

Dredging a navigation channel (depths of 10-15m or more) changes the characteristics of an estuary, as can be observed in various locations in the world, especially in harbours and industrial areas. Dredging destroys benthic habitat communities, changes water quality, and increases turbidity, all of which affects primary production (Kennish, 2000; Lehman, 2000). Benthos removal may increase the levels of nutrients and poisonous chemicals in the water column. Reclamation of the estuarine boundaries may disturb important ecological areas (e.g. salt marshes and sea grass beds), thereby affecting stocks of commercially important organisms (Kennish, 1997).

In the long term, climate change may alter estuarine topography, and ecological, physical and chemical parameters. Storms and heavy rain change riverine flow with a resulting impact on estuaries (Gillanders and Kingsford, 2002). Sea level rises due to global warming, coastal submergence and isostatic readjustment. Such a rise affects estuarine areas with concomitant drastic changes to both living and nonliving components and their intra- and inter-relationships.

1-1-2 Pelagic production and food chains

Estuaries are productive environments which include various kinds of ecosystem, such as salt marshes, sea-grass communities, benthic systems and planktonic

systems. The constituent parts depend on the topography of the estuary and tidal conditions, but may generally be divided into inter-tidal zones (salt marshes, sea-grass beds, and beaches), sub-tidal benthic zones (mud or sand) and pelagic zones.

Pelagic communities are located on the seaward edge of estuaries. Phytoplankton are the dominant primary producers and are affected by various water quality parameters. Phytoplankton is consumed mainly by zooplankton and benthic filter feeders. Zooplankton can also feed on benthic diatoms and bacteria, which are resuspended in the water column of shallow estuaries (Lalli and Timothy, 1997).

Pelagic production, mainly by phytoplankton (including suspended benthic microalgae), converts light energy (solar irradiance) and inorganic nutrients (C, N, P, and Si) into chemical energy, through photosynthesis thereby producing organic carbon that supports aquatic organisms. Primary production is to a large extent influenced by surface light irradiance. Primary production is directly proportional to available irradiance and its seasonality. The photoperiod, along with other environmental factors, initiates production. Nutrients, including nitrate, phosphate and silicate, play a very important role in estuarine production. Estuarine planktonic primary production has been calculated by many researchers to be in a range of 60-500 g C m^{-2} y^{-1} (Kennish, 1986), with a mean of 150 ±50 g C m^{-2} y^{-1} (Smith and Hollibaugh, 1993).

Secondary producers mainly consist of herbivores, carnivores and detritivores. Herbivores are phytoplankton grazers in the water column, mainly zooplankton, including the larvae of benthic organisms and fish. Carnivores are organisms which feed upon grazers as secondary consumers, such as pelagic fish and bivalves. Detritivores are benthic organisms which feed upon organic particles and detritus, such as benthic fish (flat fish), crustaceans, invertebrates and polychaetes. Thereby secondary producers could be one of the controllers of pelagic biomass (phytoplankton).

Estuarine planktonic systems may be expected to be more productive than those on the continental shelf (Smith and Hollibaugh, 1993) because environmental conditions are more favourable for phytoplankton growth as a result of the mixing of riverine

and sea water (Chan and Hamilton, 2001; May *et al.*, 2003), salinity and stratification, temperature, light attenuation and tidal cycle (Heip *et al.*, 1995; Dyer, 1997) and because of the physical effects of tides (Ketchum, 1983; Kennish, 1986; Mann, 2000). Phytoplankton composition, biomass, and productivity are affected by fluctuations in environmental, physical and chemical parameters (Calliari *et al.*, 2005).

Estuaries are stratified to varying degrees, depending on the input of fresh water and the tidal cycle, both of which influence the biology of the area (Officer, 1992). According to their salinity gradient, estuaries are divided into oligohaline, mesohaline and polyhaline zones, which govern species distribution within different zones (Ketchum, 1983; Smayda, 1983). Changes in salinity create osmotic pressure problems for estuarine organisms, thereby affecting their distribution, as well as causing variation in the phytoplankton community, and their distribution and species composition (Smayda, 1983; Muylaert and Sabbe, 1999; Muylaert *et al.*, 2000). Unlike coastal and oceanic phytoplankton species, estuarine phytoplankton species, tend to be euryhaline (Kennish, 1986). Phytoplankton size fractions vary according to salinity gradient, light and nutrients (Sin *et al.*, 2000). Estuarine organisms can adapt to wide salinity ranges more readily than marine or fresh water organisms (Vernberg, 1992).

Temperature is also an important physical parameter in estuarine ecology. The influence of temperature may be observed in relation to life cycles; for example larvae can be released into the water column during winter or spring (Vernberg, 1992) as a direct consequence of temperature. Organisms display physiological responses, such as higher metabolic rates (as a result of temperature changes). However, water column temperature influences phytoplankton growth rate, productivity and other biological processes. According to Boynton *et al*. (1982), phytoplankton biomass and productivity are higher in warm water columns. Phytoplankton metabolism is regulated by temperature (Nixon, 1981). Jorgensen (1968) noted that water temperature can affect the time needed by cells to adapt to variations in irradiance. In laboratory culture, phytoplankton production increases by two to four times with an increase in temperature, within a range favourable for

growth (Kennish, 1986). A significant correlation between climatically-related environmental variation and phytoplankton species and species group biovolume suggests a link between climate and the distribution of biovolume in the phytoplankton community (Smayda, 1998; Lehman, 2000).

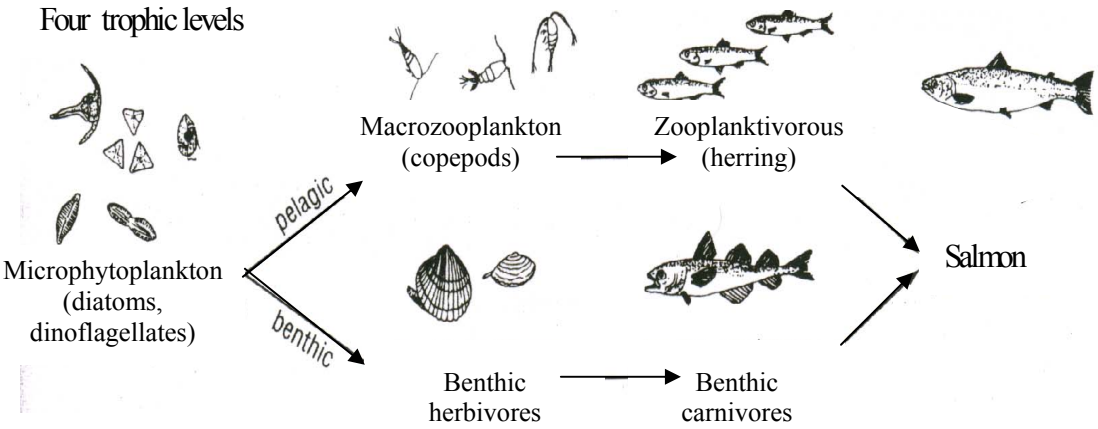
Environmental stress on phytoplankton cells causes loss of biomass. Cell lysis may also occur if cells cannot cope with environmental stresses such as incident irradiance, salinity variation or nutrient depletion. Viral infection can also cause cell lysis (Underwood and Kromkamp, 1999), thus inhibiting phytoplankton growth. Consequently, during the bloom period, viruses indirectly affect energy flux (Brussaard, 2004).

Viewed traditionally, energy flow in ecosystems is determined by the grazing pathway starting from organic detritus (Kennish, 1986; Hughes *et al.*, 2000). Self-feeders (autotrophs) are photosynthetic organisms, and these include phytoplankton, benthic microalgae (mainly diatoms) and some bacteria. They are able to convert the energy of sunlight to chemical energy, assimilating carbon dioxide and inorganic nutrients. In this way, they not only maintain themselves, but supply organic matter to dependent organisms (heterotrophs) in the water column and bottom sediment.

Autotrophs form the basis of the food chain in the water column of estuarine systems (Nixon, 1981; Kennish, 1986). The traditional food chain displays, but is not limited to, three basic trophic levels: autotrophs, herbivores, and carnivores (Lewitus *et al.*, 1998) (Figure 1-1). Algae are the principal autotrophs, with diatoms the dominant group (Hughes *et al.*, 2000).

Grazing by zooplankton and filter feeders affects phytoplankton production (Cloern, 1982), biomass and community size structure. Different phytoplankton taxa are grazed upon by different grazers including micro-meso zooplankton (copepods, ciliates, flagellates) and benthic organisms (filtering, suspension feeders). (Underwood and Kromkamp, 1999).

Figure 1-1: The four trophic levels of the pelagic food chain adapted from Lalli and Timothy (1997)

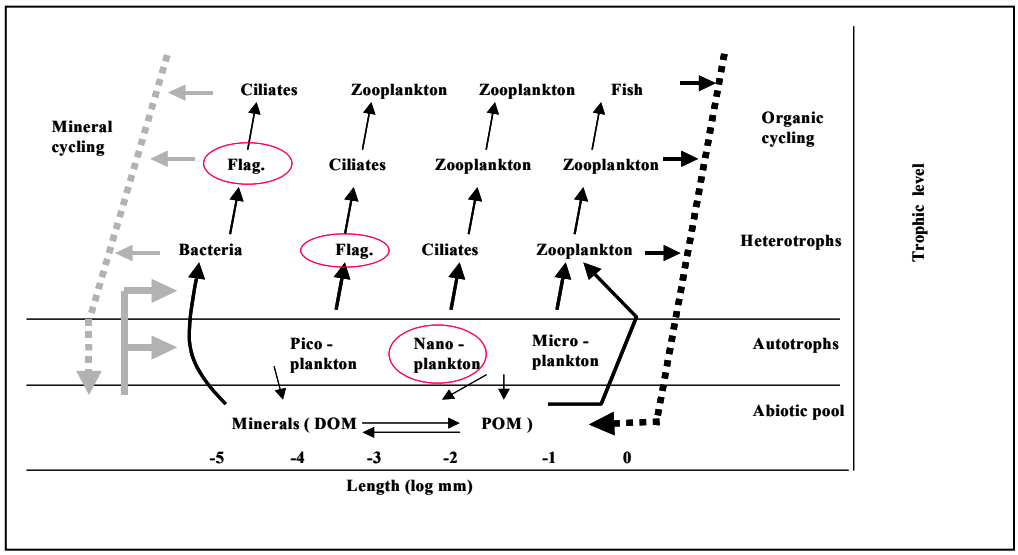


Azam *et al*. (1983) described the relationship between the higher trophic levels (larger organisms), which form the traditional food web, and the lower trophic levels (smaller organisms), which form the microbial loop (Figure 1-2). The microbial loop introduces to the food web bacteria and smaller phytoplankton (pico and nanophytoplankton of <2 µm and 2-20 µm diameter, respectively).

Bacteria are important among the smaller members of the community as they consume organic carbon (obtained from the breakdown of complex organic particles) and release inorganic nutrients (N, P, and Si) into the water column (Ducklow, 2001; Landry, 2001). The heterotrophic flagellates and ciliates prey on the bacteria, thus controlling their population. Consequently, the microbial loop is established (Ducklow, 2001; Landry, 2001).

Heterotrophs and mixotrophs also produce organic carbon through photosynthesis and help control the microbial food chain. They consume organic carbon, bacteria, pico- and nanoplankton, thus acting as controllers in the microbial web (detailed in Samuelsson, 2003). The process is dictated by environmental conditions. Estuarine food chains are linked together forming complex food webs, because every species is usually eaten by more than one predatory species (Samuelsson, 2003). The microbial food web (Figure 1-2) illustrates this. Flagellates prey on bacteria and picoplankton, and in turn they are consumed (along with autotrophic flagellates) by heterotrophic dinoflagellates and mesozooplankton.

Figure 1-2: The microbial food web (adapted from Azam et al., 1983)



The existence of a food web type depends on nutrient availability When the water column is rich in nutrients, microphytoplankton predominate; however, when nutrients are in short supply, the microbial web (nanophytoplankton) predominates (Froneman, 2001; 2004). The estuarine food chain structure and length (number of trophic levels) can be altered by changes in primary production and plankton size structure (Samuelsson, 2003).

1-1-3 Biogeochemical recycling within estuaries

Estuaries receive dissolved or particulate organic material (DOM or POM) as well as inorganic nutrients (C, N, P, Si) from rivers, detritus, and human activities (sewage and agriculture). Estuaries filter these materials and recycle them *in vivo* (Grenz *et al.*, 2000; Mann, 2000). However, estuaries and coastal areas play an extensive role in biogeochemical cycling due to considerable input of organic matter and nutrients (Gattuso *et al.*, 1998; Smith, 2003; Gazeau *et al.*, 2004).

During photosynthesis, phytoplankton, as well as benthic microalgae, use carbon dioxide and inorganic nutrients to produce DOM and POM in the water column. Phytoplankton takes up inorganic nutrients in a consistent C:N:P ratio of 106:16:1 respectively (known as the Redfield ratio) (Redfield, 1958). The ratio could be extended to include Si with a value of 16. This ratio both determines and reflects the proportional elemental chemical composition of the plankton. When sunlight is

available, phytoplankton assimilates inorganic nutrients and releases oxygen. When the essential macronutrient elements C, N, P and Si are available in the right ratios, they promote primary production. The primary production equation is shown as follows (Tyrrell, 2001):

$$106CO_2 + 16\,NO_3 + H_3\,PO_4 + 78\,H_2O \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} (C_{106}H_{175}O_{42}N_{16}P) + 150O_2$$

The phytoplankton may be consumed by grazers, flushed out of the estuary, or settle to the bottom. The settling phytoplankton is consumed by benthic organisms or bacteria during aerobic or anaerobic decomposition (mineralization) (Zehr and Ward, 2002). Nutrients (N, P, Si, C) are released back to the water column through different processes on different time scales (Heip *et al.*, 1995). When heterotrophic organisms consume phytoplankton and other POM, such as detritus, they release DOM, CO_2 and nutrients to the water column in the process of respiration, in a similar equation as the above.

Estuarine elemental budgets depend on balance between input and output on the one hand and the consumption and recycling of elements on the other. The most important elements in estuarine photosynthesis are carbon, nitrogen (in NO_3, NH_4, NO_2), phosphate and dissolved silicon (in $Si(OH)_4$). Nutrient recycling is a prerequisite for the production of new organic matter (Nedwell *et al.*, 1999). The fluxes of carbon and nitrogen budgets are exemplified in Figure 1-3 A and B.

The estuarine carbon budget is shown in Figure 1-3 A. Carbon input from rivers and estuarine edges takes the form of POC (detritus and phytoplankton), with little input of POC from the seaward side. POC input exceeds that which is consumed and recycled in estuaries. This excess carbon is lost in three ways: (1) by moving out to the sea in the form of CO_2, POC or DOC, (2) by becoming part of sediment or (3) by conversion to gases such as CO_2 and CH_4, and thus lost into the atmosphere.

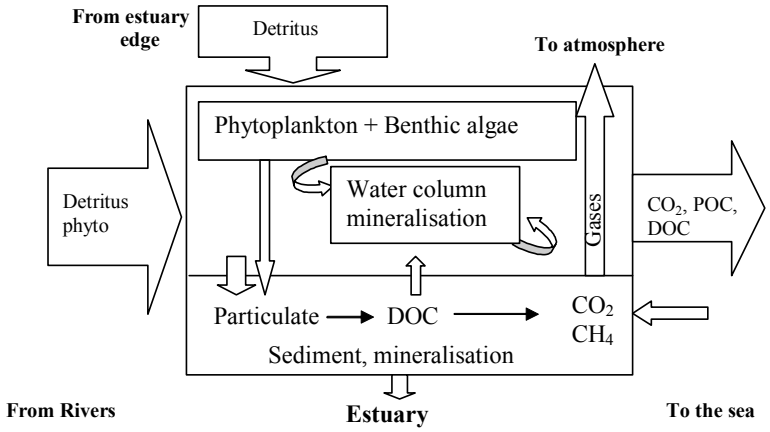
In the estuarine nitrogen budget illustrated in Figure 1-3 B, nitrogen sources are in the form of PON, NH4, and nitrate (NITR). As with POC, PON input is greater than consumption and recycling. The excess nitrogen is lost via the same three routes described above, so that estuaries emit N_2 and N_2O gases into the atmosphere, due to

the large input of PON to estuaries (Smith and Hollibaugh, 1993; Gattuso *et al.*, 1998).

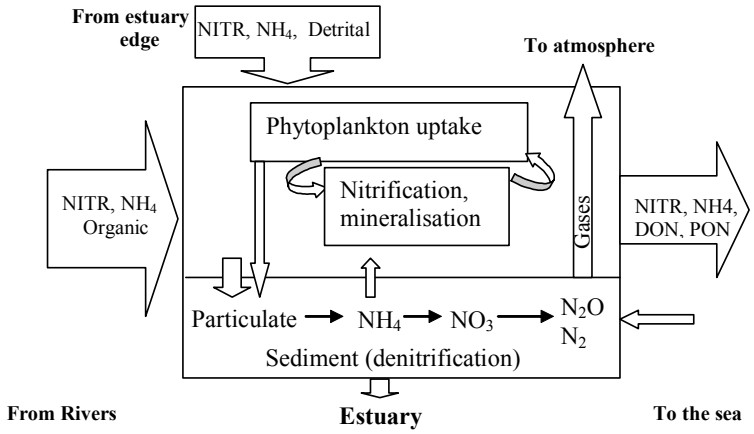
Figure 1-3: Estuarine A) carbon and B) nitrogen budgets.

[Arrows outside and to the left of the box indicate exchange with the river. Arrows above the box indicate estuarine edge discharges. Arrows to the right of the box indicate exchange with sea. Arrows inside the box (estuary) indicate material recycling between water column and sediment and gas loss to the atmosphere]

A – adapted from (Soetaert and Herman, 1995a)



B – adapted from (Soetaert and Herman, 1995b)



However, when organic materials decay, carbon is released more slowly than nitrogen and phosphorus, but faster than silicon (Tyrrell, 2001). The element recycling process (when organic materials decay) may vary, as well as the number of steps involved. The factors affecting recycling are change in temperature (seasonality) and element concentrations (e.g., after bloom, increased POM input) and location (at the head or mouth, water column or benthos) (Middelburg *et al.*, 1995; Karl and Michaels, 2001; Ragueneau *et al.*, 2002).

Material recycling can be an aerobic process occurring throughout the water column or anaerobic in the benthos. Complex organic matter (POM) is converted to low-molecular weight organic compounds (DOM). Anaerobic bacteria denitrify DOC to convert it to carbon dioxide (Mann, 2000; Tyrrell, 2001; Ragueneau *et al.*, 2002). DON is composed mostly of amino groups in protein. Through microbial activity, proteins are deaminated and ammonia nitrogen is produced. This process is called ammonification and ammonia (NH_3) is released to the environment (mineralized) or assimilated into microbial tissue. Ammonia released into water establishes equilibrium with the ammonium ion (NH_4^+) which is microbially nitrified to NO_2 then to NO_3. The other process is denitrification under anaerobic conditions. Many microorganisms can use nitrate or other oxidized forms of nitrogen instead of oxygen in respiration. This heterotrophic process is termed nitrate reduction or nitrate respiration; it is termed denitrification when gaseous forms of nitrogen (NO_2 to N_2 and N_2O) are released as metabolites and lost from the system. P cycle is less complicated than C and N, as it is an inorganic element found in both organic matter and in the water column. Its recycling does not need oxidation or reduction steps (Middelburg *et al.*, 1995; Soetaert and Herman, 1995a; b; Karl and Michaels, 2001; Ragueneau *et al.*, 2002).

1-2 Phytoplankton in Temperate Estuaries

1-2-1 Phytoplankton taxonomy and size structure

Phytoplankton in estuaries and marine waters can be categorised according to their taxonomic classes (diatoms, dinoflagellates and flagellates) (Tomas, 1997), or according to their size (micro- nano- and pico- plankton) as summarized in Table 1-1 (Jeffery and Vesk, 1997).

Diatoms are of two types: planktonic, recognisable by the central circular or dome-shaped valves and benthic diatoms which are pennate shaped. They and are present in all aquatic ecosystems. Their cell walls contain silica, which is an essential element for their growth. Diatoms have two valves (epitheca and hypotheca) joined together like a Petri dish. The cell size reduces during successive asexual generations until restored through sexual reproduction. Diatoms consist of single cells or

preformed chains, and are typically heavier than water, due to their siliceous skeleton. Diatom size ranges from <10 to 200 µm (Tomas, 1997). Diatoms are autotrophs and have fucoxanthin as a distinctive pigment in addition to chlorophyll *a* (Jeffery, 1997).

Dinoflagellates are unicellular (5-200µm in diameter), a few species having preformed chains. They possess two flagella which are used for swimming. They are found more widely in sea water than in fresh water, and at warmer temperatures. Reproduction may occur either asexually by cell division or sexually. Many are small, but efficient nutrient consumers (Kennish, 1986; Tomas, 1997). The autotrophic dinoflagellates plastids have chlorophyll *a* and the biomarker pigment peridinin (Jeffery, 1997). Heterotrophic dinoflagellates can feed upon phytoplankton and zooplankton.

Flagellates can be classified according to their trophic level. Phototrophs use light for photosynthesis. Heterotrophic flagellates take up organic matter from the water column and ingest it to support cell growth. Mixotrophic flagellates are able to use both kinds of nutrition. Mixotrophy may be a survival strategy to survive during periods of nutrient deficiency. Hetero- and mixotrophic flagellates prey upon bacteria, and are an important factor in regulating the bacterial community (Boenigk and Arndt, 2000). Since bacteria are important in organic decomposition and nutrient recycling, mixotrophic and heterotrophic flagellates together can affect nutrient turnover, and consequently the magnitude of primary production of autotrophic flagellates.

Typically, flagellates have between 1 and 4 flagella, but there may be as many as 16. They are either free swimming or attach themselves to substrates like large cellular and filamentous algae. Motile forms move by means of the whip-like action of their flagella. Three feeding mechanisms are readily recognizable among flagellates: filter feeding, interception feeding and raptorial feeding (Samuelsson, 2003). Vegetative reproduction is common in flagellates, and their life cycle may include both motile and non-motile pelagic stages.

The majority of planktonic flagellates are nanoplankton, ranging in size from 1 to 20µm. Flagellates may be divided into two classes, depending on their pigment, flagella and microanatomy (Tomas, 1997). The Chromophyta in the class Cryptophyceae lack chlorophyll *b*, whereas the Chlorophyta of classes Euglenophyceae, Prasinophyceae and Chlorophyceae have chlb in addition to chlorophyll *a* and accessory pigments (Tomas, 1997). Other accessory pigments prouduced by flagellates include alloxanthin, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin, violaxanthin and prasinoxanthin (Jeffery and Vesk, 1997).

Using the criterion of size, the following taxonomy for phytoplankton is used: ultraplankton (<5µm in diameter), nanoplankton (5-20µm), microphytoplankton (20-100µm) and macrophytoplankton (>100µm) (Kennish, 1986). Microphytoplankton are dominated by diatoms and dinoflagellates, while nanoplankton are dominated by other flagellates including members of the classes Chrysophyceae and Crypotophyceae and small species of dinoflagellates (Kennish, 1986). With the recent availability of a wider range of filter pore sizes, phytoplankton size can be further divided into picophytoplankton (<2µm in diameter), nanoplankton (2-20µm), and microphytoplankton (>20µm) (Kiorboe, 1993; Tomas, 1997; Sin *et al.*, 2000; Ansotegui *et al.*, 2003).

Table 1-1: A summary of phytoplankton taxa, cell size, and major pigments (adapted from Jeffery and Vesk, 1997)

Algal division/ class	Micro >20um	Nano 2-20 um	Pico <2 um	Chlorophyll *A*	Pigments
Diatoms	+	+	-	+	Fucoxanthin
Dinoflagellates	+	+	-	+	Peridinin
Chlorophyta	+	+	+	+	Chlorophyll b, Violaxanthin
Cryptophyta	+	+	-	+	Alloxanthin
Cyanophyta	+	+	+	+	Zeaxanthin
Euglenophyta	+	-	-	+	Chlorophyll b
Prasinophyta	+	+	+	+	Chlorophyll b, Prasinoxanthin
Prymnesiophyceae	-	+	-	+	19 -hex-, 19but-anoyloxyfucoxanthin
Raphidophyceae	+	-	-	+	Fucoxanthin, Diadinoxanthin

Chemotaxonomy: Chlorophyll *a* pigment concentrations in phytoplankton are normally measured by a spectrophotometer or a fluorometer, which indicates the density of phytoplankton in the water column (Parsons *et al.*, 1984). Phytoplankton species and this spatio-temporal distribution can be shown by microscopic identification. Nowadays, high performance liquid chromatography (HPLC) is used to identify the different photosynthetic pigments present in organisms as taxonomic markers (Barlow *et al.*, 1997; Jeffery, 1997). Chemotaxonomy and microscopy can match phytoplankton groups with their respective pigments. Therefore, the availability of chlorophyll *a* and the main accessory pigment can be correlated to the presence of particular groups available in the microscopic analyses. Due to the spring bloom, diatoms and prymnesiophytes are able to increase their marker pigment, fucoxanthin, in lower estuaries, while in upper estuaries prymnesiophytes are replaced by symbiotic dinoflagellates with alloxanthin and peridinin as pigment markers. Chlorophyll *b* signifies the presence of euglenophytes, chlorophytes and prasinophytes. Microscopy and chemotaxonomy can be used to identify accurately phytoplankton assemblages to species level (Garibotti *et al.*, 2003), while HPLC analyses can identify microalgal sediment pigments (Lucas and Holligan, 1999 a). Karlson (1995) studied pico- and nanoplankton, using HPLC pigment analysis to quantify the relative contribution of different algal groups to total phytoplankton biomass.

The size structure of phytoplankton assemblages reflects responses to environmental conditions (Tamigneaux *et al.*, 1999). The size of an organism at any trophic level can be a determining factor in the length of the food chain (Parsons and Takahashi, 1973). Smaller algae are more effective than larger ones in competing for light and nutrients. However, larger algae suffer less predation pressure than small ones; consequently, they may bloom, not because of growth, but because they escape size-selective grazers (Riegman *et al.*, 1993).

To understand biomass production and food web dynamics, the full spectrum of plankton size must be investigated. In the past, the lower portion of the size spectrum was overlooked. Techniques developed in the last 20 years or so have shown that bacterial biomass is related to phytoplankton concentration, and that bacteria utilise

10 to 50% of the carbon fixed by photosynthesis (Azam *et al.*, 1983). Nanoplankton are an important component of the microbial loop, containing a diverse taxonomic assemblage which is often overlooked. In the Celtic Sea, large phytoplankton (>5μm) was significant only for a short period during the spring diatom bloom. In winter, small nanoplankton (< 5 to > 1μm) and picoplankton (<2μm) accounted for almost 70% and for 13%, respectively, of the daily primary production (Joint *et al.*, 1986). The above information high light to the importance of including different phytoplankton size fractions in primary production studies. The inclusion of such information about size fractions should help to explain variation in the structural components of communities and their spatio-temporal contribution to primary production.

1-2-2 Effect of environmental factors on phytoplankton growth

1-2-2-1 Irradiance

In temperate estuaries, phytoplankton biomass is generally low in winter and autumn and high in spring and summer. Cole (1989) reported that 90% of the variation in phytoplankton productivity is attributable to variations in light irradiance. Phytoplankton composition in temperate estuaries is influenced by light intensity (Smayda, 1998; Cloern and Difford, 2005).

The relationship between photosynthesis (P) and irradiance (E) is described by the P *vs* E curve and is shown in Figure 1-4. Photosynthesis increases linearly with increasing light intensity until it reaches a maximal value, $P_{max,}$ at which the system becomes light saturated. The slope (α) equals $\Delta P/\Delta E$ before light saturation and depends on light intensity. At higher levels of irradiance, photosynthesis may significantly decrease due to photoinhibition (β) resulting from physiological reactions (e.g. chloroplasts shrink in high light intensity). The light saturation parameter, E_K, can be calculated as P_{max}/α. Values for α and E_K differ from species to species, and over time for a given species. The final parameter is Ec, the compensation point, where the photosynthetic rate equals the amount of oxygen consumed in respiration (Lalli and Timothy, 1997).

The response of a species to light intensity determines its predominance or otherwise. Two species (species 1 and 2) are presented in the P vs. E curves (Figure 1-4) which differ in their number and size of photosynthetic units (PSU). Species 1 (high number of PSU) has E_{K1} higher than E_{K2} of species 2 (large PSU size). This suggests that species 2 can compete under lower light intensity, whereas species 1 requires higher light levels to be dominant (Kiorboe, 1993).

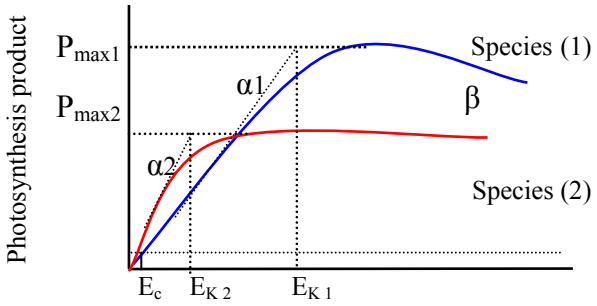


Figure 1-4: Relationship between photosynthesis (P) and irradiance (E) curves.

Light intensity decreases exponentially with water depth. Light attenuation in the water column can be measured by the extinction coefficient (K_d), which can be calculated using the following equation:

$$K_d = (\ln E_0 - \ln E_D)/ (D)$$

Where E_0 is surface irradiance and E_D is irradiance at depth D (m).

The primary production profile for phytoplankton in the water column is illustrated in Figure 1-5. This shows the distribution of photosynthesis (areas A, E, C) and respiration (R) (area A, B, C, D), with depth. Figure 1-5 also defines the compensation depth (Dc) (see P-E curve), when the oxygen production rate equals the amount of oxygen consumed, and the critical depth (D_{cr}), where total photosynthesis (P_w) equals total respiration (R_w) in the water column. The D_{cr} varies, depending on water column depth and its mixing or stratification status.

Variations in irradiance availability in the water column euphotic zone (down to the 1% incident light level) alter the productivity and species of phytoplankton (Goosen *et al.*, 1999). The phytoplankton growth rate increases towards the mouth of an estuary because of increased water transparency (Cloern, 1987; Cole, 1989; Kocum

et al., 2002b). Accordingly, light penetration governs phytoplankton distribution within nutrient-rich estuaries (Kocum *et al.*, 2002b).

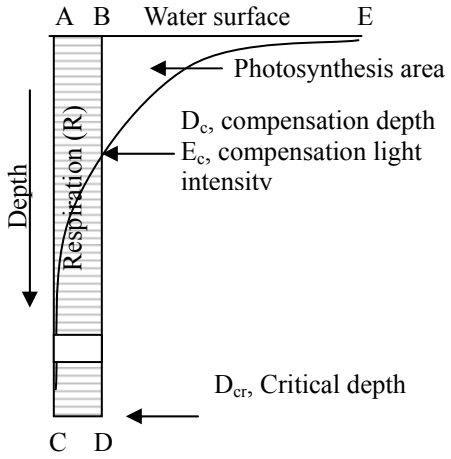


Figure 1-5: Definition of compensation and the critical depths.

The compensation depth in estuaries is less than that in oceanic water because of greater light attenuation in estuaries, resulting largely from the increased load of suspended particles (Smayda, 1983; Gallegos *et al.*, 2005). According to Goosen *et al*. (1999), there is a strong correlation between phytoplankton primary production and the depth of the euphotic zone in different European estuaries. Phytoplankton tend to be light limited in both highly turbid water and in the nutrient-rich oligohaline zone (Nedwell *et al.*, 1999).

The water column in estuaries is affected by tidal cycles and river inputs affect light attenuation, nutrient availability and column properties (Westeyn and Kromkamp, 1994; Gillanders and Kingsford, 2002). Fluctuations in salinity, mixing and stratification allow nutrients and phytoplankton to move up and down or remain longer within the photic zone. Turbidity causes a shading effect, scattering light. However, flushing time increases or decreases the residence time of nutrients, which in turn influences their uptake and recycling within the estuary (Nedwell *et al.*, 1999; Underwood and Kromkamp, 1999). High flow rates can be detrimental to phytoplankton by creating a turbid environment which inhibits light penetration (Westeyn and Kromkamp, 1994).

1-2-2-2 Inorganic nutrients

Inorganic nutrients, especially phosphate (P), nitrate (N), and silicate (Si), have a major influence on phytoplankton community structure and biomass in aquatic environments (Heip *et al.*, 1995; Hessen, 1999; Underwood and Kromkamp, 1999). N, P and Si levels increase during autumn and winter, due to low consumption and high riverine input and regeneration (Hessen, 1999).

Phytoplankton growth rate (μ) is affected by nutrient concentration within the water column. Figure 1-6 shows the relationship between growth rate and nutrient concentration for two algal species. The growth rate, μ (d^{-1}), varies with different species, and maximum growth rate (μ_{max}) depends upon the species nutrient half saturation constant K_N (μM), which is equal to the concentration of nutrient at 1/2 μ_{max} response of a species to nutrient uptake. This varies also according to species size, due to the variable ratio of cell area to volume (Riegman *et al.*, 1993; Lagus *et al.*, 2004).

There is a relationship between species dominance and nutrient level. In figure 1-6, species 1 is larger than species 2. When nutrient level is low (μ2 >μ1), species 2 predominates. In contrast, where the nutrient level is high, species 1 predominates due to its higher μ (Riegman *et al.*, 1993; Lalli and Timothy, 1997; Ornolfsdottir *et al.*, 2004b).

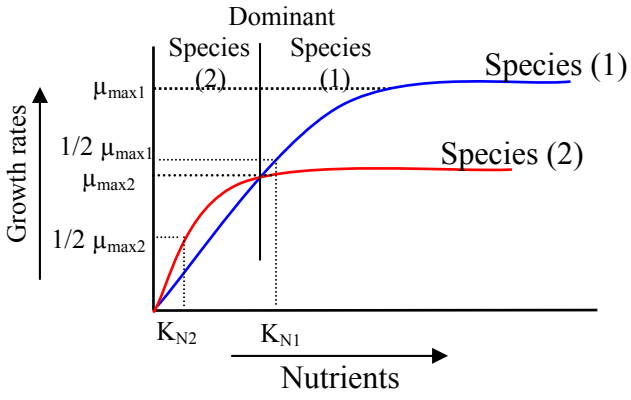


Figure 1-6: Relationship between growth rate (μ) and nutrient level for two species.

The composition of the cells, in terms of the major elements are C, N and P, whose ratio is defined by Redfield ratio of 106: 16: 1, respectively (Redfield, 1958; Tyrrell, 2001). The nutrient ratio of particulate matter in the water column (mainly N: P) can be used to indicate which element is deficient (limiting).

Rising nutrient loads (especially N and P) in the water column tend to increase the phytoplankton biomass (Smayda, 1989; Cloern and Difford, 2005). Si is especially important to diatoms. Nutrients exhibit seasonal patterns (with higher values in winter and lower ones in summer) associated with the intensity of biological removal (Underwood and Kromkamp, 1999). Boynton *et al*. (1982) found a linear relationship between dissolved inorganic nitrogen and annual primary production.

After the spring bloom, Si is depleted, mainly by diatoms (Underwood and Kromkamp, 1999). Thereafter, the phytoplankton community is dominated by flagellates, which do not require Si (Garnier *et al.*, 1995), e.g. *Phaeocystis* (Prymnesiophyceae) (Peperzak *et al.*, 1998).

It is assumed that nitrogen limits biomass in marine systems and that phosphorus has a similar effect in inner estuaries (Pitkanen and Tamminen, 1995). P may also limit phytoplankton growth (Heip *et al.*, 1995), and is accepted as a phytoplankton-controlling factor (Kocum *et al.*, 2002a; Nedwell *et al.*, 2002), especially in the short term (Fisher *et al.*, 1988). It has been suggested that nutrient limitation in UK estuaries may occur in a decreasing order of limitation: P> Si >N (Nedwell *et al.*, 2002). However, nitrogen (or nitrogen and phosphorus) limitation is associated with periods of low river flow, with balanced N:P ratios of sea water (Fisher *et al.*, 1992).

According to Nedwell *et al*. (2002), in many European estuaries, nutrients are anthropogenically elevated. Nitrogen (N) and phosphorus (P) tend to regulate the coastal phytoplankton biomass in spring before it becomes dependent on regenerated nutrients. Silica (Si) loads are relatively independent of anthropogenic influences (Hessen, 1999), and fluctuate seasonally, primarily as a result of variations in biological removal, rather than seasonal variations in fluvial loads (Ficheza *et al.*, 1992).

A strong correlation has been found between the maximum values of chlorophyll *a* and the annual input of nutrients (particularly nitrogen) in 40 estuaries (Monbet, 1992). That same result was found by Boynton *et al*. (1982) at Chesapeake Bay. The phytoplankton community growth rate also responds to nutrient enrichment pulses in changing estuarine environments (Pinckney *et al.*, 1999; Pinckney *et al.*, 2001;

Ornolfsdottir *et al.*, 2004a). Beman *et al*.(2005) found that the highest community growth rates occurred with high nitrate levels, in calm conditions. Nutrient supply or depletion tends to influence biological processes, smaller algae being more successful competitors for nutrients than larger ones (Riegman *et al.*, 1993).

Nutrient ratios in the water column are modified by change in input or uptake. For example, a diatom-dominant community shifts to a dinoflagellates dominant community given a low Si:P ratio (Smayda, 1989). The results of an enrichment experiment show that phytoplankton biomass, determined as chl or carbon biomass, is generally limited by N across various individual species. *Skeletonema costatum* is P-limited; the N:P ratio strongly affects mixotrophic chrysophytes, which grow exponentially at higher ratios (Lagus *et al.*, 2004). Variations in the N:P and Si:P ratios can also change phytoplankton composition and size structure where decreasing Si concentrations become a limiting factor (Justic *et al.*, 1995a; Justic *et al.*, 1995b). A higher N:Si ratio enhances the proliferation of all microbial groups, including phototrophic nanoflagellates, autotrophic dinoflagellates, bacteria and heterotrophic flagellates and ciliates (Roberts *et al.*, 2003).

1-2-3 Effect of environmental factors on species succession and population structure

Heip (1995) reported that species composition varies with estuarine gradient. In temperate estuaries, diatoms generally dominate year round, contributing up to 80% of the total (Smayda, 1983; Kennish, 1986). The balance is composed of *Cryptomonas*, dinoflagellates, and classes such as Chlorophyceae and euglenoids (*Eutreptiella*) (Raymont, 1980).

Diatom blooms occur in late winter to late spring as light increases, and they are accompanied by nutrient depletion. The population remains relatively small in summer due to low nutrient levels and high grazing pressure. At this time, occasional blooms of dinoflagellates may occur. Other groups are able to flourish during periods of low nutrient levels and produce organic material, such as picoplankton and nanoplankton (<2 and 2-20 μm in diameter, respectively) (Little, 2000; Mann, 2000).

For example, the phytoplankton community structure in the lower Urdaibai estuary (Spain) is dominated by diatoms (microphytoplankton > 20µm size) during winter and spring, while nanophytoplankton <8µm in size including dinoflagellates and euglenophytes, are present during the rest of the year (Ansotegui *et al.*, 2003). By comparison, in Chesapeake Bay, diatoms are present year around, the species *Skeletonema costatum* and *Chaetoceros socialis* being more abundant in autumn and winter. The dominant summer species are *Coscinodiscus marginatus* and *Rhizosolenia calcara-vis*. Dinoflagellates (such as *Gymnodinium danicans*, *Ceratium furca* and G*yrodinium estuariale*) are more abundant in the summer month. Nanoplankton (<35µm diameter) account for 57-90% of total phytoplankton productivity in warm periods (Kennish, 1986).

The River Seine system (France) is mainly dominated by diatoms in early spring, with a consequent increase in biomass and Si depletion. Chlorophyceae succeed diatoms, becoming dominant by the end of May (Garnier *et al.*, 1995).

Between 1975 and 1993, the phytoplankton community structure in Northern San Francisco Bay, was characterized by a noticeable decrease in diatom biovolume, as opposed to an increase in green, blue-green algae and flagellate biovolume (Lehman, 2000). However, in 2005, the phytoplankton population of the Bay was dominated by diatoms, which accounted for 81% of the total biomass, while dinoflagellates and cryptophytes contributed 11% and 5% of the total biomass, respectively. Chlorophyta, Cyanophyta, Chrysophyta and Euglenophyta were minor components, although they may be important in specific samples (Cloern and Difford, 2005). Evidently, there are shifts in community structure at certain periods. High temporal frequency shifts in population structure were reported in the temperate estuary of the Narragansett Bay (US), and these were attributed to habitat variability (Smayda, 1998).

A part of the phytoplankton community consists of nanophytoplankton, which are soft organisms, mainly of smaller cell size (<5 µm) in diameter (Tomas, 1997). They can not be distinguished in light microscopic analyses (Booth, 1993). They are a little clearer using an epifluorescence microscope, but without the identification to species, which can be done with an electron microscopy (Maclsaac and Stockner,

1993). No relationship has been found between green algae and their pigment signature (chlb) and the microscopic count due to uncertainties in the numeration of small sizes (<5 μm) (Breton *et al.*, 2000). However, a combination of different techniques may be helpful in terms of cell numeration and species identification such as pigment analyses and using a flow cytomeyer counter, and fluorescent *in situ* hybridisation (Simon *et al.*, 1994; Not *et al.*, 2002). The flow cytometer can also characterize the community cell size structure (Tarran *et al.*, 2001).

1-2-4 Occurrence of blooms

A bloom may be defined as a population explosion of a particular species of phytoplankton, which is often confined to a definite part of the water column. It is brought about by an excess of primary production over loss resulting from respiration, grazing and advection (Legendre, 1990). However, blooms may manifest themselves other than in numbers, e.g. in colour change, high chlorophyll and noticeable toxin production. Dinoflagellate blooms normally stain water red (Smayda, 1989). Blooming diatoms and ciliates result in green or brown coloration (Crawford *et al.*, 1997). Some blooms are toxic, and are associated with massive fish mortality (Smayda, 1989). Blooms occur at different times in different estuaries, depending on the environment and meteorological conditions (Lucas *et al.*, 1999a; Pinckney *et al.*, 1999). Within a single estuary, the bloom can occur yearly, but at different times and with varying magnitude (Boynton *et al.*, 1982; Li and Smayda, 1998). Li and Smayda (1998) studied chl distribution in Narragansett Bay for two decades, reporting that blooms can occur all year round, with maximum values (major bloom) during winter–spring and minimum ones during summer. The spring blooming of diatoms in temperate estuaries is well known. Normally, the spring diatom bloom is succeeded by flagellates and dinoflagellates, depending on light, nutrient availability and water column mixing (Heip *et al*., 1995).

1-3 Southampton Water

1-3-1 Description of Southampton Water

Southampton Water is a shallow coastal plain estuary located in the southern part of the United Kingdom. It is 15km long, 2.5km at its widest part and about 10m deep in

a centrally dredged channel (Dyer, 1973; 1982). The northern end of the estuary is fed by the Rivers Test and Itchen which have a mean annual discharge of 8.81 and 3.26 m^3s^{-1}, respectively (Sylaios and Boxall, 1998). It is a macrotidal estuary (i.e. mean tidal range >2m) and is defined as partially mixed (Dyer, 1973). The tidal range varies between 1.5 and 5.0m. However, the estuary has a semidiurnal tidal regime, which means that it has a low ebb tide and a double high tide some 2 hours apart (Dyer, 1973).

The mean annual water temperature is 10.9^o C. The salinity on the surface exceeds 30 at Dock Head (upper estuary) during high tide in the summer and autumn, while surface salinity during low tide at Calshot (lower estuary) is less than this (Carr *et al.*, 1980). Raymont (1972) described Southampton Water at high tide as a marine environment with salinity reaching 34 at Calshot and >31 in the upper estuary.

Southampton Water receives 10% of its fresh water input in the form of treated water from both domestic and industrial discharges (Soulsby *et al*., 1985, cited in Kifle and Purdie, 1993). The discharges increase nutrient levels (for more details see Hydes, 2000). Nutrients in water have been widely studied over the last two decades in both the Test and the Itchen rivers, which are the main nutrient suppliers (Hydes and Wright, 1999; Hydes, 2000). The nutrient input of NO_3 and PO_4 in the two rivers has witnessed a significant increase. The NO_3 input increased from 650µM in 1974 - 1979 to 815µM in 1990 - 1997. During the same period, the PO_4 input increased from an average of 5.3µM to 10.7µM. Nutrient observations on the estuary showed a nitrate concentration range of 0.5-40 µM.

1-3-2 Phytoplankton in Southampton Water

Phytoplankton biomass (chlorophyll a) ranges from 1-2 mg m^{-3} to 10-20 mg m^{-3} in winter and summer, respectively (Iriarte and Purdie, 2004; Ali, 2003). Therefore, it shows a wide seasonal fluctuation. During bloom time, chlorophyll *a* may exceed 40 mg m^{-3}, as recorded in the 1999 spring bloom (Holley and Hydes, 2002). The timing of major spring blooming events (>10 mg chlorophyll a m^{-3}) was found to be correlated with mean water column irradiance (Iriarte and Purdie, 2004; Ali, 2003). A five-year study from 1998 to 2003 showed that major spring blooming events are

usually dominated by large chain-forming diatoms (usually in May). The mean water column photosynthetic active radiation (PAR) averaged for one week prior to the sampling date was >380 W h m^{-2} d^{-1} (Iriarte and Purdie, 2004). Light irradiance is a major factor influencing phytoplankton growth in Southampton Water, particularly because of the ample supply of nutrients (Ali, 2003; Kifle, 1992; Hydes, 200).

Southampton Water has a characteristic phytoplankton species succession. In the spring, diatoms accumulate under favourable conditions of high turbulence and increased nutrient concentration. Dinoflagellates succeed diatoms under conditions of reduced turbulence and lower nutrient levels (Kifle and Purdie, 1993; Ali, 2003). Conditions intermediate between these two periods favour flagellates such as *Eutreptiella* and *Cryptomonas* spp. and the ciliate *Mesodinium rubrum* (Crawford *et al.*, 1997; Ali, 2003). Blooms with maximum algal density and variety do not appear until early summer (Raymont, 1980). *Mesodinium rubrum* (autotrophic ciliate) has been reported as a yearly event (Kifle, 1992; Lauria, 1998). Iriarte (1991) recorded a bloom of small flagellates, a prymnesiophycean (*Phaeocystis* sp.) seaward. Table 1-2 lists the phytoplankton species succession.

Grazing by zooplankton is a controlling factor on phytoplankton biomass. A positive correlation has been found between chlorophyll *a* and zooplankton abundance (Muxagata, 2005). Zooplankton as secondary producers are a component of the estuarine food chain. The mean annual secondary production is estimated at 33mg C m^{-3} y^{-1} (Muxagata, 2005).

Table 1-2: Phytoplankton species succession in Southampton Water (source: Ali, 2003).

Months	Dominant group
Mid April / May	Diatoms (*Skeletonema costatum*, *Thalassiosira* spp.)
April / May-June	Diatoms (*Guinardia delicatula*)
Mid/late May	Euglenoid (*Eutreptiella marina*)
Late May / late August	Ciliates (*Mesodinium rubrum)*
Mid June-early August	Dinoflagellates (*Scrippsiella trochoidea, Prorocentrum*
Late August	Diatoms (*Chaetoceros spp., Skeletonema costatum)*

Table 1-3 summarizes information about dominant phytoplankton species and chlorophyll *a* concentrations in Southampton Water since 1973. Data (adapted from

Ali, 2003) have been compiled from various studies carried out in Southampton Water (Iriarte, 1991; Kifle, 1992; Lauria, 1998; O'Mahony and Weeka, 2000; Ali, 2003).

During a study conducted at Southampton Water during 1991, Kifle (1992) observed that chlorophyll size fraction >10 μm was the most important fraction, contributing 60% of the total phytoplankton biomass, while smaller nanoplankton, 3-10 μm in diameter, which contained micro-flagellates of the class Cryptophyceae, contributed between 16.5 and 20.4%. The picoplankton peak appeared at different times of the year, with the highest contribution in spring at Calshot and in summer at NW Netley.

Phytoplankton chlorophyll *a* size fractions and picoplankton abundance were studied in Southampton Water at NW Netley and Calshot, as a comparison with marine North Sea water by Iriarte (1991). Chlorophyll *a* was fractionated with (3 (5), 1, and <1μm membrane filters). A relative chlorophyll *a* concentration of >3 (5) μm contributed the highest, with values of 85.7% and 81.7%, 1-3 with value 9.5% and 13.8%, while the fraction <1μm contributed the lowest with values of 4.8% and 4.5% values at NW Netley and Calshot, respectively (Iriarte and Purdie, 1994). Subsequent research on the size fractions of chlorophyll and picoplankton cell density has shown that chlorophyll concentration is correlated inversely with small size fraction (Iriarte, 1993).

Mean annual productivity in Southampton Water was estimated at 150 g $m^{-2}y^{-1}$, falling to 100 g $m^{-2}y^{-1}$ at the mouth of the estuary (Williams, 1980). Average primary production at NW Netley and Calshot was estimated at 157.5g m^{-2} yr^{-1} (Iriarte and Purdie, 1994).

The phytoplankton taxonomy of Southampton Water has been well documented by microscopic (Kifle, 1992; Lauria, 1998; Ali, 2003) and chemotaxonomic analyses (Ali, 2003). However, apart from a few studies (Iriarte, 1991; Kifle, 1992; Iriarte, 1993; Iriarte and Purdie, 1994), phytoplankton size structure has not been thoroughly investigated in terms of carbon biomass, species taxonomy or relationship with environmental parameters. Phytoplankton community size structure needs further research, particularly its relation to biomass distribution and the effect of

environmental parameters. More details will be given in the next section (Aims of the thesis).

New techniques (e.g. HPLC and flow cytometry) are widely used to identify phytoplankton groups in the seas (Rodriguez *et al.*, 2002; Llewellyn *et al.*, 2005) and in estuaries (Ansotegui *et al.*, 2001; Lemaire *et al.*, 2002; Ansotegui *et al.*, 2003). However, apart from chemotaxonomic analysis using HPLC (Ali, 2003), these techniques have not been applied to Southampton Water, particularly for smaller fractions. The use of this technique will provide a useful comparative tool in taxonomical analysis.

Table 1-3: Summary of the dominant phytoplankton species and chlorophyll a in Southampton Water since 1973

Time of survey	Location	Chl-a mgm^{-3}	*Dominant species*	Reference
June,1973	Netley (surface)	26	*Scrippsiella trochoidea*	Diwan, 1978
June,1973	(5m)	27	*Prorocentrum spp.*	
July, 1973	Calshot (surface)	13	*Gonyaulax spinifera*	
July, 1973	(5m)	13		
February 1974	Calshot (lm)	3	*S. costatum; Navicula spp*	Burkill, 1978
May 1974	Calshot (lm)	4	*Odontella spp; Asterionella*	
August 1974	Calshot (lm)	6	*Preidinium spp; Gonyaulax*	
			Prorocentrum spp. _	
July/August 1974	Inner	44.8 /130		Bryan, 1979
	Mid	166 /25.6		
	Outer	14.9 /20		
	Lower Test	22.4 /596		
	Solent	14.4 /46.5		
May, 1985	Netley (Mean)	ND	*Mesodinium rubrum*	Crawford, 1992
June, 1985	Netley (Mean)	ND		
May,1986	Calsho t(lm)	27	*S. costatum; M. rubrum*	Leakey, 1986
Augst,1986	Netley (1m)	74		
May, 1987	Netley (0 -1m)	39	*Guinardia delicatula*	Antai, 1989
August, 1987	Netley (0 -1m)	50	*M. rubrum*	
May, 1988	Netley (0 -1m)	10	*G. delicatula*	
August, 1988	Netley (0 -1m)	<5		
May12,1988	Netley (1m)	12	*G. delicatula; E. marina*	Kifle.1992
June17,1988	Netley (1m)	50	*Chaetoceros spp.*	
June27, 1988	Netley (1m)	73	*S. costatum*	
June SO, 1988	Netley (1m)	36		
5&12May1988	Netley (1m)	17		
May, 1990	Netley (surface)	11	*Phaeocystis; Chaetoceros*	Iriarte,1991
EarlyAug.,1990	Netley (surface)	19	*M. rubrum*	
LateApril, 1990	Calshot (surface)	16		
May.1992	Netley (surface)	22	*G. delicatula; Phaeocystis*	Anning, 1995
May, 1993	Netley (surface)	15	*G. delicatula; Phaeocystis*	
June-July1992	Upper Test	> 40***	*S. costatum; R. dlicatula;*	Proenca, 1994
May, 1992	NW Netley	> 20***	*Chaetoceros spp.*	
May, 1992	Calshot	> 20***		
July, 1993	Calshot	5.46		Hirst, 1996
	NW Netley	10.92		
	Hamble	13.26		
	Cracknore	33.93		
	Bury Buoy	58.9		
Late August, 1994	Netley (surface)	> 50	*M. rubrum*	Ryan,1994
Early August, 1996	Transect along estuary	11	*Asterionella japonica; Gyrosigma sp;*	Lauria,1998
			Prorocentrum micans; M. rubrum	
during 1996	Hamble (surface)		*Skeletonema costatum*	Mahony &
EarlyApril 1996			*Asterionella glacialis; Thalassiosira spp*	Weeks, 2000
May, 1999	Upper estuary	21	*Guinardia delicatula*	Ali, 2003
June, 1999	Transect along estuary	2.24-6	*Ditylum brightwellii;R shrubsolei*	
July,1999	Transect along estuary	1.3-5.4	*Scrippsiella trochoidea*	
Auguest,2000	Transect along estuary	6.3-25.7	small flagellate*; Cryptomonas sp*	

*** Chlorophyll a measured using HPLC method

1-4 Aims of the thesis

Investigations of phytoplankton and its primary production have shown that chlorophyll size fractions determine the contribution of each size fraction to total phytoplankton biomass. The studies referred to relate size fraction to primary production and phytoplankton abundance. Earlier studies at Southampton Water have concentrated on large cell size (diatoms and dinoflagellates); some studies (Iriarte and Purdie, 1994 and Kifle, 1992) have examined smaller sizes (picoplankton). The present study also brings into the picture nanoplankton in the range 2-5 μm, and mainly takes into account carbon biomass distribution. The thesis will examine unresolved questions mainly about the contribution of nanoplankton <5μm in size, but in particular the 2-5 μm size range in terms of its biomass and chlorophyll, and its taxonomy, as well as the effects of environmental parameters on it.

Two broad subdivisions (<5 and >5 μm sizes) are selected for investigation, and emphasis is placed on assessing the impact of several environmental parameters on these fractions. This is significant in the light of growing concerns over global climatic changes, local spatio-temporal changes and increasing anthropogenic input into Southampton Water. Different techniques are employed to assess community structure. Data are analysed using multivariate methods involving the PRIMER (Plymouth Routines in Multivariate Ecological Research) software package to identify biotic and environmental relationships.

The aims of the thesis are:

1- To extend knowledge of the phytoplankton community in Southampton Water in terms of species composition and cell size (allometry). This study is important because it takes into account the whole phytoplankton community, in terms of its carbon biomass and also in relation to total chlorophyll (Tchl), size fractionated chlorophyll (Fchl) and cell size.

2- To relate variations in community structure to environmental parameters, especially nutrients and light. Irradiance varies with season according to water column stability. Nutrients are also variable according to external inputs

(eutrophication) and rate of regeneration. Using PRIMER to categorize biotic and environmental relationships

3- To assess the respective photosynthetic capacity of different size fractions of phytoplankton.

4- To investigate the taxonomy of phytoplankton cells <5µm in diameter, using advanced techniques. No detailed taxonomical study has been conducted on this group of organisms in Southampton Water estuary. They are likely to be important for their contribution to the food chain in the estuary.

1-5 Plan of the thesis

In order to contribute to research, to add further data and to update the characteristics of Southampton Water, the thesis presents the following chapters. Chapter 2 gives details of the four stations sampled and measurement methods, physical parameters recorded; analyses of inorganic nutrients, particulate organic carbon and nitrogen, pigment analyses, photosynthesis measurements, flow cytometer as well as statistical methods. Chapter 3 deals with physical, chemical, pigment and taxonomic data for Empress Dock collected on a weekly basis in 2002. Chapter 4 describes an equivalent set of monthly data for 2002 from three different stations N W Netley, Calshot and Horse Elbow for spatial comparison. Chapter 5 includes data from weekly samples from N W Netley in 2003, for temporal comparison with 2002 data. Analysis and characterisation are given in Chapter 6 of (for) nanophytoplankton, with further discussion in Chapter 7.

Chapter 2- METHODS

2-1 Station locations and sampling

Sampling was carried out at four stations, three in Southampton Water: (1) Empress Dock (Dock) 50° 54 `N, 1° 24` W, (2) NW Netley (NWN) 50° 52` N, 1° 22` W and (3) Calshot 50° 48`N, 1° 17`W and one station in the Solent, (4) Horse Elbow (HE) 50° 44`N, 1° 03`W, as shown in Figure 2-1. Samples were collected at high tide from the Dock at weekly intervals between January-November, 2002, while at other stations samples were collected on monthly intervals using a boat within 2-3 hours after high water. In 2003, NWN and Dock stations were sampled at weekly intervals between April-September, 2003.

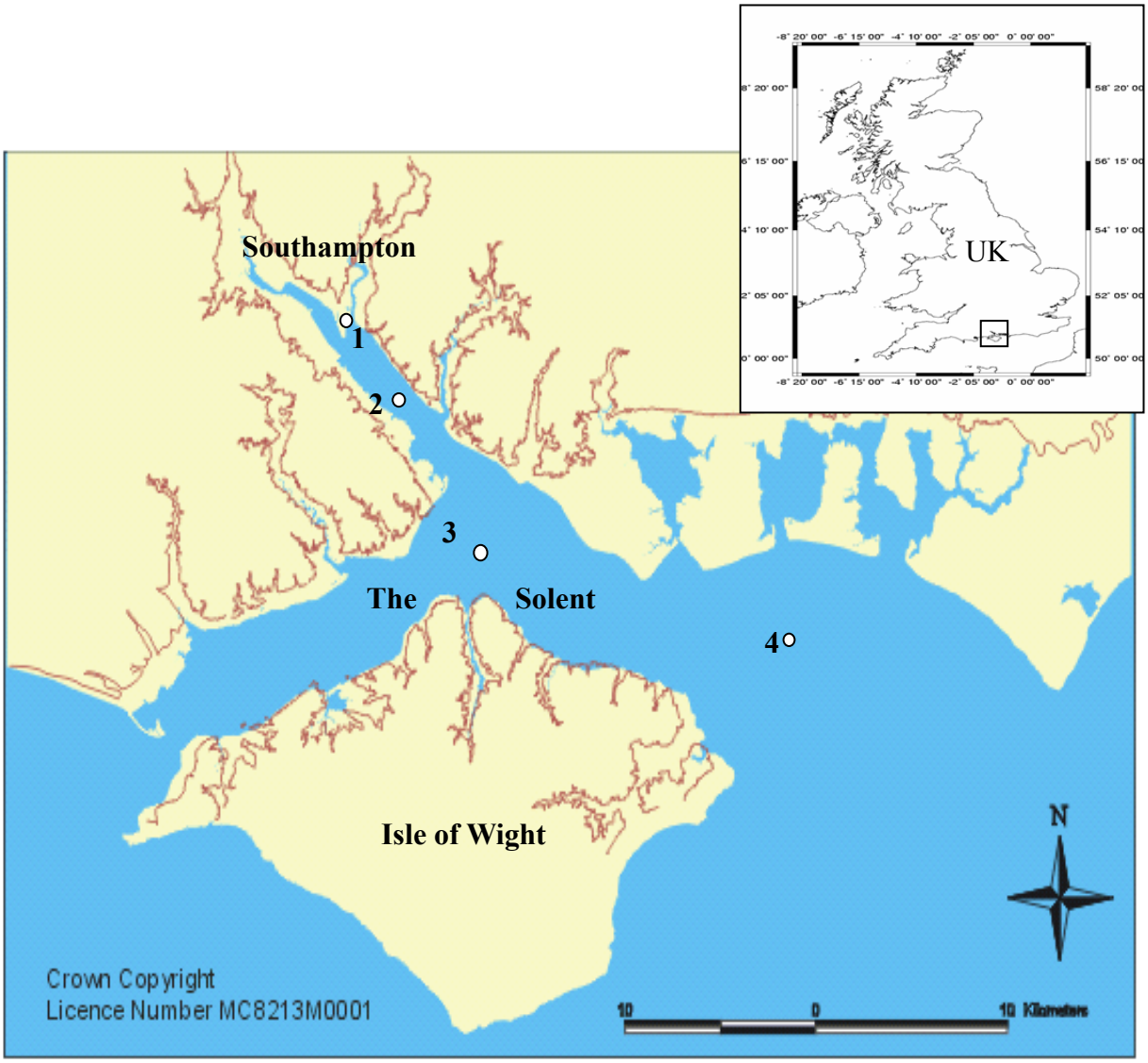
2-2 Meteorological data

Solar irradiance and air temperature data were obtained from the meteorological station at Southampton Oceanographic Centre (50°54`N, 1°24`W) (http://www.soc.soton.ac.uk/INTRANET/metstation), which employs a Solar Radiation Sensor Model CM3, Temperature Probe Models 107 and 108, and Temperature and Relative Humidity Probe Model CS50 (Compdell Scientific INC).

Hourly irradiance values were summed for each day and the weekly mean daily values calculated. Measured values in KJ $h^{-1}m^{-2}$ were converted to W m^{-2} and then multiplied by 0.46 to give photosynthetically available radiation (PAR) expressed as W h m^{-2} d^{-1} (Peperzak, 1993).

Hourly air temperatures were averaged to give daily mean temperatures. These values were then used to calculate weekly mean temperatures.

Figure 2-1: Station locations: 1) Empress Dock (Dock), 2) N. W. Netley (NWN), 3) Calshot in Southampton Water, and 4) Horse Elbow (HE) in the Solent.



2-3 Water column measurements

Vertical profiles of water column properties were made using a YSI 650 MDS logger with an Environmental Monitoring Systems 6600 multi-parameter water quality monitor probe for temperature (^{o}C), salinity, chlorophyll florescence (µg/l), turbidity (UNT) and oxygen (% saturation). When the YSI logger was not available, water temperature (^{o}C) and salinity were measured using a WTW salinity and temperature meter, with analogue outputs (LF597-S).

Water column photosynthetically available radiation (PAR 400-700 nm, μE m^{-2} s^{-1}) was recorded using a Li-COR LI-1000 (Glen Spectra) cosine-corrected sensor. The data were recorded simultaneously from a surface sensor (I_0) and from an underwater sensor at 1, 2, 3 or 4 metres below the sea surface (I_d). Values for the vertical diffuse attenuation coefficient, K_d (m^{-1}), were determined from the regression of ln (I_d/I_0) against depth.

Water samples for laboratory analyses were collected from the dock using a plastic beaker, and from the other stations in clean two-litre plastic Nansen water bottles. Sample bottles were placed in a cool box with ice bricks and taken back to the laboratory for further processing.

2-4 Pigment measurements

2-4-1 Chlorophyll a by fluorescence

Chlorophyll (chl) concentrations in water samples were measured by fluorescence using the method of Welschmeyer (1994). Water samples (25ml) were filtered under light positive pressure through 25 mm GF/F (Whatman) filters using a syringe attached to an in line filter holder.

Alternatively, chlorophyll was size fractionated (Fchl) by passing 25 or 50ml water samples through a sequence of 25mm polycarbonate filters with pore sizes of 20, 5, 2 and 0.2μm. Filtration through the 20 and 5μm filters was done under gravity, but through the 2 and 0.2 μm filters under gentle vacuum (<20cmHg) using a hand vacuum pump (NALGENE Brand products), following the method of Iriarte and Purdie (1994). All filters were stored at –20 ^{o}C prior to extraction.

To measure the chlorophyll *a*, each filter was placed in a 15-ml centrifuge tube and 5ml of 90% acetone was added and shaken thoroughly to extract the pigments. The samples were than immediately sonicated for 30 seconds, or they were allowed to stand in the dark (4^{o}C) overnight. The samples were then centrifuged at 3000 rpm for 10 minutes at room temperature, and the supernatant fluorescence measured in a 10-Av fluorometer (Turner Designs). The fluorometer was calibrated regularly using standard chlorophyll *a* solution (Sigma Chemical Co) in 90% acetone. The

concentration of the chlorophyll standard was determined by spectrophotometry according to the equation given by Jeffery and Humphrey (1975):

$$chla = 11.85\ E664 - 1.54\ E647 - 0.08\ E630$$

Where E = the absorbance readings of the spectrophotometer at 664, 647 and 630nm, using a 1cm cell, after correction for turbidity by subtracting the reading at 750nm. The sample chlorophyll *a* concentrations were determined from the following equation:

$$chl\ (mg/m^3) = R * v/V$$

Where R = Calibrated fluorescence value, v = Volume of acetone used for extraction (ml) and V = Volume of sample filtered (ml) (Parsons *et al.*, 1984).

In this study, all samples of chl were measured in triplicate and mean values calculated; the standard deviation was <5%, although maximum deviations up to 25% were found when chlorophyll was lower than 1mg m^{-3}.

In addition to total chlorophyll *a* (Tchl) measurements with different water sample volumes, an experiment was carried out for size fractionated chlorophyll *a*. Three (a-c) 25 ml water samples were size fractionated through four different polycarbonate filters, with pore sizes 20, 5, 2, 0.2 µm, as described above. The results are shown in Table 2 -1.

The total chlorophyll a (Tchl) obtained from the different volumes (25 and 50 ml) of sample water gave similar values (2.2, 2.3 mg m^{-3}, respectively) to the average of summation of size fractionated chlorophyll *a* (Fchl) (see Table 2-1). The highest variability was found for the 5-2 and < 2µm fractions.

Table 2-1: Results of the size fractionated chlorophyll a experiment

size fract.	A	B	C	range	mean	%
> 20µm	1.07	1.04	1.06	1.04-1.07	1.06	46
5-20 µm	0.43	0.41	0.47	0.41-0.47	0.44	19
2-5 µm	0.36	0.28	0.50	0.28-0.50	0.38	17
< 2 µm	0.38	0.41	0.47	0.38-0.47	0.42	18
Sum	2.23	2.15	2.50	2.15-2.50	2.30	100

2-4-2 High Performance Liquid Chromatography (HPLC) of phytoplankton pigments

High Performance Liquid Chromatography (HPLC) was used to analyse the pigment content of samples, using the method of Mantoura and Llewellyn (1983), as described by Barlow *et al* (1993). A Thermoquest HPLC system (gradient pump, vacuum degasser, autosampler, UV/V photodiode array and Fluorescence Detector) incorporating a 3µm C-18 HPLC column, was used. The solvents were A: 80% methanol and 20% 1M ammonium acetate and B: 60% methanol and 40% acetone, with a decreasing gradient from 100% A to 100% B for 10 minutes, followed by an isocratic stop at 100% B for 7.5 minutes. A second gradient over 2.5 minutes was then used to return to the initial condition of 100% A. The total run time per sample was approximately 17.5 minutes.

Chlorophyll and carotenoids were measured by absorbance at 440 nm, and detected phaeopigments were measured by fluorescence using an excitation wavelength of 410nm and emission at 670nm. Data collection and integration utilised the Chromquest software on a Dell 1100 computer.

Pigment identities were established by co-elution with authentic pigment standards (Sigma Chemical Co, DHI). Peak identity was further confirmed by on-line photodiode array spectroscopy. The consistency of the HPLC values are within an error of ±5%, and the correlation (R^2) for chl standards was 0.99, as shown in Figure 2-2. Figure 2-3 shows a chromatogram for a pigment mixture standard, with different concentrations of each pigment, used as a standard before sample analysis.

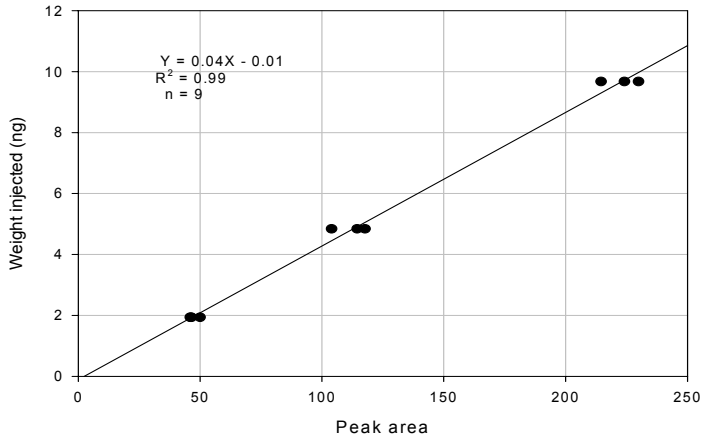
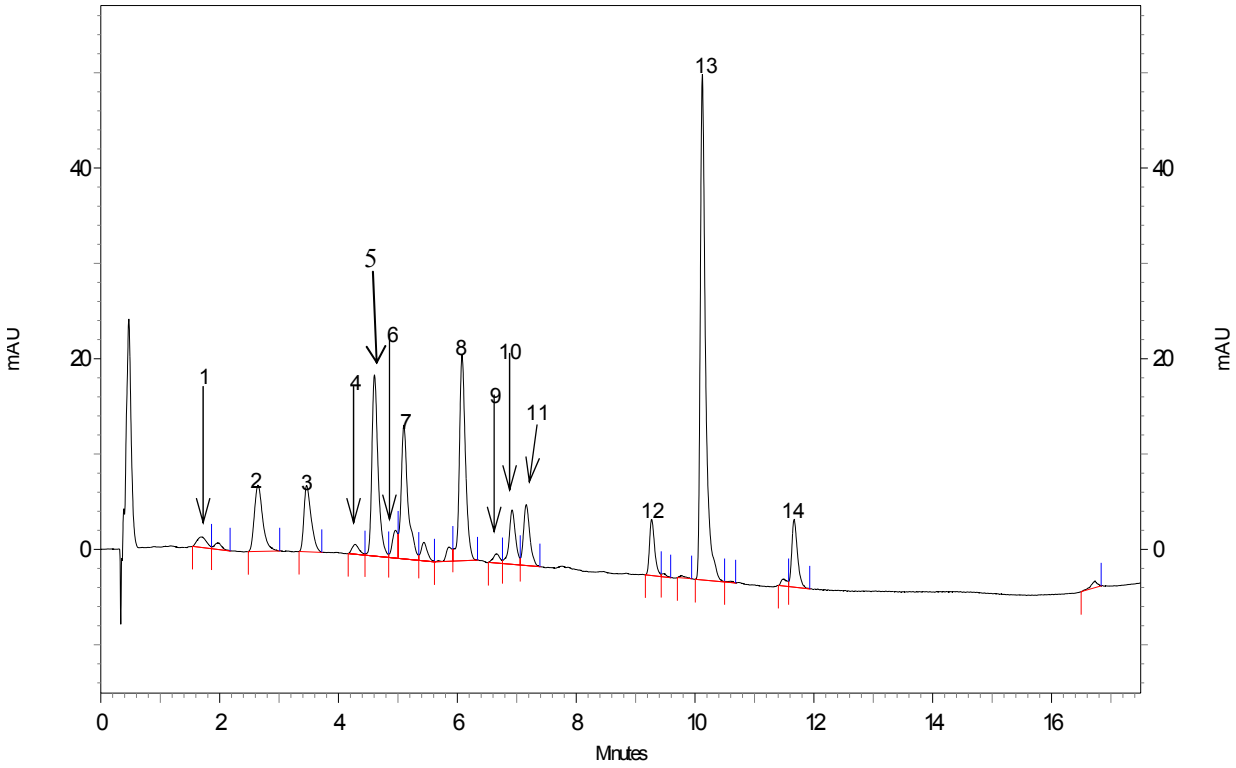


Figure 2-2: HPLC chla calibration.

Figure 2-3: HPLC chromatogram for a standard pigment mixture.

(Pigment identification after solvent peak: 1= chlorophyll *c3*, 2=chlorophyll *c1+c2*, 3=peridinin, 4=19-butanoyloxyfucoxanthin, 5=fucoxanthin, 6=19-hexanoyloxyfucoxanthin, 7=brasinoxanthin, 8=violaxnthin, 9=diadinoxanthin, 10=alloxanthin, 11=zeaxanthin, 12= chlorophyll *b*, 13= chlorophyll a, 14=*ß*-carotene).



Four 250ml water samples were filtered onto four 25mm GF/F filters using a Millipore glass filtration system, giving duplicate pairs of samples. The filters were stored at -70 to -85^{o}C for up to three months. One pair of filters (the other pair is stored as back-up) was placed in 3ml HPLC grade 90% acetone, sonicated in a 15ml centrifuge tube for 30 seconds, centrifuged at 3000 rpm for 10 minutes to remove cellular debris, and then filtered through a 0.2µm nylon filter. 1ml of the extract was transferred to a small glass vial and placed in the HPLC auto-sampler. Then an aliquot of 500µl of clarified extract was automatically mixed with 500µl of 1M ammonium acetate. A mixture of 100µl of that was injected into the HPLC.

Pigment concentrations (Pc) were calculated according to the following equation (Barlow *et al.*, 1993):

$$Pc\ (\mu g\ l^{-1} or\ mg\ m^{-3}) = (Pa\ .\ v\ .\ 1000\ /\ Pr\ .\ Vi\ .\ V\ 0.5)/1000$$

Where *Pa* = Peak area at 440nm, *v* = Volume of extracted acetone (ml), *Pr* = Pigment response factor, *Vi* = Volume injected in the column (100 µl), *V* = Volume of filtered sample (l) and 0.5 = the buffer dilution factor

2-5 Particulate Organic Carbon and Nitrogen (POC/N) measurements

Triplicate 25 or 50ml water samples for POC/N analysis were filtered on the same day of collection through 13mm GFF filters pre-combusted at 460^{o}C for four hours using a syringe and inline filter holder. The filters were then dried in a 60 ^{o}C oven for two hours then stored in a desiccator for up to three months.

The POC/N analyses were performed with a Carlo ERBA EA1108-Elemental Analyser. The dry filters were folded, placed inside tin capsules and compressed to a size suitable for the instrument, then transferred to the auto-sampler of the analyser. The analyser was standardised using sulphanilamide, together with a pre-combusted filter, which was used as a blank. After standardisation, the field samples were run. A standard was also run after every ten field samples in order to constrain machine drift and allow estimation of relative and absolute analytical errors (Wilkinson, 1991).

The concentrations of carbon were calculated (on a volume basis) from the peak area, with correction for any baseline drift. The carbon peak area (*A*) was found from:

Carbon peak area $A = (T. S)/P$

Where *T* is the theoretical standard peak based on the manufacturer's specification, *S* is the sample area, and *P* is the practical standard area (mean of standard before and after each batch of 10 samples). Particulate organic carbon values were obtained as mg l^{-1} from:

$POC = (A . W . \%C / Cs . V) 1000$

Where *A* is sample carbon area, *W* is standard weight (mg), *%*C is a standard carbon percent, *Cs* is the standard carbon area and *V* is the sample volume filtered (ml). Particulate organic nitrogen values were obtained (mg l^{-1}) in a similar way as particulate organic carbon.

2- 6 Inorganic nutrient measurements

Nutrient water samples (50ml) were syringe filtered through 25mm diameter GF/F (Whatman) glass fiber filters into two plastic vials. One of them for nitrate + nitrite and phosphate analyses was kept frozen at –20 ^{o}C; the other one for silicate analysis was kept in the dark at room temperature. Samples were analyzed within three months.

Inorganic nutrients were determined with an autoanalyser from Burkard Scientific, model SFA-2, with an 80 plus autosampler connected to a chart recorder and a computer as described by Hydes (1984) and Hydes and Wright (1999), using colorimetric methods that relate colour density to the concentration of nutrients. It has an automated Analytical system linked to a digital-analysis Microstream data capture and reduction system.

The autoanalyser was standardised with sodium silica-fluoride for silica (Si), potassium nitrate for nitrate (NO_3) and potassium dihydrogen phosphate for inorganic phosphate (PO_4). Milli-Q water was used to prepare all reagents, blanks and standards.

The volume of sample used in each analysis was 2ml and each sample was analysed in duplicate. Colour density was measured using a photometer containing filters corresponding to the colour developed in the solution. The equipment was set up to measure nitrate concentrations up to 80µM, silicate concentrations up to 40µM and phosphate concentrations up to 3µM. Variable working standard concentrations were prepared, according to the expected range of sample concentrations. A full calibration curve was run at the beginning of each batch of 64 samples, and drift standards, intermediary concentrations and blanks were used in the middle and at the end of each run, every 30 samples. NaCl (20 g l^{-1}) was used as the wash, blank and matrix for the working standards. Some samples needed to be diluted with NaCl (20 g l^{-1}).

The analysis of nitrate requires the reduction of nitrate to nitrite. After passing on line through the cadmium column, the sample was mixed with sulphanilamide and

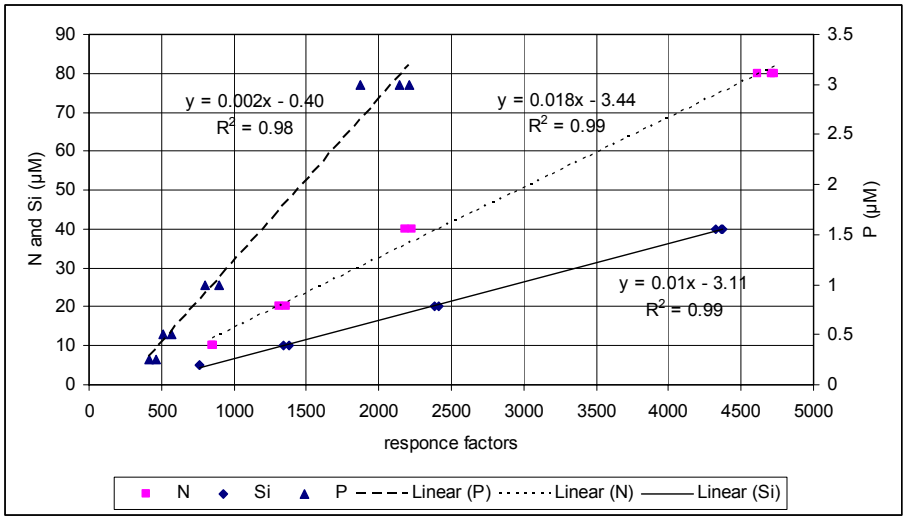
naphthylethelynedihydrochloride (NED) to produce a pink compound. Values were presented for the nitrate plus nitrite concentrations in the original sample.

Phosphate reacts with a molybdate reagent in an acidified medium to give a phosphomolybdate complex, which is then reduced to a highly coloured blue compound. Ascorbic acid is used as the reducing agent and antimonyl tartrate speeds this reaction.

Dissolved silicate reacts in acidic molybdate solutions to form yellow silicomolybdic acid, which is then reduced with ascorbic acid to form a blue compound. Oxalic acid is added prior to the reduction step to avoid phosphate interference, as this nutrient also reacts with the molybdate to form a yellow compound.

The concentrations of nutrients (µM) were calculated by computer programme. A plot can be used for calculation if there is any uncertainty in the computer reading. However, the nutrients in this study were averaged from duplicate analyses. The consistency of the auto analyser error is <2%, with a correlation (R^2) of > 0.98 for different standard curves as shown in Figure 2- 2.

Figure 2-4: Calibration of a nutrient auto analyser.



2-7 Light microscope counts

100ml water samples from the surface of each station were preserved with 1ml of acidic Lugols solution in tightly-stoppered bottles for phytoplankton cell counts and identification (Parsons *et al*, 1984).

Microscope counts were made with an inverted Leica microscope (DMIRB 1/97) after shaking the bottles. A 10ml sample was placed in a settling chamber for 24 hours. The whole floor of the chamber was scanned to be sure of the even distribution of cells. Two transects of the counting chamber were examined and phytoplankton cells >10 μm diameter were counted under 200x. This was followed by a complete count of the chamber again under 100x to examine for large species not yet counted. Next, 5 to 10 fields of view (FOV) chosen randomly were counted under 400x, for >5 and <5μm diameter cells; at least 200 cells were counted. The numbers of cells per ml^{-1} were calculated using the following equation:

Cells $ml^{-1} = C.\ a / A\ (mm^{-2}).V\ (ml)$

Where C is the number of cells counted in the area examined, a is the area examined (mm^{-2}), A is the whole chamber area (mm^{-2}) and V is the volume (ml) of sample settled. The value of a was varied according to the magnification used. Phytoplankton identification was undertaken with the assistance of the following references: (Hendey, 1964; Tomas, 1997; Horner, 2002). Species nomenclature follows Tomas (1997).

2-8 Phytoplankton biomass estimation

Phytoplankton biomass was calculated by estimating the cell volume for each species. The mean dimensions of 10-20 cells of abundant species were converted to volume, depending on a standard spreadsheet algorithm provided by Derek Harbour (Plymouth Marine Laboratory) based on the algorithms given in Kovala and Larrance (1966). The cell volumes were converted to cell carbon content using the relationships given by Menden-Deuer and Lessard (2000): pg C cell 1^{-1} = 216 x volume$^{0.939}$ for taxonomically diverse protist plankton, and pg C cell 1^{-1} =0.288 x volume$^{0.811}$ for diatoms.

The effect of preservatives on measured cell volume is dependent on the type and strength of fixative (Montagnes *et al.*, 1994; Menden-Deuer *et al.*, 2001), but no correction for this effect has been applied to the data presented here.

2-9 Fluorescent in–situ hybridisation

Water samples (50 ml) were filtered through 20 and 5μm Millipore filters, and the filtrates fixed with 2.5 ml of 20% paraformaldehyde (PFA) to a final concentration of 1% and stored at room temperature for one hour. The fixed samples were then filtered through a 1.0 μm polycarbonate 47mm filter (Whatman), pre-rinsed with Milli Q water (Millipore), and finally dehydrated with 5ml of 96% ethanol. The filters were then dried at room temperature, labelled, divided into small segments and stored at –20 °C until further analysis. The procedure was adapted from that described by Not *et al.* (2002). The protocol used for *in-situ* hybridisation with fluoresce in isothiocyanate (FITC) mono labelled probes was adapted from Amann *et al.* (1995). On the day of analysis, the filter samples were thawed and the surface supporting the cells was marked with a pencil.

For hybridisation with FITC-labelled probes, the filters were covered by 20 μl of formamide hybridisation buffer containing 2 μl of oligonucleotide probes (stock at 50 ng μl^{-1}) and incubated at 46˚C for 2 hours. In order to enhance the probe specificity of CHLO01, Simon *et al* (1995) used 2.5ng of competitor. After one successive washing step of 15 minutes at 48˚C in a wash buffer, the filters were dried, before being mounted in anti-fading reagent (Citifluor-DAPI). The hybridisation buffer and the washing buffer used for the different oligonucleotide probes are shown in Table 2 -1.

Probes Euk516 and Eub338 were used to distinguish between the eukaryote and prokaryotic cells, CHLO01 for Chlorophyta, Prym02 for Haptophyta, Pela01 for Pelagophceae and Boli02 for Bolidophyceae (Simon *et al.*, 1995; Not *et al.*, 2002).

The hybridised and DAPI-stained filters were observed with a Zeiss epifluorescence microscope, equipped with a mercury light source and a 40xUV fluorescence objective. Excitation/ emission filters were 360/420 for DAPI and 490/515 for FITC. For each natural sample, 300 cells were visually counted per sorted sample. Probe

positive cells were presented as fractions of cells stained with the general nucleic acid dye DAPI.

Table 2-2: Chemicals used to make up the hybridisation buffer and the washing buffer for the different oligonucleotide probes (in µl).

[sodium dodecyl sulphate (SDS); Ethylene diamine tetra acetic acid (EDTA); Milli-Q (Millipore) water M.Q].

	Oligonucleotide probes		
	Chemicals	**Euk516 &Eub338**	**Chlo01, Prym02, Pela01 & Boli02**
1. Hybridisation buffer	**Formamide**	400(20%)	700(35%)
	5M NaCl	360	360
	1M Tris HCl	40	40
	M.Q.	1200	900
	10% SDS	2	2
2. Washing buffer (Made in 50 ml MQ)	5M NaCl	2150	700
	1M Tris HCl	1000	1000
	EDTA	500	500
	10% SDS	50	50

2-10 Flow cytometry

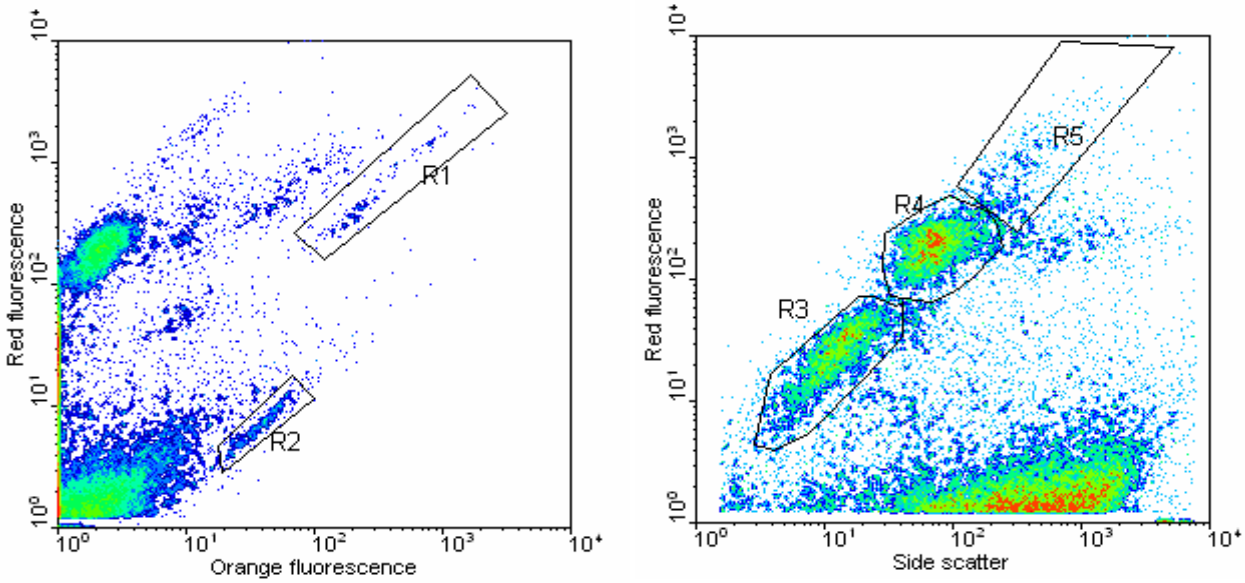
Cell concentrations were estimated using flow cytometry following the procedure of Tarran *et al.* (2001). For flow cytometry analysis, 1.5 ml of 20, 5, 3 and 2µm filtered water samples were fixed with 75µl of 20% paraformaldehyde acetic acid (PFA) and stored at –2 0°C until further analysis.

Picoeukaryotic phytoplankton (PEPP) and nanophytoplankton (NPP) were counted by flow cytometry (AFC) using a Becton Dickinson FAC Sort flow cytometer, which also measured chlorophyll fluorescence (> 650 nm), phycoerythrin fluorescence (585 ± 21 nm), and side scatter (light scattered at 90° to the plane of a vertically polarised argon ion laser exciting at 488 nm). Data acquisition was triggered on side scatter with secondary triggering on red fluorescence using laboratory cultures to set the rejection gates to measure PEPP and NPP simultaneously.

Samples were analysed for 4 min at a flow rate of 75µl min^{-1} ±3µl. The flow rate was calibrated using 3.4µm fluorescent beads (Beckmen–Coulter FlowSET) of known concentration. With this approach, it was possible to resolve cryptophytes

(*Cryptomonas* sp (R1)), cyanobacteria (*Synechococcus* spp (R2)), picoeukaryotic phytoplankton (<2µm (R3)), and two to three other unidentified NPP (>5 µm (R5) and <5µm (R4) diameter) groups (Fig. 2- 5).

Figure 2-5: Nanophytoplankton with different size identified by flow cytometer.



Measurements of light scatter and fluorescence were made, using cell Quest software (Becton Dickinson) with log amplification on a four-decade scale with 1024 channel resolution. Data were stored in list mode format on the flow cytometer's computer hard disk and then transferred to personal computer software. Data analysis was carried out on a personal computer using Win MDI software 2.8 (Joseph Trotter). Bivariate scatter plots of phycoerythrin against chlorophyll fluorescence were used to discriminate the *Synechococcus* spp. and cryptophytes from other phytoplankton. The NPP were resolved using bivariate plots of side scatter against chlorophyll fluorescence. The size categories studied in this thesis are R3 (<2 µm picophytoplankton, including *Synechococcus* spp), R4 (2-5 µm nanophytoplankton) and R5 (>5 µm nanophytoplankton , including *Cryptomonas* sp)

2-11 Photosynthesis measurements.

2-11-1 Oxygen

Primary production measurements in natural populations were made by the light and dark bottle technique (Gaarder and Gran, 1927) and using the Winkler titration method. Reactions involved addition of manganous chloride and alkaline-iodide to a sample of water leading to production of manganous hydroxide:

$Mn^{2+} + 2(OH) = Mn\ (OH)_2$

The manganous hydroxide reacts with the dissolved oxygen to form a dark brown higher valent hydroxide:

$Mn(OH)_2 + ½\ O_2 = MnO(OH)_2$

When acidified with 10N H_2SO_4, the hydroxide precipitate dissolves and initiates a reaction between the manganese and the iodide, which forms a soluble tri-iodide:

$2Mn(OH)_3 + 6H^+ + 3I^- = 2Mn_2^+ + I_3^- + 6H_2O$

The iodine is chemically equivalent to the oxygen at the start of the reaction. The amount of iodine released was determined by photometric end-point titration with sodium thiosulphate (Bryan *et al*., 1976).

$I3 + 2S_2O_3^- = 2I^- + S_4O_6^-$

Filling of oxygen bottles: Unfiltered and 5µm filtered water was used for measuring oxygen production, corresponding to total community and nanophytoplankton production. Bottles (50 ml) were placed in two rows in a long rectangular box (110*16 cm) with a perspex window at one end (Wyman *et al.*, 1998) for incubation experiments. The oxygen bottles were filled with water through a plastic tube and each bottle was flushed with 2 to 3 times its own volume of water in order to avoid any introduction of bubbles. After filling, the stoppers were carefully replaced on each bottle.

Incubation of oxygen bottles: The bottles were submerged in the water bath. The water enters the box via the transparent box between the light source and the perspex

window, at a constant flow, so as to incubate the samples at near *in situ* temperature. Illumination was provided by a 500 watt tungsten halogen lamp placed in the box, the irradiance gradient being produced by a combination of distance from the light source and attenuation through the bottles. The irradiance values ranged from ~20 to1800 μE m^{-2} s^{-1}, simulating a range comparable to that observed in the field. The light was measured with a submersible probe connected to a quantum meter (Biospherical Instruments Inc), which was placed centrally behind each bottle. Four bottles were incubated in the dark inside a black bag and kept at the end of the incubation box.

Fixation of oxygen in the samples: The reagents required for analysis were prepared according to Carritt and Carpenter (1966). The dissolved oxygen was fixed using manganous chloride and alkaline iodide reagents. 0.5 ml of each reagent was added to the bottom of each bottle using a positive displacement-repeating syringe (Jencons Scientific LTD). The bottles were carefully stoppered, mixed by inversion, and stored underwater to prevent evaporation. Four zero time bottles were fixed immediately, while the remaining samples were placed in the incubator. The samples in the incubator were fixed after three hours and stored as above.

Standardisation of thiosulphate**:** Standardisation of the thiosulphate with potassium iodate was carried out prior to the sample titrations. Replicates of five 125-ml BOD bottles were filled with distilled water. To each, concentrated sulphuric acid, alkaline iodide and manganous chloride were added in revese order to their addition in sample fixation as described by Parsons *et al*. (1984). The normality of the thiosulphate was calculated as follows (Iriarte, 1991):

$NT = (VI.NI)/VT$

Where NT is the normality of the thiosulphate solution, VT is the volume of thiosulphate added (ml), NI is the normality of the iodate solution and VI is the volume (i.e the weight) of iodate (ml).

Titration: The apparatus for the Winkler titration consisted of a photometric endpoint reactor connected to a chart recorder (J.J. Instruments CR55) and an automated microburette (Auto titrator, Metrohm Dosimat 665) for addition of thiosulphate. The

titration was monitored by an increase in light transmission through the bottle, and detected by the chart recorder.

After addition of 0.5 ml of 10N H_2S0_4 to liberate the tri-iodide ion, the bottle was placed in a water bath in the centre of the light path. The contents were stirred using a magnetic stirrer bar throughout the titration. This ensured that the precipitate formed on addition of the manganous chloride and alkaline iodide was fully dissolved before titration commenced, and the end point on the chart recorder was set. This was achieved by placing an oxygen bottle filled with seawater in the light path and setting the deflection to 70% of the chart recorder scale. The burette tip was placed in the neck of the bottle in the sample and thiosulphate added. As the titration end point was approached, light reaching the photocell after passing through the bottle increased until further addition of thiosulphate caused no further change. The volume of thiosulphate added up to this endpoint was taken as the titrate volume. The oxygen concentration was calculated using the following equation (Iriarte, 1991):

Dissolved oxygen (μmol l^{-1}) = 10^6 ($VT.NT$) / (4[V-VR])

Where VT is the volume of thiosulphate added (ml), NT is the normality of the thiosulphate solution (N), V is the bottle volume (ml), and VR is the volume (ml) of the reagents added (manganous chloride and alkaline iodide).

Gross photosynthesis was estimated as the difference between the mean oxygen concentration in light and the mean oxygen concentration in the dark incubation bottles. Respiration in the dark was calculated as the difference between the mean zero-time oxygen concentration and the mean dark oxygen value. Net photosynthesis was the difference between the gross photosynthesis and dark respiration values.

Mathematical formulation of the photosynthesis (gross) versus irradiance (P *vs* I) relationship**:** The photosynthesis *vs* light (P *vs* E) curve can be used to derive parameters of physiological interest, and has been described using a mathematical model that accommodates the possibility of photoinhibition of photosynthesis at high irradiance (Platt *et al.*, 1980). The equation representing the model is given by:

P = Ps*[1-exp (-ά*I/Ps)] exp (-β*I/Ps)

Where P is the photosynthetic rate at a given irradiance, Ps is maximum potential photosynthetic rate in the absence of photoinhibition, ά is the initial light-limited slope of the curve, β is photoinhibition and I is irradiance. However, in this study, any photoinhibition is ignored, so that the equation representing the model is:

$P = Ps*[1-\exp(-ά*I/Ps)]$

This model was used to fit experimental P vs I data, using a non-linear curve fitting routine in the program Sigma-plot for Windows, as described by Webb *et al* (1974).

2-11-2 Fast Repetition Rate Fluorometer (FRRF)

A Fastracka FRRF (Chelsea Scientific Instruments), constructed to measure photosynthesis-irradiance response curves (P *vs* I), was used, as described by Suggett *et al* (2001). The FRRF together with a light projector and a fan were mounted in a wooden box to exclude extraneous light. Power was provided to the instrument using a standard Chelsea Instruments deck box. Samples (total, fractioned <5 μm) for FRRF *P vs* E curves were stored in a dark bottle until laboratory analysis. All samples were analyzed within 4 hours of collection. Samples were placed in a non-toxic plastic chamber attached to the FRRF light sensor. The light projector provided 10 different light levels to the FRRF light chamber, controlled by MATLAB software. Data from this instrument were recorded internally and downloaded to a PC. The FRRF was programmed to generate ST (single turnover) saturation from 100 flashlets of 1.1μs duration and applied at 2.4μs intervals. Excitation light was emitted from a bank of blue LEDs. Each ST saturation curve was used to calculate the values of minimal and maximal fluorescence, σ_{PSII} and the connectivity parameter (ρ). Irradiance intensity, or photosynthetically available radiation (PAR), was measured using a CI 2 π (400-700 nm) PAR sensor interfaced with the FRRF. All data were fitted to the biophysical model (Kolber *et al.*, 1998). Data downloaded from internally recording instruments were typically analysed using the custom software provided by the instrument manufacturers (FRS version 1.4) and MATLAB software, based on original codes provided to the FRRF community by S. Laney (V4).

2-12 Statistical methods (PRIMER)

The computer software package PRIMER (Plymouth Routines In Multivariate Ecological Research) version 5.1.1, was implemented for statistical analyses, as recommended by Clarke and Warwick (1994). Multivariate methods, cluster and non-metric multidimensional scaling (MDS) were used to resolve the complexity of the phytoplankton community and its relationship to environmental parameters.

Data on species biomass and environmental variables were examined for each pair of samples using similarity coefficients. The Bray-Curtis coefficient test of similarity was used for the phytoplankton community to reflect the difference between samples in different stations or similar stations at different times. Blocks of zero counts were ignored.

Phytoplankton species contributing >1% of the total biomass were included, but other species (<1%) were excluded as they are considered as being too rare. Transformation of the data was important for non-parametric multivariate representation in order to identify the dominant and rare species, and to balance rare and common species. The transformation methods range through square root, fourth root (4^{th} root), logarithmic (log x) and standardised (%). Phytoplankton biomass data in this study were transformed using square root or 4^{th} root transformation (suggested for biomass or number of species by Clarke and Warwick (1994), which enhances the mid to rare species, giving them more attention with respect to total biomass. Physical data (water salinity, temperature, light irradiance (PAR), chemical data (NO_3, Si, PO_4, POC, PON), and biological data (chl) were standardised and transformed as log (v+1) for dissimilarity (hierarchical) cluster analysis or Euclidean distance, followed by MDS analysis.

MDS was used as an interactive procedure to describe the samples in two or three dimensions, with consideration of all conditions imposed by the rank (dis)similarity matrix (hierarchical cluster analysis). Stress values show how easy or difficult it is to plot data in such a way as to measure the distance (similarity) between the samples. The MDS algorithm calculates the Senses values iteractively, and values of <0.05

represent excellent ordination, <0.1 good ordination, <0.2 a useful two-dimensional cross check on clusters and > 0.2 a random placement in two dimensions.

Similarity percentage (SIMPER) species contribution data were standardised and transformed along with biological data, to find similarity or dissimilarity between pairs of groups. SIMPER analysis reveals species contribution within a group, as well as weight biomass and cell number.

The BIOENV routine of PRIMER analysis was used to examine the relationship between environmental and biological data. This routine calculates a measure of agreement between two similarity matrices, biotic and abiotic matrices, to give best matching and highest degree of combination (rank correlation, ρ); the highest value of ρ will give the best matching environmental parameters, using a standard Spearman rank correlation (value between -1 and 1). A value of ρ around zero corresponds to absence of any match between two parameters, but typically ρ is positive (Clarke and Warwick, 1994). PRIMER's programme flexibility allowed superimposition of the parameters of the MDS plot to show the correlation between samples and different environmental parameters.

Chapter 3- EMPRESS DOCK OBSERVATIONS IN 2002

3-1 Physical data

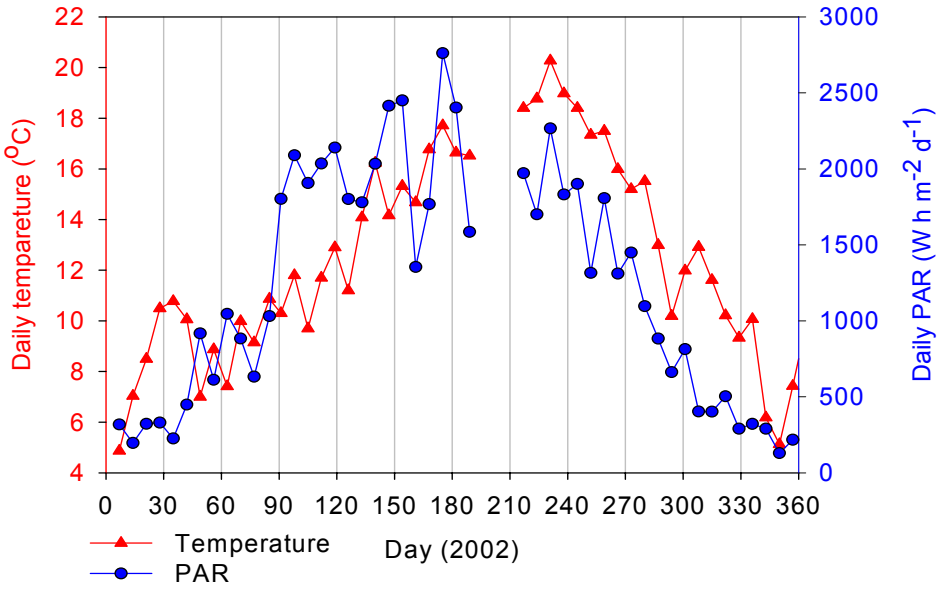
3-1-1 Meteorological and tidal data

Variations in the weekly means for daily solar irradiance (Photosynthetically Active Radiation (PAR)) and air temperature during 2002 are shown in Figure 3-1. Weekly mean values for PAR were in the range 129-2761 W h m^{-2} d^{-1}, with the lowest value in mid December (Day 350) and the highest value at the end of June (Day175).

The trends in mean air temperature were similar. Weekly mean values ranged between ~5 to 20 ^{o}C, the minimum being observed at the beginning of January (Day 1) and the maximum at the end of July (Day 209).

Figure 3-1: Weekly mean values for daily Photosynthetically Active Radiation (PAR) and daily mean air temperature in 2002.

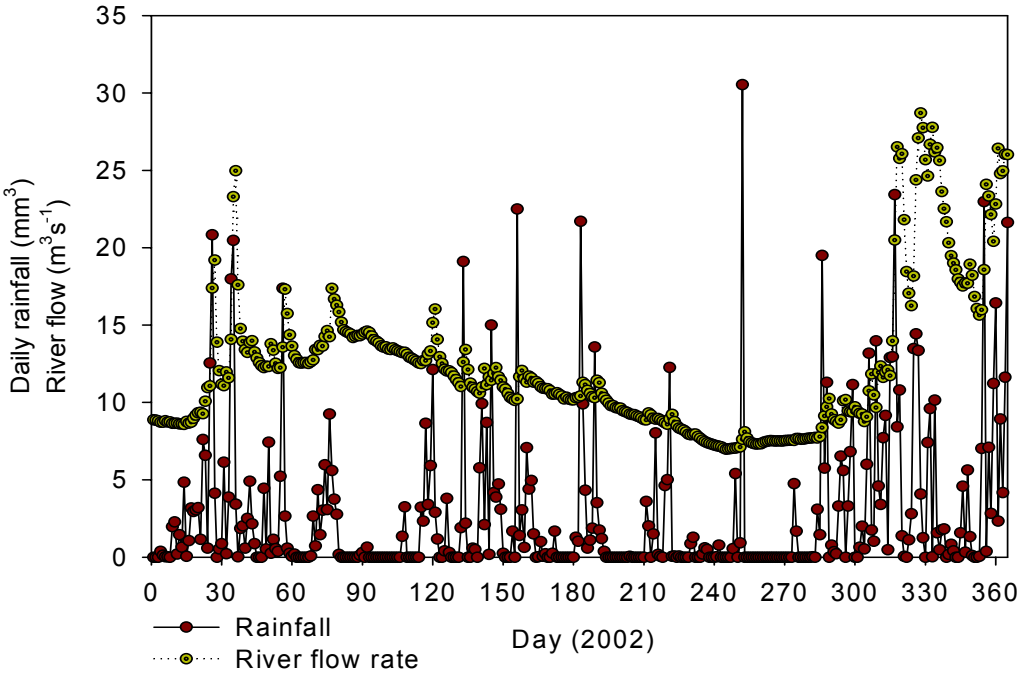
[There was a failure in the recordings at SOC the meteorological station for two weeks in July].



The mean rainfall data for 2002 for five stations in the Southampton area (Otterbourne Water Works, Dean, Corhampton Road, Abbottswood, Southsea and Everton) were obtained from the Meteorological office, and Test River flow rates at Broadland station from the HYDROLOG Data Management System, Winchester (Figure 3-2). The maximum daily rainfall, ~30mm, occurred in August (Day 251). The maximum river flow was 27 m^{-3} s^{-1} in October (Day 329) and the minimum flow was 7.3 m^{-3} s^{-1} in the middle of August. Note that the maximum flows in the early part of the year were very much lower than late in the year, reflecting the high autumn rainfall.

Figure 3-2: Mean daily rainfall in the Southampton Water area and the Test river flow rate.

[The stations are Otterbourne WWks, Dean, Corhampton Road, Abbottswood, Southsea and Everton. The Test river flow rate at Broadland station was measured by the HYDROLOG Data Management System Winchester, 2002.]



Changes in tidal range (spring/neap cycle) as predicted in the Southampton Tidal Table (Admiralty charts and publication, Tidal Tables V1 2002) are shown in Figure 3-3. The lowest tidal range (<2m) occurs in the summer period, associated with relatively low river flow; as a result, the residence time of water in the estuary will be greatest at this time of year. The tidal range (prism) affects water column parameters,

such as maximum current velocity, and sediment suspension and light penetration which, in turn affect the phytoplankton population. Also, high kinetic energy at spring tides (Lauria *et al.*, 1999) will tend to prevent large non-motile phytoplankton cells (mainly diatoms) from settling and affect the composition of the phytoplankton population in the water column.

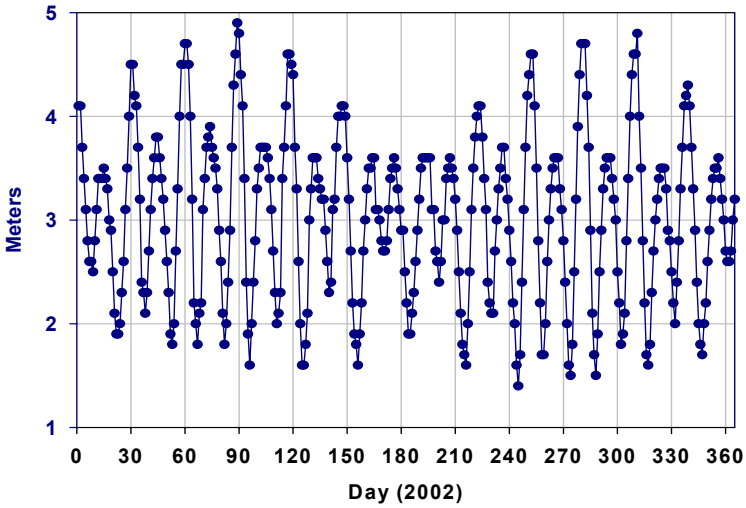


Figure 3-3: Southampton tidal range 2002.

3-1-2 Water column salinity and temperature

Salinity and temperature measurements at the surface and at 4m are shown in Figures 3-4 and 3-5. Water column salinity showed greater variability at the surface than at 4m depth, due to changes in riverine water input (Figure 3-2). Surface salinity values ranged from 25 to 32, the lowest value being in mid-May. At 4m depth, salinity was generally 31-33, apart from two readings of 29 and 30 in mid-May.

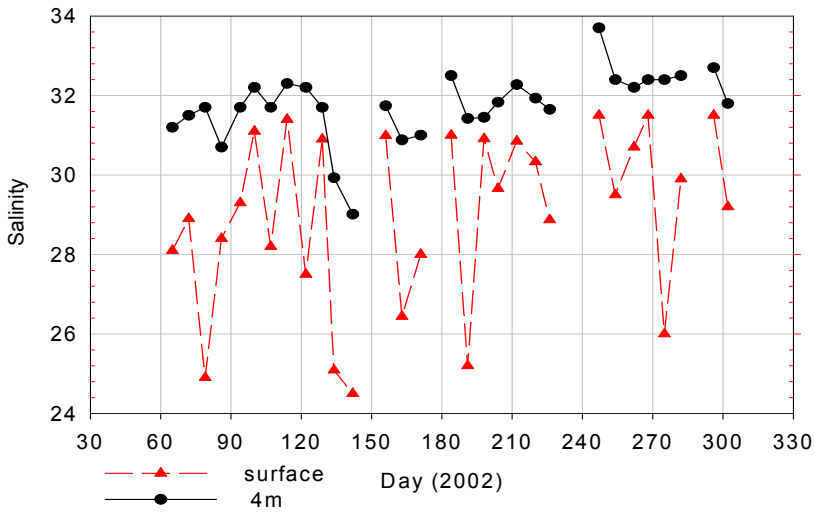


Figure 3-4: Water salinity at surface and 4m depth, Dock 2002.

[Data are missing for Days 150, 178, 232, 240 and 290 due to not function of instrument].

Surface water temperature increased from less than 10°C in winter to ~21°C in summer (Figure 3-5) and it followed the general pattern of the air temperature. Differences in water temperature between the surface and 4m were usually < 1°C, with the deeper water generally being colder.

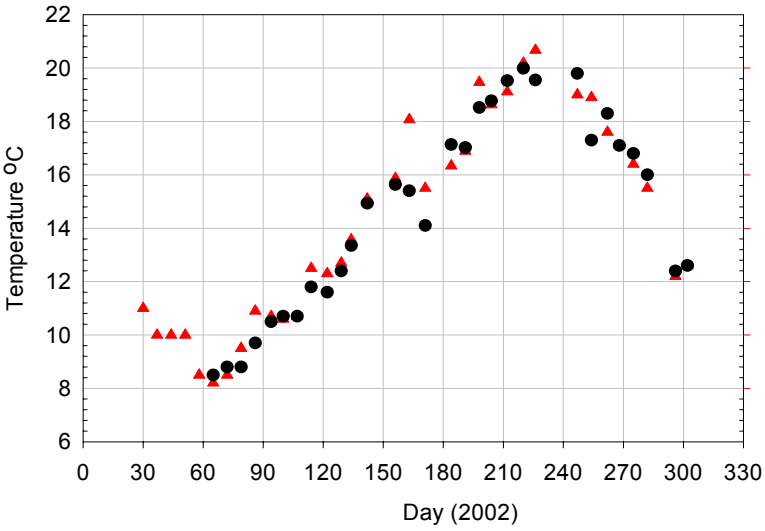


Figure 3-5: Water temperature at surface and 4m depth, Dock 2002.

3-1-3 Water column light attenuation

Values of K_d ranged from 0.6 to ~1.9 m^{-1} during 2002 as shown in Figure 3-6, the greatest variability being in November (ranging from 1.85 m^{-1} on D290, to 0.61 m^{-1} on D302) during the period of heavy rainfall (Figure 3-2).

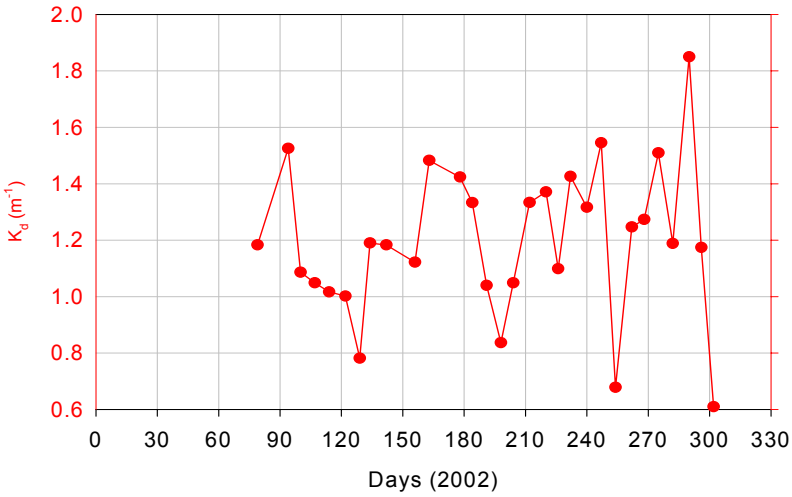


Figure 3-6: Light attenuation coefficient (K_d), Dock 2002

The K_d value is affected by various physical and biological parameters that change water column transparency, including concentrations of total suspended matter (TSM), chlorophyll, and dissolved organic matter. However, K_d does not show strong correlation with the tidal range and is not correlated with chlorophyll concentration (see Figure 3-10 in Section 3.3), suggesting that the processes determining light attenuation in the Dock are complex.

3-2 Chemical Parameters

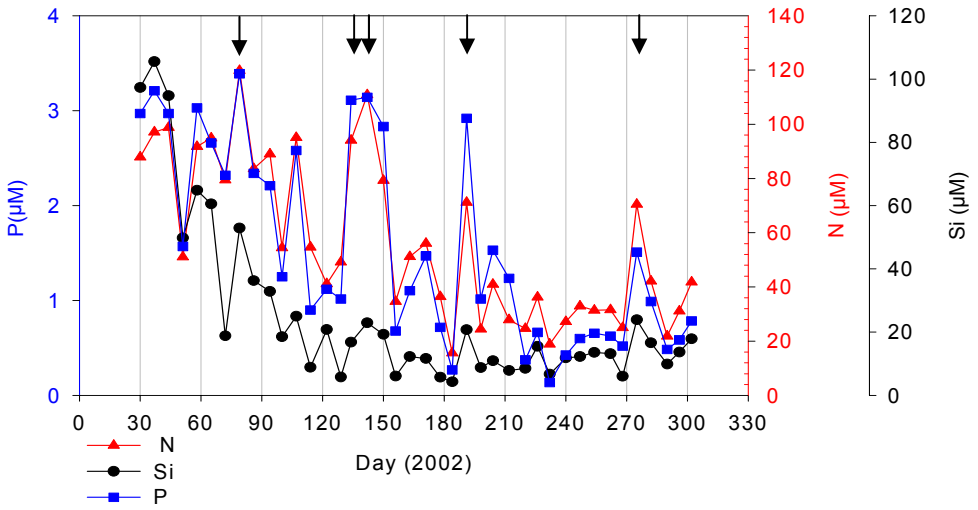
3-2-1 Inorganic nutrients

The distribution of inorganic nutrients (nitrate (N), phosphate (P), silicate (Si)) in general followed a pattern during 2002 of relatively high concentrations in winter and low ones in summer (Figure 3-7).

The three nutrients were above detection levels throughout the sampling period, which means that nutrient depletion never occurred. Phosphate concentrations ranged from 3.40µM in mid March to 0.13µM in late August, nitrate from 119µM (February) to 15.6µM (July), and silicate from 105µM (February) to 4.2µM (July). The highest N, P and Si values in spring and summer were found when surface water salinity was low (< 26, see Figure 3-4).

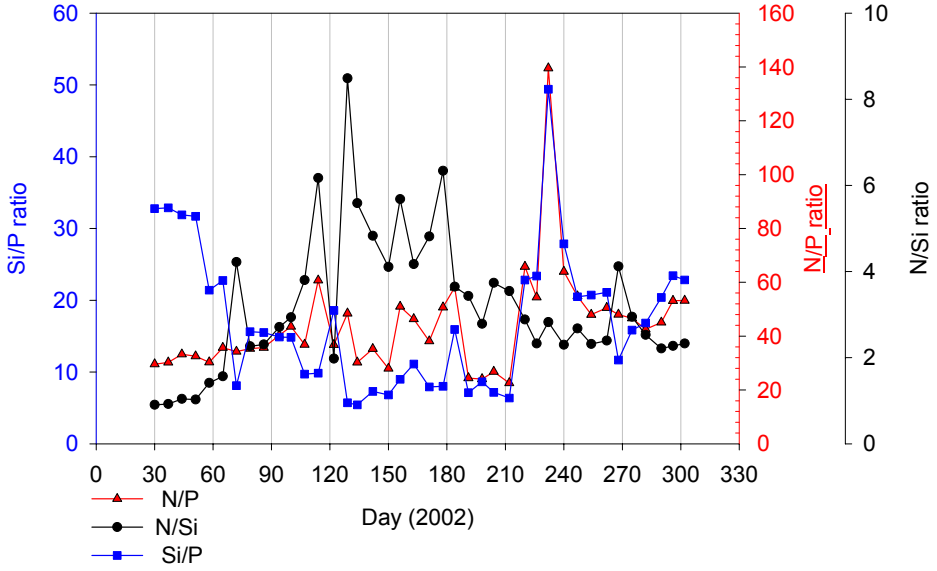
Figure 3-7: Surface nutrient distributions, Dock 2002.

[The black arrows indicate where low surface salinity occurred (see Figure 3-4)]



The nitrate to phosphate ratios (N/P) generally ranged between 20 and 140 (Figure 3-8), significantly greater than the Redfield ratio of 16 for typical marine water. Silicate to phosphate ratios (Si/P) were generally between 10 and 30, and the nitrate to silicate ratios (N/Si) between 1 and 9. Both N/P and Si/P ratio increased to 140 and ~50, respectively in late August, when the lowest nitrate and phosphate concentrations were observed. By contrast, the N/Si ratio did not vary significantly at this time (Figure 3-8).

Figure 3-8: N/P, N/Si and Si/P ratio distributions, Dock 2002.

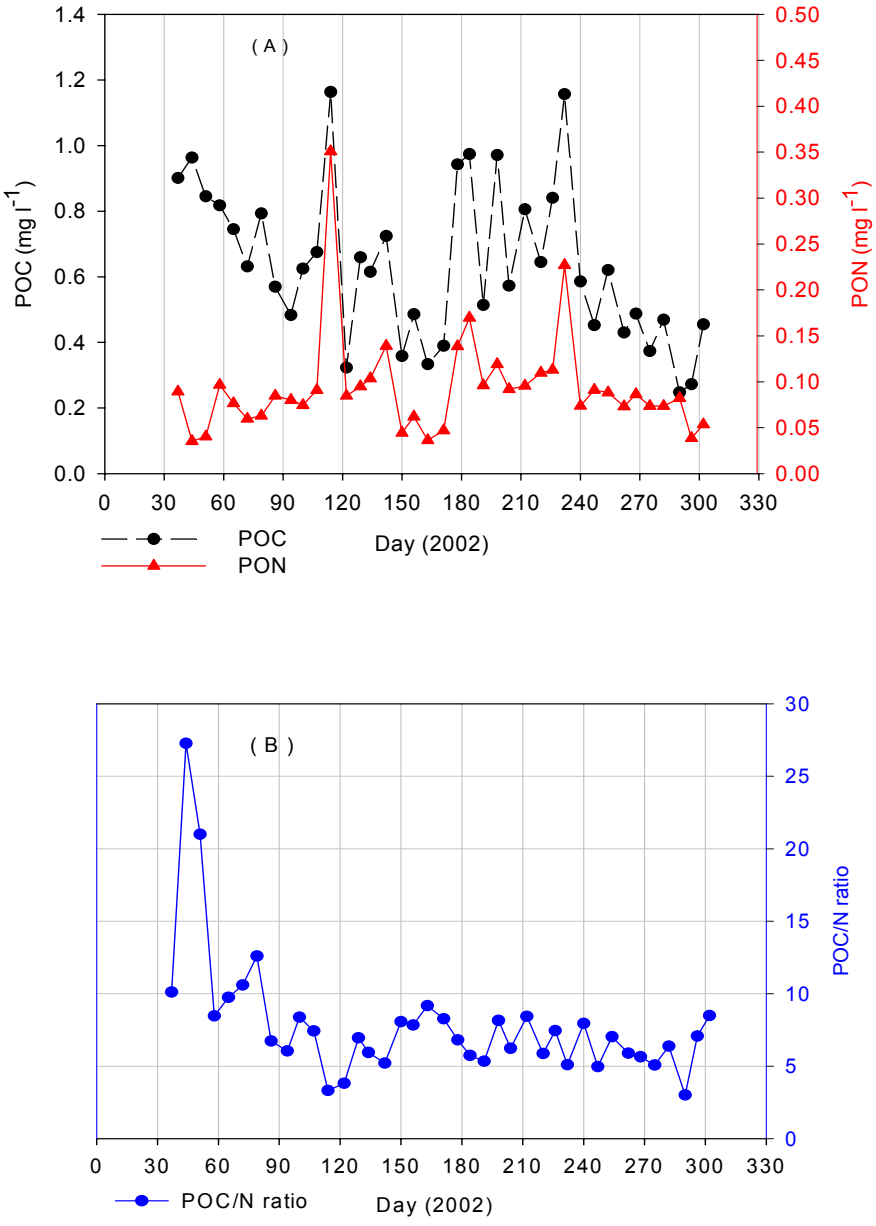


3-2-2 Particulate organic carbon and nitrogen (POC and PON)

Organic particulate carbon (POC) concentrations were relatively high in winter and low in the spring and summer seasons (Figure 3-9A). Particulate organic nitrogen (PON) concentrations fluctuated within a relatively narrow range of approximately 0.02 to 0.35 mg l^{-1}, with the highest value at the end of April.

By contrast, the C/N ratios for particulate organic matter were at their highest in late winter, gradually decreasing through spring and summer to values between 5 and 10 (Figure3-9B), matching the Redfield ratio of ~7. The regression between POC and PON for the productive season from the end of March to the end of the summer showed a good correlation (R^2 = 0.82, with slope of 4.98 and n = 31). High POC/N ratios during the late winter may have been due to detritus inputs from the river.

Figure 3-9: Distribution of A) Particulate Organic Carbon (POC) and Nitrogen (PON), and B) C/N ratio, Dock 2002.



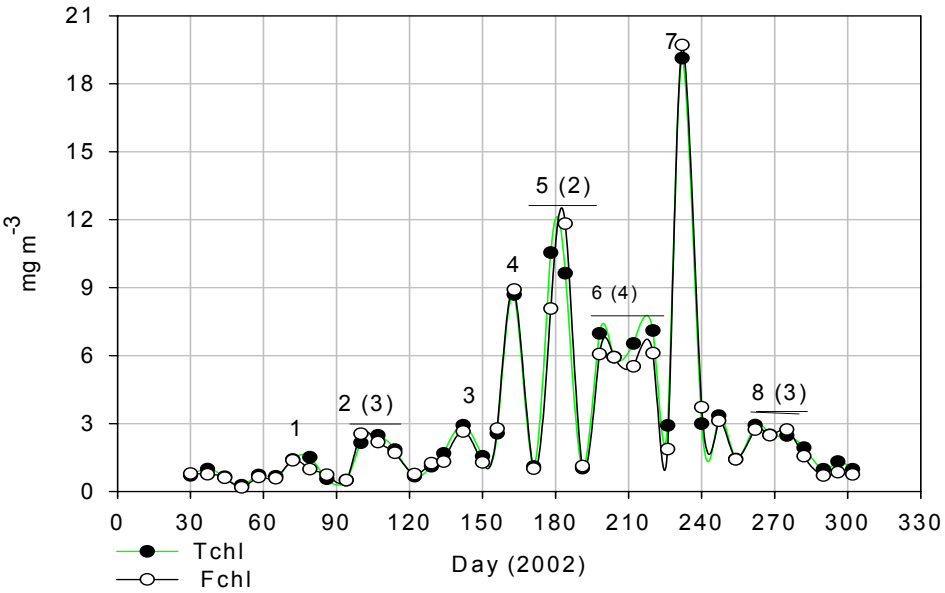
3-3 Phytoplankton pigments

3-3-1 Total chlorophyll a and chlorophyll a size fractions

Changes in total surface chlorophyll *a* concentrations (Tchl) and the sum of chlorophyll *a* size fractions (Fchl) are shown in Figure 3-10. Tchl values ranged from 0.3 mg m^{-3} in February (Day 51) to 19 mg m^{-3} in August (Day 232), with three small peaks of ~2 to ~3 mg m^{-3} during March-May (Days 79, 100, 142), followed by larger peaks of ~ 6 to 19 mg m^{-3} between June–August (Days 163, 184, 198, 220, 232), and a final small peak at the end of September (Day 265). Chlorophyll *a* events (peaks) are numbered in series (1-8) in Figure 3-10 below. The chl events may involve a single sample or more than one; for example the second chl event has 3 samples, the fifth event has 2 samples, the sixth event has 4 samples and the eighth event has 3 samples.

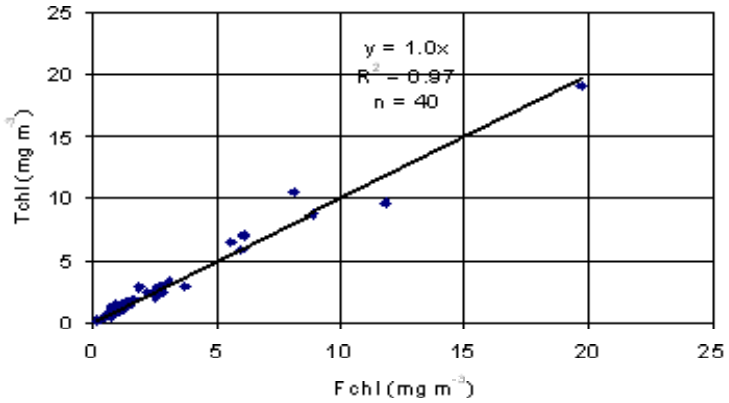
Figure 3-10: Seasonal distribution of total chlorophyll a *(Tchl) and sum of size fractionated chlorophyll* a *(Fchl), Dock 2002.*

[The numbers shown above the chl curve identify a series of chl events with, in brackets, the number of samples contributing to each event.]



Good agreement between Tchl and Fchl (Figure 3-11), with slope 1.0 and an R^2 value of 0.97, gave confidence that the size fractionation procedure was not associated with significant loss of phytoplankton cells.

Figure 3-11: Correlation between Tchl and Fchl, Dock 2002.



The distribution of the chlorophyll *a* size fractions is illustrated in absolute units and as percentages in Figures 3-12 and 3-13, respectively. The mean percentages for each size fraction were: >20 µm, 32% (maximum ~76% in D 232); 5-20 µm 26% (maximum ~44% in D 65); 2-5µm 20% (maximum 39% in D 226); <2 µm 22% (maximum 43% in D 191).

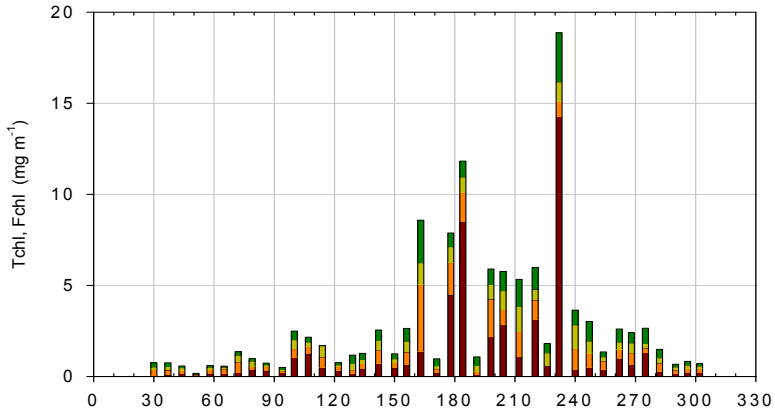


Figure 3-12: Seasonal distributions of chlorophyll a *size fractions, Dock 2002.*

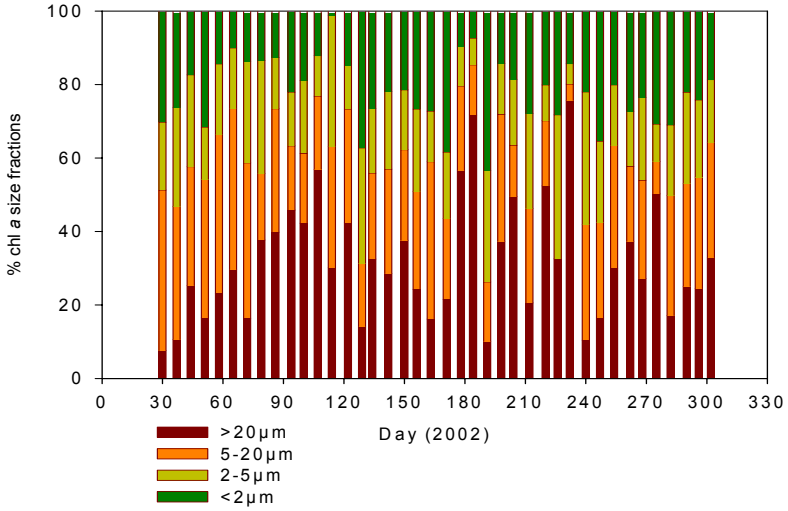
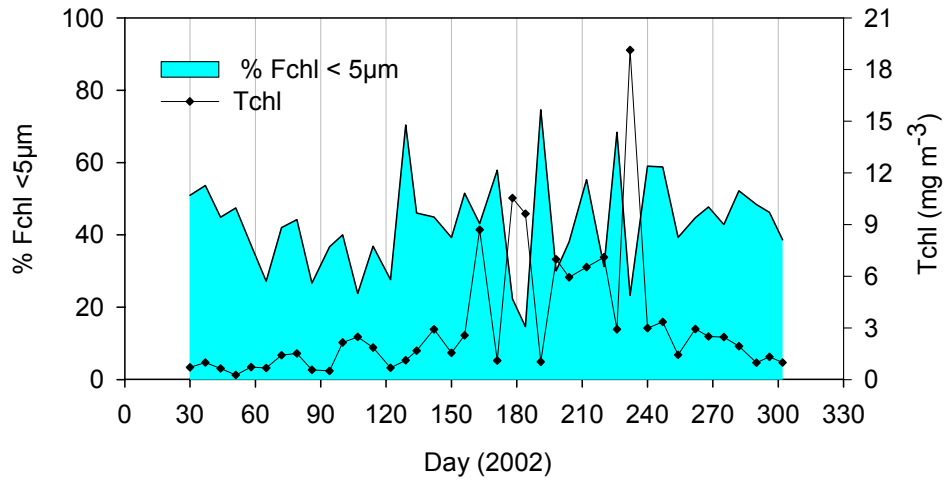


Figure 3-13: Seasonal distribution of chlorophyll a *size fractions expressed as percentages, Dock 2002.*

The four size fraction classes shown in Figure 3-12 and 3-13 can be grouped into two size fractions, >5 µm and <5 µm (Figure 3-14), corresponding to larger cells identifiable with the light microscope and the smaller flagellates, respectively. In general, when Tchl increased, >5 µm Fchl also increased. The mean percentage of Fchl >5 µm fraction was found to be 57% (range 25-85%) and the correlation between Tchl and >5 µm gave R^2= 0.91(n = 40).

Figure 3-14: Total chlorophyll a *and percentages of >5 and <5 µm chlorophyll* a *fractions, Dock 2002*



3-3-2 Phytoplankton accessory pigment

High Performance Liquid Chromatography (HPLC) was used to measure all phytoplankton pigments including chla. Fluorometer determinations of chl were consistently higher than HPLC determinations. On the other hand, there was good agreement between the two readings, as shown in Figure 3-15. Lower HPLC values reflect interference by accessory pigments in the determination of chlorophyll *a* by fluorescence (Trees *et al.*, 1985; Trees *et al.*, 2000).

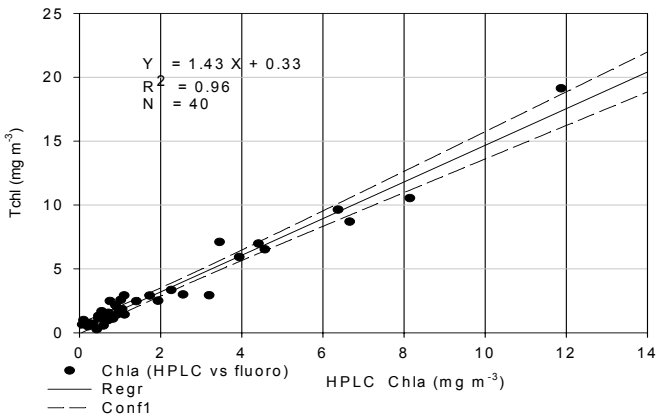
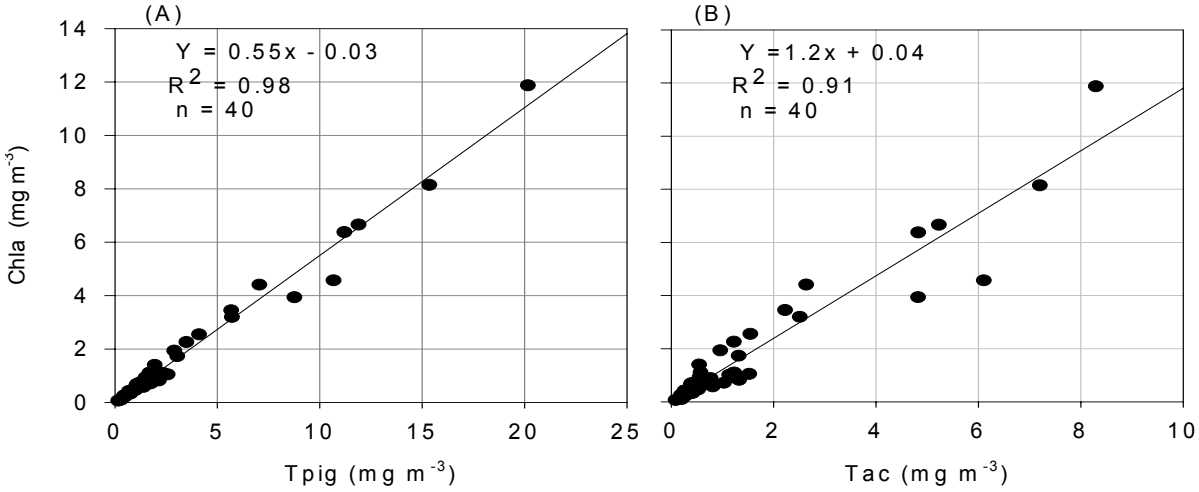


Figure 3-15: Comparison of chlorophyll a *measurements by HPLC and fluorometer*

[The dashed line indicates the confidence interval (99%)]

HPLC detected up to 15 pigments some of which can be used as biomarkers to distinguish between phytoplankton classes (See Figure 2-3). As also shown by Trees *et al*. (2000) and Ali (2003), there were good correlations between chlorophyll *a* and both total pigments (Tpig) (all pigments including all chla) or total accessory pigments without chla (Tac), as shown in Figure 3-16, which was irrespective of phytoplankton composition and pigment content.

Figure 3-16: Relationship of chlorophyll a to total pigments (Tpig) and total accessory pigments (Tac), Dock 2002



The temporal successions of major pigments, namely chlorophyll *a* (chla), fucoxanthin (fuco), peridinin (perid), chlorophyll *b* (chlb) and alloxanthin (allo) as well as two minor, but taxonomically diagnostic, pigments 19-butanoyloxyfucoxanthin (19but) and 19-hexanoyloxyfucoxanthin (19hex) are shown in Figure 3-17. Variations in the ratios of accessory pigments, to chlorophyll *a* (Figures 3-18) reflected changes in the taxonomic composition of the phytoplankton population.

The major pigments fuco and perid are biomarkers for diatoms and dinoflagellates, respectively. Each showed maximum values >1 mg m^{-3}, but at different times and corresponding to different chl events. The two major peaks in perid corresponded to maxima perid to chla ratios, suggesting the phytoplankton population was dominated by dinoflagellates at these times. By contrast, the peaks in fuco were not linked with

particularly high fuco to chla ratios, suggesting that the increases in diatom abundance were accompanied by similar increases in other phytoplankton groups.

The minor pigments chlb and allo are biomarkers of euglenoids, and of *Cryptomonas* spp and the ciliate *Mesodinium rubrum* (*MR*), respectively. Each showed maximum values (<1 mg m^{-3}), but at different times, and corresponded to different chl events. The two early peaks in chlb corresponded to the highest chlb to chla ratios, suggesting the phytoplankton were dominated by green algae (*Eutreptiella* sp.) as well as other later peaks. The highest allo peak corresponded with a high allo to chla ratio, suggesting *Cryptomona*s spp and *MR* were contributing to the phytoplankton community. Chlb was used as a biomarker for many classes (green algae, Chlorophyceae, Prasinophyceae and the land plants).

19But and 19 hex could be considered as biomarkers for both Prymnesiophyceae and Chrysophyceae. Each pigment showed a maximum value <0.08 mg m^{-3}, but at different times and corresponding to different chl events.

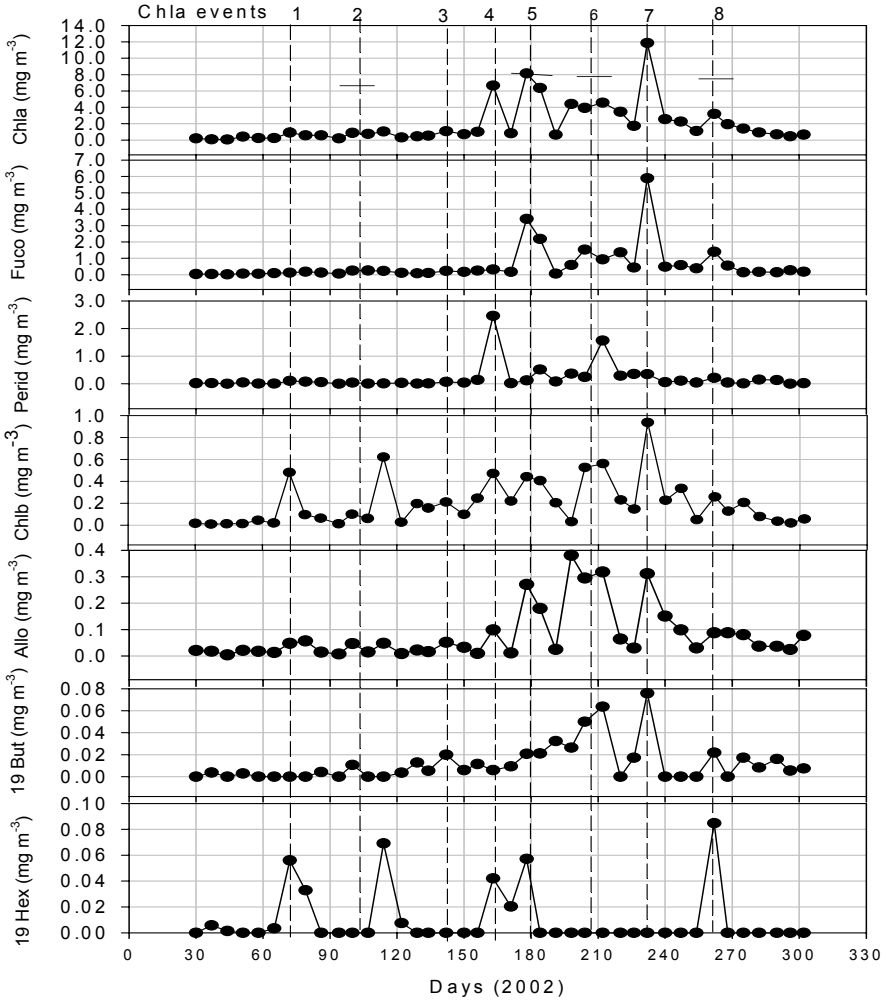
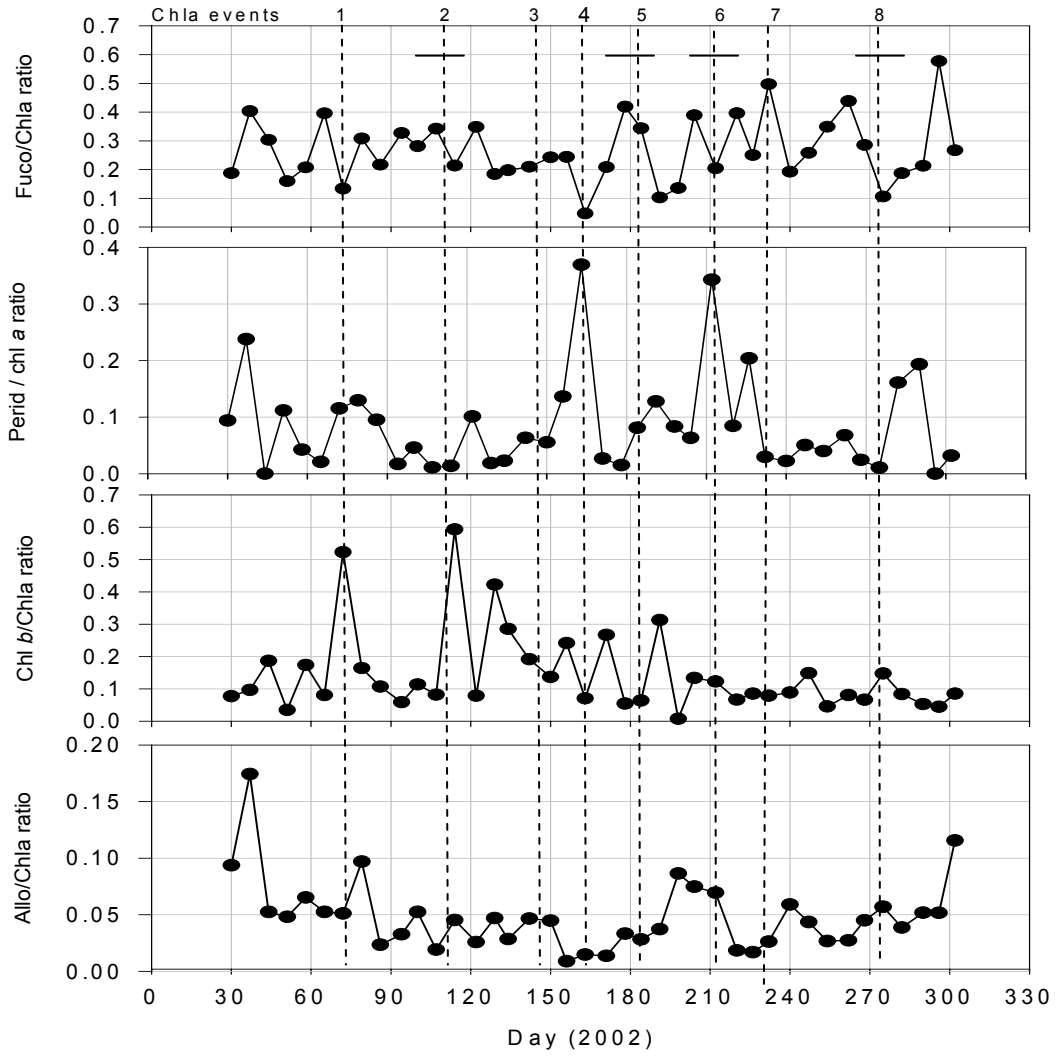


Figure 3-17: Temporal distributions of phytoplankton chlorophyll a *and accessory pigments, Dock 2002.*

Figure 3-18: Accessory pigment to chlorophyll a *ratios, Dock 2002.*



3-4 Phytoplankton taxonomic data

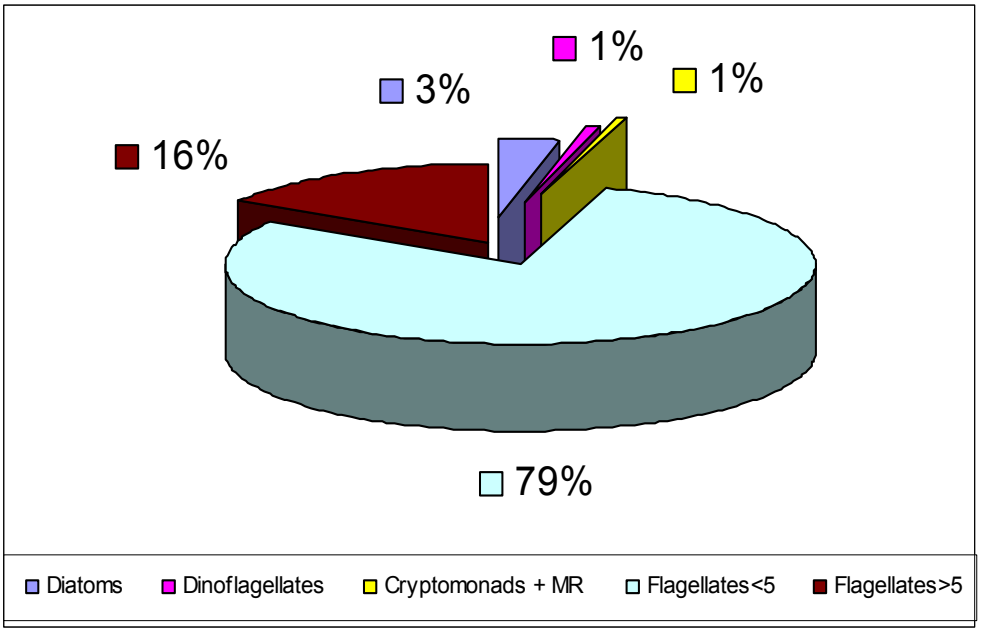
3-4-1 Phytoplankton cell abundance and carbon biomass.

During the sampling period, more than 30 diatom species, 4 photosynthetic dinoflagellate species, 1 autotrophic ciliate (*Mesodinium rubrum* with a cryptophyte endosymbiont) and 2 taxa of autotrophic flagellates (*Cryptomonas*, *Eutreptiella*), were recorded in addition to small flagellates, divided into <5 and >5 μm diameter sizes. The complete data are shown in Appendix 1.1.

The phytoplankton counts for the Dock 2002 samples are summarised in Figure 3-19 for taxa representing more than 1% of the total counts. The dominant group was

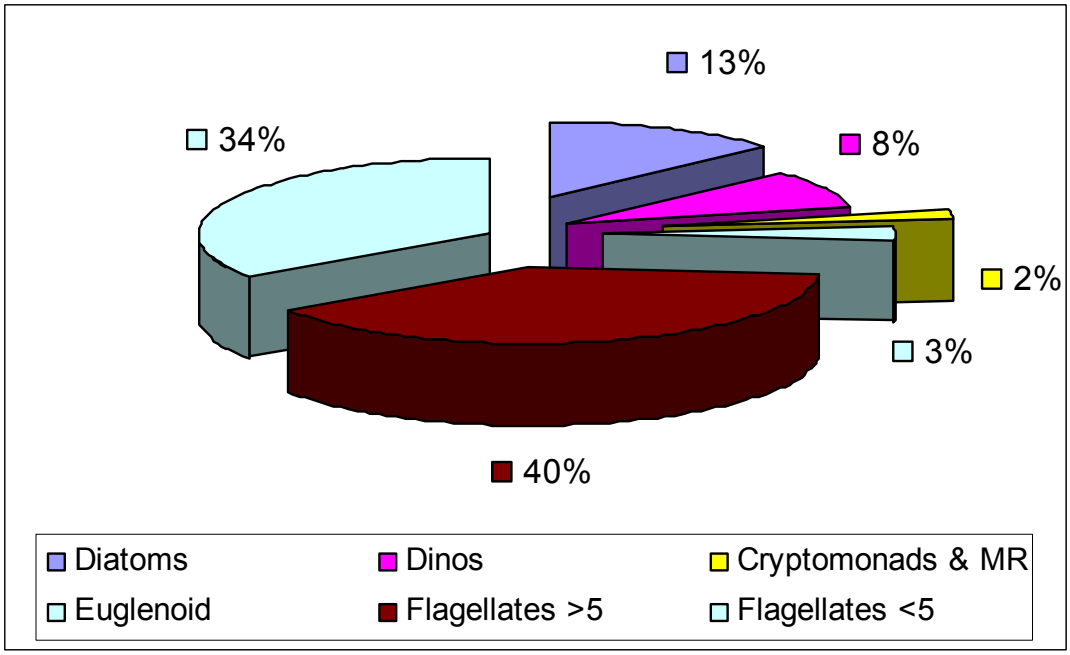
<5µm flagellates, represented about 79% of the total phytoplankton counted, followed by >5µm flagellates which accounted for 16% of the total phytoplankton. Other large phytoplankton cells accounted for the remaining percentages and they consisted mainly of diatoms and dinoflagellates.

Figure 3-19: Cell count percentage for main phytoplankton groups, combined data for whole year, Dock 2002.



Phytoplankton biomass (mg C m^{-3}) was calculated from cell volumes for each species (see Methods). The average distribution of carbon biomass during the whole sampling period is shown in Figure 3-20. This analysis shows that the larger cells are all more important than indicated by the cell counts and that the >5µm flagellates and diatoms are on average, the larger components of biomass. The increase in diatom carbon biomass was accompanied by increases in other large cells (dinoflagellates, euglenoids, cryptophytes) and also by higher total biomass, as measured by chlorophyll.

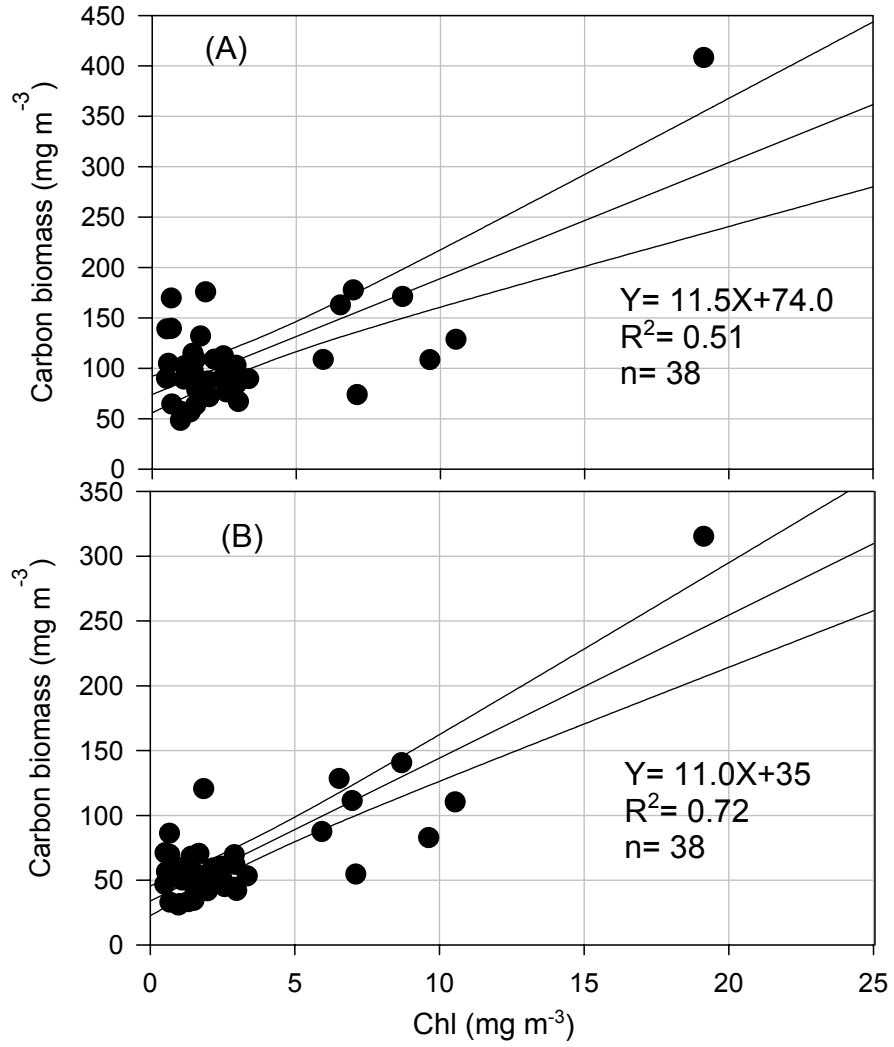
Figure 3-20: Biomass percentage of the main phytoplankton groups, combined data for whole year, Dock 2002.



As expected, measured chlorophyll *a* concentration and estimated phytoplankton carbon were positively correlated. A proportion of the >5 and <5 µm flagellates are known to be heterotrophic, and the best fit between chlorophyll and phytoplankton carbon is found when it is assumed that 50% of each group of flagellates is heterotrophic (Figure 3-21). The slope of the relationship gives the C/chl ratio and is relatively independent of the proportion of flagellates assumed to be heterotrophic. The C/chl ratio of 11.0 shown in Figure 3-21 is lower than would be expected from the literature (eg. chl/C ratio is 51 at San Francisco Bay (Wienke and Cloern, 1987), and a range of 20-70 for Southampton Water (Ali, 2003), and may reflect an underestimation of cell abundance in samples containing much non-living particulate material from a turbid environment. Other factors that could account for a low C/chl ratio are underestimation of cell dimensions (see Methods), and /or a high cellular chlorophyll content for an environment with low mean irradiance (high K_d).

Figure 3-21: Correlation between phytoplankton biomass and chl, Dock 2002.

[A) 100% flagellate biomass assumed to be autotrophic; slope when regression is forced through zero is 21.6. B) 50% flagellate biomass assumed to be autotrophic; slope when regression is forced through zero is 15.7. The dashed line indicates the confidence interval (99%]



3-4-2 Phytoplankton species composition

Four out of the eight chl events identified in Figure 3-10 included samples with a chl concentration >5mg m^{-3}. The phytoplankton cell counts and biomass values for these samples are summarised in Tables 3-1 and 3-2.

Apart from the flagellates (>5 and <5 μm), by far the most numerous cell types were dinoflagellates and diatoms in chl events 4 and 7, each with a single species,

respectively. Other chl event populations were mixed. Diatoms had a highest number of species contributions on Day 204 than on other days, although some species appeared only one time, while others appeared on many days.

When phytoplankton cell numbers are converted to carbon biomass (Table 3-2) a different picture emerges of the community composition. The phytoplankton biomass contributions to different chl events are shown in terms of the highest species contributors within the group, and the dominant groups within similar samples.

The diatoms were highly dynamic in terms of shifting from one species to another over a short period (one week in between samples). The diatom carbon biomass was high, but varied from time to time. The diatoms were composed of *Cerataulina pelagica*, *Coscinodiscus sp*, *Rhizosolenia setigera* and *Pleurosigma, Guinardia delicatula,* and *Skeletonema costatum*. The highest biomass was obtained at the last peak in late summer and was dominated be *S*. costatum

The dinoflagellates contributed to all peaks at different biomass values and times. Dinoflagellates comprised few species, such as *Gymnodinium sp2* which dominated the early samples as well as *Scrippsiella trochoidea,* and *Prorocentrum micans* which dominated the late samples. Other groups, such as the <5, >5µm flagellates, were present at all peaks, with comparable biomass values.

Table 3-1: Phytoplankton species counts (cell ml^{-1}) for the high chl samples, Dock 2002.

[All species counted from settled 10 ml samples are listed]

Chlorophyll a events	4	5	5	6	6	6	6	7
Days	163	178	184	198	204	212	220	232
chl a (mg m^{-3})	8.69	10.54	9.63	6.98	5.93	6.53	7.11	19.13
Diatoms								
Asterionella glacialis	0.4			0.9	7.5	13.8	4.3	41
Asterionella kariana					0.5			
Biddulphia pulchella				2	0.4			
Cerataulina pelagica		45.8						
Chaetoceros sp				12.9	20.8	1.0	21.2	94.0
Coscinodiscus	0.2	4	8.1		1.4	4		3
Cylindrotheca closterium	0.2			0.7	1.1	3.2	10.6	124.5
Ditylum brightwelli		0.1		0.6	0.1	1		
Guinardia delicatula		4			7.2			7
Lithodesmium undulatum	0.8			2	7.9	3		
Navicula sp			1.2		4.5			
Odontella aurita					0.2			
Odontella mobiliensis					0.1			
Pennate				0.8	0.3	19	4	7
Pleurosigma	0.7	5.3		1	0.4	13.8		
Pseudo-nitzschia sp				3	2.3			2
Rhizosolenia setigera		0.1		7.0	2.0	18.0	0.0	1.0
Skeletonema costatum				47.3	601.9	388	1669	16189
Thalassionema nitzschioides			7.4	1.2				
Thalassiosira hyalina		6.4						10
Thalassiosira rotula		0.9		0.7			16.2	
Total diatoms	2	67	17	80	659	465	1725	16479
Dinoflagellates								
Gymnodinium		0.7		0.4	8	10		
Gymnodinium sp2	2189		63	360	96	869		88
Prorocentrum micans			0.3		10	6.4	9	2
Scrippsiella trochoidea	90	7.5	69	25	8	8	1	8
Total dinoflagellates	2279	8	132	385	122	893	10	98
Other groups								
Mesodinium rubrum	5	20	15	16	8	11		14
Cryptomonas spp	20	150	100	535	210	275	6	323
Eutreptiella sp	39	16.9	2	6	3.1			25
Flagellates<5 µm	10667	6095	6481	19294	5882	7852	2283	13705
Flagellates>5 µm	1857	1143	1889	4529	1500	2667	1787	8235

Table 3-2: Phytoplankton species biomass (mg C m^{-3}) for the high chl samples, Dock 2002.

[Only species contributing 0.1 mg C m^{-3} or more are listed]

Chlorophyll a event	4	5	5	6	6	6	6	7
Day	163	178	184	198	204	212	220	232
chl (mg m^{-3})	9	11	10	7	6	7	7	19
Total carbon (mg m^{-3})	171	128	108	178	109	163	74	408
Diatoms								
Asterionella glacialis				0.1	0.8	1.5	0.5	4.4
Cerataulina pelagica		53.7						
Chaetoceros sp		0.3		2.8	2.7	0.9	1.1	7.3
Coscinodiscus	0.5	10.9	22.1		3.8	10.9		8.2
Ditylum brightwelli				0.2		0.3		
Guinardia delicatula		7.8			14.1			13.7
Lithodesmium undulatum	0.2			0.5	2.0	0.8		
Odontella mobiliensis					0.8			
Pleurosigma	0.4	3.4		0.6	0.3	8.9		
Rhizosolenia setigera		0.3		8.4	6.2	18.0		3.1
Skeletonema costatum				0.5	6.0	3.9	16.6	161.3
Thalassionema nitzschioides			0.7	0.1				
Thalassiosira hyalina		2.1						3.3
Thalassiosira rotula		0.2		0.1			3.4	
Total diatom	1.2	78.8	22.8	13.4	36.9	45.2	21.7	201.7
Dinoflagellates								
Gymnodinium		0.3		0.2	3.8	4.8		
Gymnodinium sp2	64.6		1.9	10.6	2.8	25.6		2.6
Prorocentrum micans	0.0		0.4		14.4	9.2	12.9	2.9
Scrippsiella trochoidea	35.0	2.9	26.8	9.7	3.1	3.1	0.4	3.1
Total dinoflagellates	99.6	3.2	29.1	20.5	24.1	42.7	13.3	8.6
Other groups								
Mesodinium rubrum	1.2	4.8	3.6	3.9	1.9	2.7		3.4
Cryptomonas sp	0.2	1.7	1.1	6.0	2.4	3.1	0.1	3.6
Eutreptiella sp	7.4	3.2	0.4	1.1	0.6			4.8
Flagellates<5 μm	27.5	15.7	16.7	49.7	15.1	20.2	5.9	35.3
Flagellates>5 μm	34.0	20.9	34.6	82.9	27.4	48.8	32.7	150.6

3-4-3 Seasonal succession of phytoplankton taxa and pigments

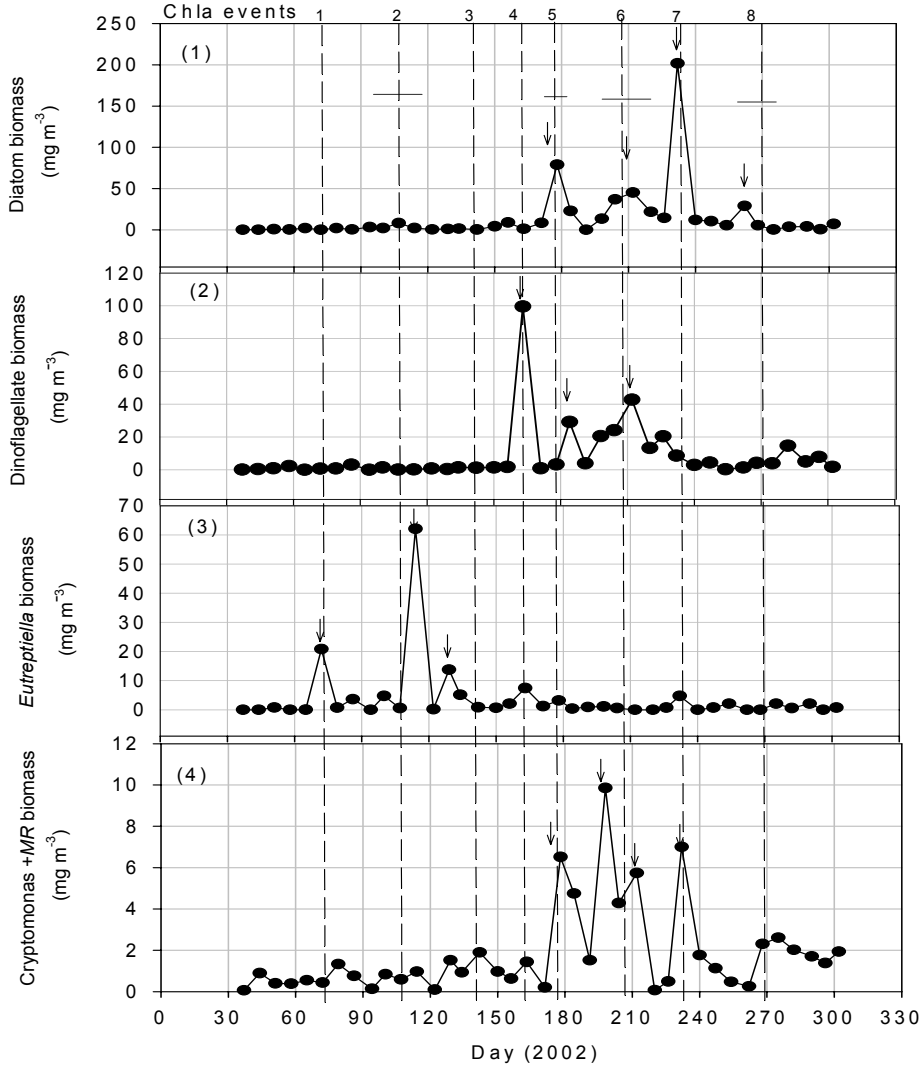
Phytoplankton seasonal succession is shown in Figure 3-22. It shows the absolute contributions of different phytoplankton groups, over the whole sampling period rather than selected days, with the four chl events indicated by dashed lines and the signatures of particular taxa by arrows. The diatom and dinoflagellate populations are represented by the species data Table 3-2. The main biomass peak for dinoflagellates (chl event 4, *Gymnodinium* sp2) was earlier than the peaks for diatoms, while

euglenoids (Day 113, *Eutreptiella* sp) and *Cryptomonas* / *Mesodinium* (Day 189) occurred when levels of chl were < 3 mg m^{-3}.

Consequently, group succession is presented as phytoplankton percentage biomass as shown in Figure 3-22. The phytoplankton succession was initiated by the large flagellate *Eutreptiella*, in early spring, with two peaks. The dinoflagellates peaked in late spring, followed by a diatom peak. The summer peaks were mixed diatoms, dinoflagellates, together with *Cryptomonas* sp and *Mesodinium rubrum*. The last and highest diatom biomass peak was at the end of summer.

Figure 3-22: The succession of phytoplankton groups, Dock 2002.

1) Diatoms, the arrows indicate fuco/chla ratios >0.4; 2) Dinoflagellates, the arrows indicate perid/chla ratios >0.3; 3) *Eutreptiella*, the arrows indicate chlb/chla ratios >0.4, 4) *Cryptomonas* and *Mesodinium rubrum* (MR), the arrows indicate allo values > 0.25 mg m^{-3}.



3-5 Multivariate data analysis and interpretation

The physical, chemical and biological data collected during 2002 were analysed by multivariate analysis. The analyses were carried out in several steps: 1- grouping of environmental parameters by similarity or dissimilarity (Euclidean distance); 2- grouping of phytoplankton species using the Bray-Curtis Similarity Index; and 3- correlation of environmental variables to phytoplankton groups (BIOENV analysis). For each type of analysis, SIMPER analysis was used to calculate the percentage similarity of each sample group and the dissimilarity between each pair of groups.

Two types of plot are shown, dendrograms for hierarchical clustering of samples and Multi-Dimensional Scaling (MDS) to show group similarity and distance between sample groups in two-dimensional space. The MDS plots can be superimposed with the environmental variables to give an indication of important relationships between phytoplankton distribution and environmental parameters. The stress level for each MDS ordinal plot is used as an indicator of how the plot manages the sample distribution. Stress levels of <0.1 indicated good ordination, 0.1-0.2 a useful two-dimensional display of clusters and > 0.2 a random placement in two dimensions (see Clarke and Warwick (1994) for further details).

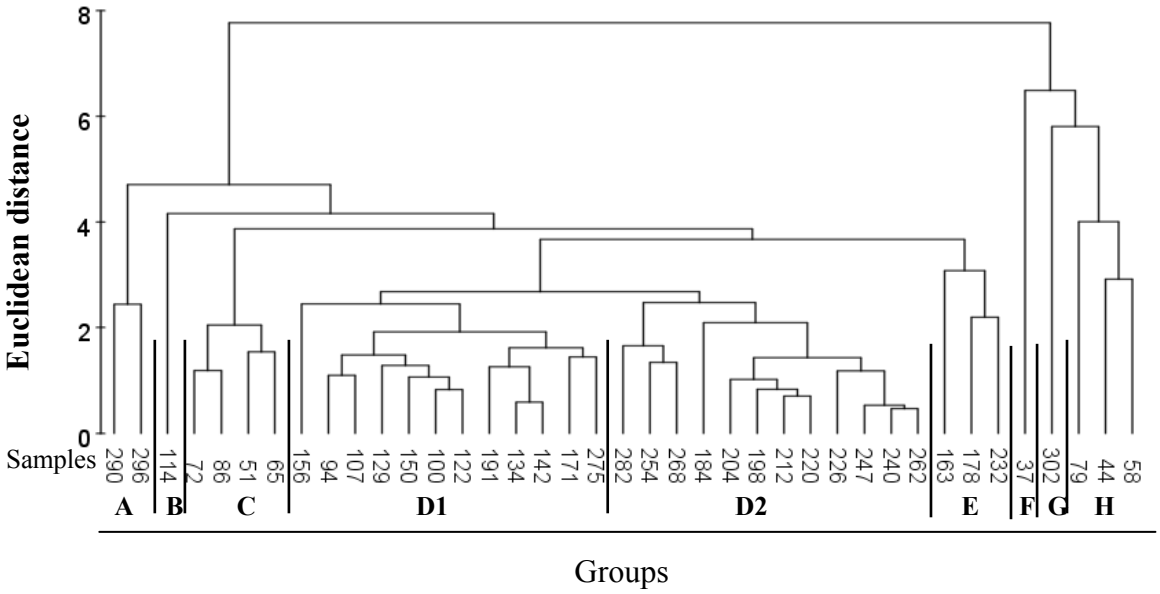
3-5-1 Environmental data analyses

The environmental parameter data were normalised as log (1+x) to calculate the Euclidean distance. The environmental variables (air temperature, PAR, water salinity, water temperature, tide range, K_d, nutrients (N, P, Si), POC, PON and chl) were clustered to give groups with the lowest distance between pairs of samples.

Groups A-H were defined from the dendrogram for hierarchical clustering (Figure 3-23). The Euclidean distance between most of the groups A-E was <4.5%, while the distance between groups F-H was <8%. A majority of samples were in group D, which could be sub-divided in to D1 and D2, while groups B, F, and G had only one sample and groups A, C, E and H had 2-4 samples.

The data were then analysed by Multi-Dimensional Scaling (MDS) to provide a two-dimensional distance plot (Figure 3-24) which shows more clearly how the samples are grouped. The plot stress (<0.08) indicates a very good two-dimensional representation of the data. A seasonal pattern to group distribution can be distinguished from the plot: groups C, H and F represent the early spring, group D1 the late spring and early summer, group D2 the late summer, and groups A and G the autumn. Group E was intermediate between groups D1 and D2.

Figure 3-23: Dendrogram for hierarchical clustering of samples defined by environmental parameters.



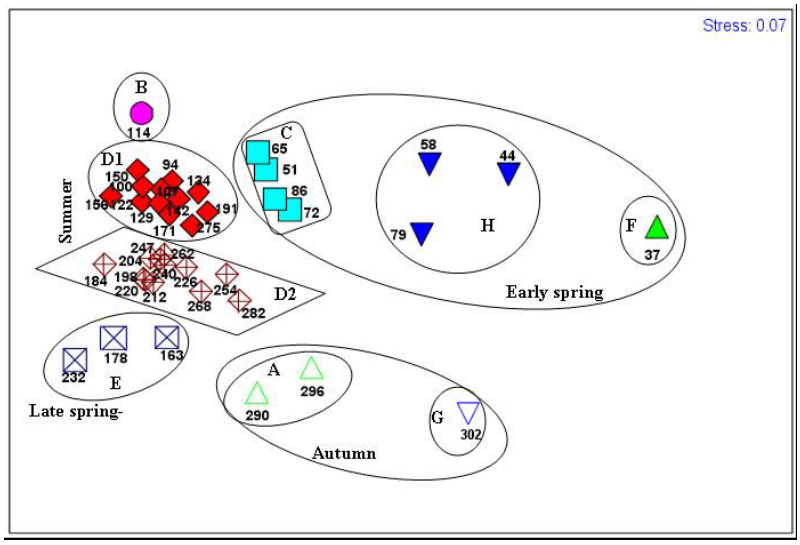


Figure 3-24: MDS plot of environmental parameter groups. Numbers indicate the sample days.

PAR and water temperature were the most important environmental parameters for defining the sample groups. Both follow similar seasonal cycles, with temperature dependent upon and slightly lagging behind PAR (Figures 3-1 and 3-5). For this reason, water temperature was omitted from a quantitative analysis of the relative contributions of environmental parameters to group definition, the results of which are summarised in Table 3-4.

Table 3-3: Main characteristics of sample groups defined by environmental parameters.

[The high chlorophyll samples (Table 3-1) are identified by sample days in bold].

Group	Samples day no	Parameters % contribution	% of similarity
A	290, 296	PAR (45), Salinity (13.8), N (12).	93
B	114	High PAR and low nutrients	
C	51, 65, 72, 86	PAR (43), N (19), Si (12), Salinity (11).	95
D1	94, 100,107, 122, 129, 134, 142, 150, 165, 171, 191, 275	PAR (55), N (15), Salinity (10)	94
D2	**184, 198, 204, 212**, **220**, 226, 240, 247, 254, 262, 268, 282	PAR (55), Salinity (11), N (10)	94
E	**163, 178, 232**	PAR (55), Salinity (10)	92
F	37	Low PAR and high nutrients	
G	302	Low K_d and PAR	
H	44, 58, 79	PAR (34), N (21), Si (16), Salinity (11)	95

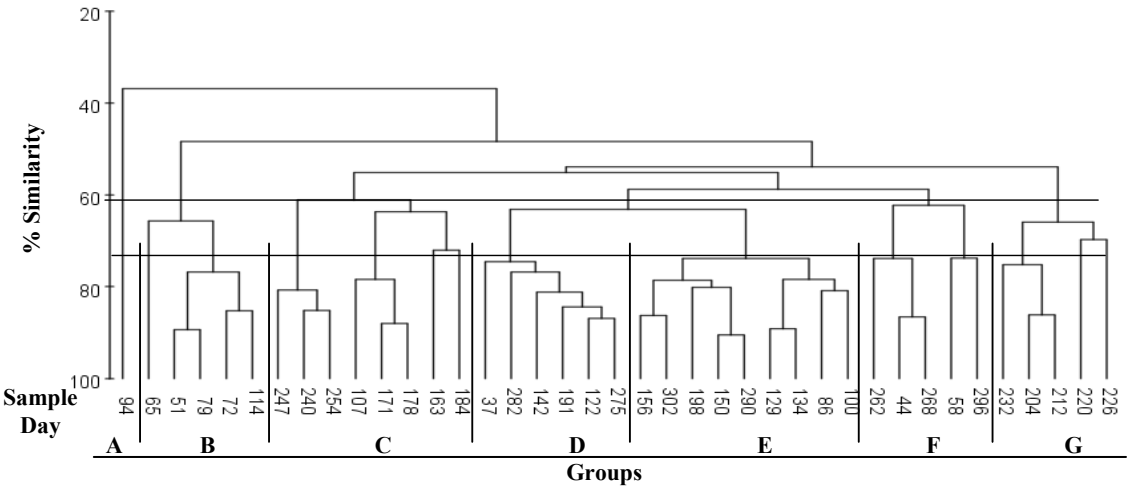
Overall, seasonal change in PAR (low in winter, high in summer) was the dominant factor in defining the environmental groups. The early spring groups (F, H, C, B) were characterised by low/increasing PAR and by high/decreasing nutrients, with Si as well as N being relatively important. By early summer, PAR was generally high, Si was relatively low and N continued to decrease (group D1). All the high chlorophyll samples were in groups E and D2 when nutrient levels were at a

minimum. In the autumn (groups A, G) PAR was decreasing, and nutrients were beginning to increase, probably through a combination of regeneration and enhanced river inputs. The relatively low but persistent contribution of salinity is likely to reflect associated changes in nutrient levels and water column turbidity (K_d). The single sample groups were distinguished by particular environmental conditions at the start and end of the sampling period or relatively anomalous conditions on a particular date.

3-5-2 Phytoplankton species biomass data analyses

The phytoplankton data were normalised and transformed to the fourth root before calculating the Bray-Curtis Similarity Index. Only species/taxa representing >1% of total biomass were considered. The results of hierarchical clustered analysis, illustrated as a dendrogram (Figure 3-25), showed seven phytoplankton groups (A-G) at a similarity level range of about 60-70% (a slightly higher similarity threshold was used to distinguish groups D and E).

Figure 3-25: Dendrogram for hierarchical clustering of samples defined by phytoplankton species/taxon biomass.

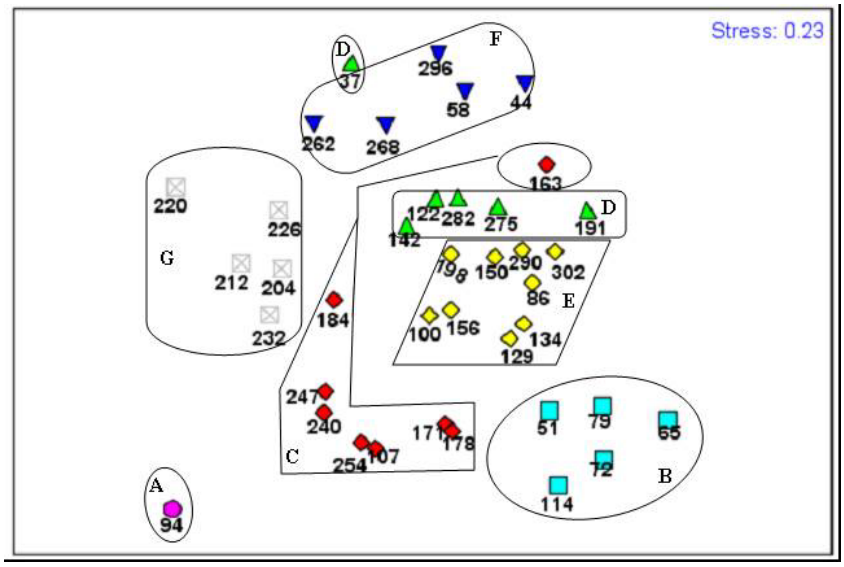


The lowest dissimilarity was between groups D and F at 31%, and the highest dissimilarity was between groups A and B at 71%. The average dissimilarity between the seasonal groups was 62%, for autumn-winter (B and F), 69% for spring groups (A and E) and 44% for the mixed groups (C and D).

The two-dimensional MDS plot (Figure 3-26) with a stress of 0.23 indicates a relatively poor two dimensional representation of the data, which is not surprising, as many species/taxa are widely distributed over time. However, the groups are relatively well defined in three-dimensional space (not shown) with a stress of 0.15.

The groups follow a seasonal pattern: groups A and B consist of spring samples, groups C, D and E overlap and are mainly mid-year, group G is a set of late summer samples, and group F includes both spring and autumn samples.

Figure 3-26: MDS plot of samples defined by phytoplankton species/taxon biomass. Numbers indicate the sample day.



The results of the SIMPER analysis summarised in Table 3-5, show how the groups are distinguished in terms of particular species/taxa. The diatom, *Coscinodiscus*, and the euglenoid, *Eutreptiella* dominated spring groups (A and B) when nutrient levels are still high. In the late summer (group G, mainly high chlorophyll samples), the diatom, *Skeletonema*, and the dinoflagellate, *Prorocentrum*, are the main species. These and other species/taxa occur during mid-year (groups C, D, and E) in various combinations. Group F is characterised by a high component of dinoflagellate species and a low component of diatoms. Another notable feature is the significant contribution of *Cryptomonas* to all groups except A. No obvious relationship can be

seen between the reduction in silicate levels by day 90 and the relative importance of diatoms.

Table 3-4: Main characteristics of sample groups defined by phytoplankton species/taxa.

[The high chlorophyll samples (Table 3-1) are identified by sample days in bold]

Group	Samples (Day No.)	Species (average biomass as % contribution)	% of similarity
A	94	*Coscinodiscus*	
B	51, 65, 72, 79, 114	*Eutreptiella* (57), *Gymnodinium* sp2 (14), *Cryptomonas* (27)	74
C	107, **163**, 171, **178**, **184**, 240, 247, 254	*Coscinodiscus* sp (30), *Eutreptiella* (25), *Cryptomonas* (13), *Gymnodinium* sp2 (8)	67
D	37, 122, 142, 191, 275, 282	*Scrippsiella* (43), *Cryptomonas* (27), *Eutreptiella* (18), *Skeletonema costatum* (13)	79
E	86, 100, 129, 134, 150, 156, **198**, 290, 302	*Eutreptiella* (25), *Rhizosolenia setigera* (23), *Scrippsiella* (21), *Cryptomonas* (21)	41
F	44, 58, 262, 268, 296	*Scrippsiella* (38), *Cryptomonas* (28), *Gymnodinium* 2 (26)	68
G	**204, 212, 220,** 226**, 232**	*Skeletonema costatum* (22), *Prorocentrum* (21), *Scrippsiella* (14), *Cryptomonas* (14), *Gymnodinium* sp2 (11).	70

Phytoplankton species were well distributed thought out the year, but some of them increased rapidly, to become dominant species on a particular day. The phytoplankton species successions are not clear between the phytoplankton species/taxa groups. However, *Eutreptiella* sp and *Coscinodiscus* sp which contributed highly to the low chl groups A and B, and can be considered as early spring species that are associated with the environmental spring groups C, G and H (Table 3.3) *Skeletonema costatum* and *Prorocentrum* sp contributed highly to group G (most samples are high chl) and are classified as summer species, mixed with other

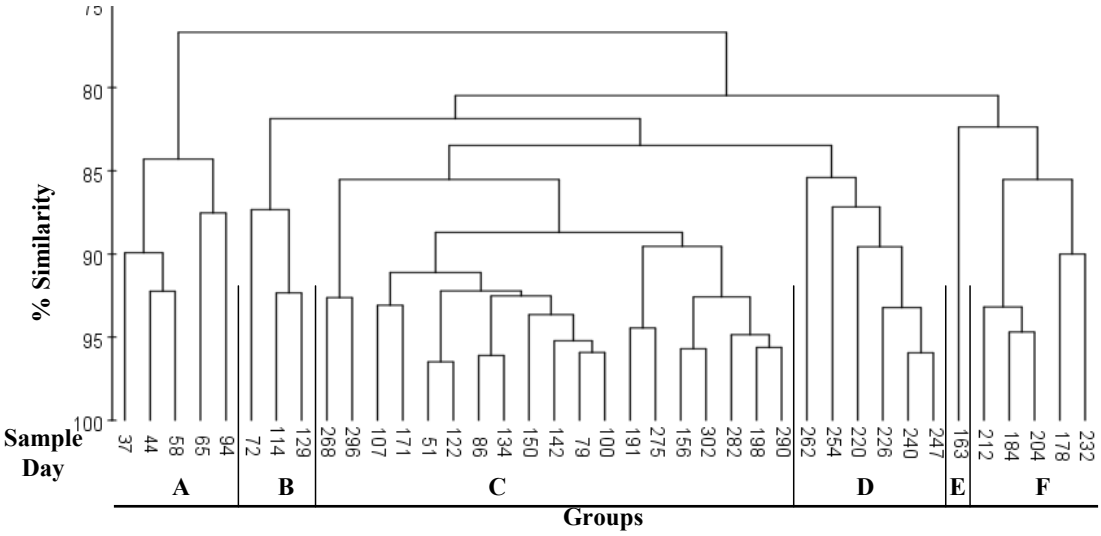
species, and associated with environmental groups D2 and E, which are characterised by high PAR and low nutrients in spring-summer time. Other groups overlap in terms of time and numbers of important species. Group C has many samples with high chl, and had similar important species as groups A and B, but *Gymnodinium* sp2 and *Cerataulina pelagica* are more important to the samples 163 and 178, respectively. Also *Scrippsiella* sp contributed highly to groups D and F, indicating the importance of this species during the year. Group E has one sample of high chl and with high contribution from *Gymnodinium* sp2 than other species recognised by the programme.

Phytoplankton group biomass analyses

Biomass data for the main phytoplankton taxonomic/size groups (diatoms, dinoflagellates, *Eutreptiella*, *Cryptomonas*, and flagellates <5µm and >5µm) were analysed in terms of the Bray-Curtis Similarity Index, after a 4^{th} root transformation. The hierarchical cluster dendrogram (Figure 3- 27) identified six sample groups at a level of similarity of about 84%.

MDS ordination for the same data gives an acceptable two-dimensional representation (stress value 0.15) of the groups, as shown in Figure 3-28.

Figure 3-27: Dendrogram for hierarchical clustering of samples defined by phytoplankton group (taxon, cell size) biomass.



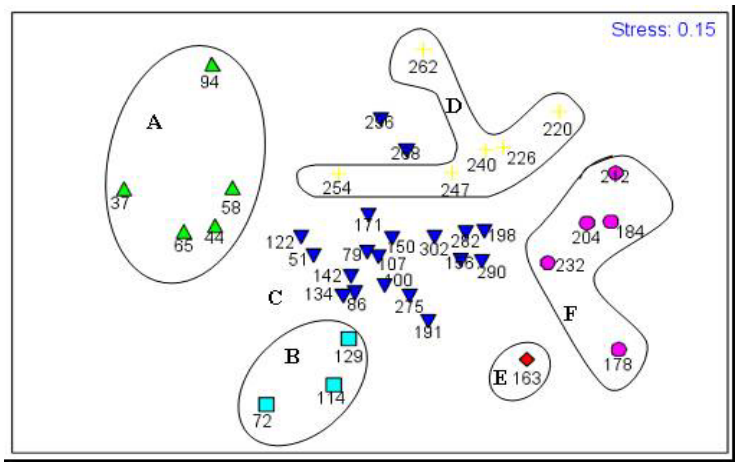


Figure 3-28: MDS plot of samples defined by phytoplankton group (taxon, cell size) biomass. Numbers indicate sample days.

The sample group specifications are summarised in Table 3-5. As expected flagellates (<5 and >5µm) are a major component of all groups. Early samples (groups A and B) included a minor contribution from diatoms and a more significant one from *Eutreptiella*. Diatoms and dinoflagellates were generally more important during the summer months, with diatoms the main component of several high chlorophyll samples (group F) and dinoflagellates of one high chlorophyll sample (group E).

Table 3-5: Main characteristics of sample groups defined in terms of phytoplankton group (taxon, cell size) biomass.

[The high chlorophyll samples (Table 3-1) are identified by sample days in bold].

Group	Samples (Day no.)	Group (% contribution)	% of similarity
A	37, 44, 58, 65, 94	Flagellates >5µm (40), Flagellates <5µm (38), Diatoms (8)	86
B	72, 114, 129	Flagellates >5µm (29), Flagellates <5µm (25), *Eutreptiella* (22)	89
C	51, 79, 86, 100,107, 22, 134, 150, 156, 171, 91, **198**, 268, 275, 282, 90, 296, 302	Flagellates >5 µm (27), Flagellates <5µm (25), Dinoflagellates (11), Diatoms (11)	90
D	**220**, 226, 240, 247, 254, 262	Flagellates >5 µm (28), Flagellates <5 µm (24), Diatoms (20), Dinoflagellates (13)	89
E	**163**	Dinoflagellates	
F	**178**, **184**, **204**, **212**, **232**	Diatoms (22), Flagellates >5 µm (21), Flagellates <5 µm (17), Dinoflagellates (14), *Cryptomonas* (10.6), *Mesodinium rubrum* (10)	88

Accessory pigment analyses

Data for the main HPLC pigments (excluding the ubiquitous chlorophyll *a*) were normalised by square root transformation to calculate the Bray Curtis Similarity Index, and hierarchical clustering (not shown) used to define groups with similarities in the range of 79-88%. Each group had >3 samples, except group G with just one sample and groups A and C with two samples each. The group specifications are summarised in Table 3-6.

Table 3-6: Main characteristics of sample groups defined in terms of photosynthetic pigment composition.

[The high chlorophyll samples (Table 3-1) are identified by sample days in bold].

Group	Samples (Day no.)	Pigment (% contribution)	% of similarity
A	**163**, **212**	peridinin (43), chlb (24), fucoxanthin (19), alloxanthin (11)	80
B	**178**, **184**, **204**, **220**, **232**, 262	fucoxanthin (46), chlb (20), peridinin (17), alloxanthin (13)	78
C	72, 114	chlb (43), fucoxanthin (22), 19 hex (15), alloxanthin (13)	88
D	**198**, 226, 240, 247, 268	fucoxanthin (45), chlb (20), peridinin (18), alloxanthin (17)	81
E	107, 254, 296	fucoxanthin (61), chlb(20), alloxanthin (16)	82
F1	129,134, 142, 156, 171, 191, 275	chlb (39), fucoxanthin (29), peridinin (12), alloxanthin (11)	84
F2	79, 86, 100, 150, 282, 290, 302	fucoxanthin (37), chlb (23), peridinin (19), alloxanthin (16)	85
G	44	single sample	
H	37, 51, 58, 65, 94, 122	fucoxanthin (42), chlb (21), alloxanthin (18), peridinin (16)	79

Groups C, E, F2, G and H include almost all the early (Day 122 and before) and late (Day 282 and later) samples characterised by fuco and chlb in varying proportions. The source of the fuco in samples before Day 150 cannot readily be distinguished from the cell count data (Fig 3-22), and this anomaly may be indicative of under sampling of large diatoms in the relatively small volume (10 ml) water samples used for cell counts compared to the large volumes (500ml) used for HPLC analysis.

Groups B and D are very similar and cover the period of the *Skeletonema* bloom, with fuco as the main accessory pigment as well as generally high chlorophyll. Group F1 is similar to F2 but characterised by chlb rather than fuco and samples more restricted to the early summer period. Group A has perid as the dominant accessory pigment.

3-5-3 Relation of environmental parameters and biological parameters

Multivariate analyses based on environmental parameters and on phytoplankton characters have shown how samples are grouped in terms of similarity. Examination of the three phytoplankton characters (species/taxon biomass, taxon/cell size group biomass, accessory pigments) gave different groupings. Seasonal changes could be clearly distinguished on the basis of environmental parameters, but were less well defined by the phytoplankton data. To address more directly the question of how environmental conditions affected the phytoplankton, a further analysis was performed using the BIOENV routine of PRIMER to calculate the best matching rank correlation (ρ) between environmental parameters and phytoplankton species biomass, taxonomic group biomass and chlorophyll *a* size fraction.

The BIOENV analysis attributed best correlation values of 0.22, 0.37 and 0.1 for species biomass, for taxonomic group biomass and for chlorophyll *a* size fraction data, respectively as summarized in Table 3-7.

Table 3-7: BIOENV analyses, the correlation factors (best variables) for different biotic data

Group	Single variable (ρ) value	Best correlation ρ
Species carbon biomass	N (0.136), PAR (0.126) + Si (0.142)	Two variables (0.216) Three variables (0.208)
Taxon carbon biomass	Si (0.354), N (0.285)	Two variables (0.374)
chl size fraction	P (0.1)	One variable (0.1)

These results indicate that seasonal changes in total phytoplankton biomass are affected by both light and nutrient availability, whereas changes in taxonomic

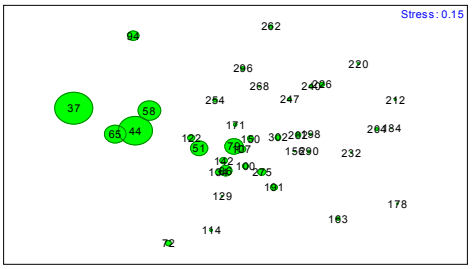
biomass are best explained by varying nutrient levels, in particular of silicate. By contrast changes in the size fractions of chlorophyll were correlated, albeit weakly, with varying phosphate concentrations, and suggest that low phosphate in summer may restrict the growth of larger cells (i.e. diatoms and dinoflagellates)

The MDS plot of phytoplankton group (taxon, cell size) biomass was superimposed with the best correlated environmental parameters (Si, N, PAR and P) to show how different parameters influence biomass distribution (Figure 3-29). The patterns of seasonality with Si (early spring), N and P (late spring and early summer) and PAR (mid summer) are clearly shown. The differences between the N and P plots are slight and appear to relate specifically to changes in cell size distribution (Table 3-7).

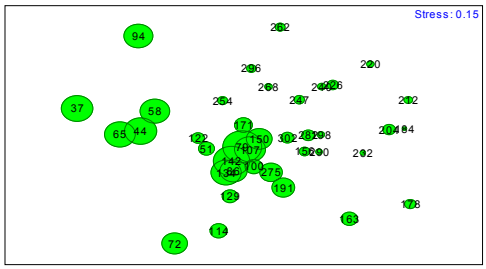
Figure 3-29: Phytoplankton taxon carbon biomass groups MDS plot, superimposed with bubble values of (A) Si, (B) N, (C) PAR, and (D) P.

[The bubble size indicates the value of particular variable from maximum large, to minimum small bubbles]

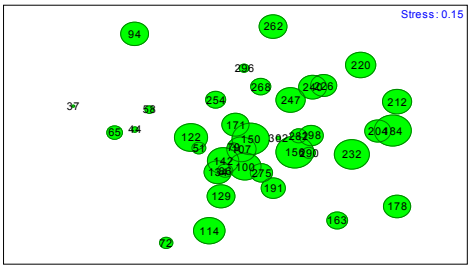
(A)



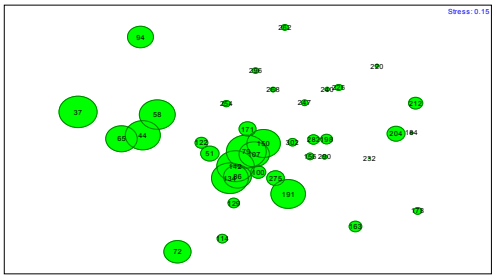
(B)



(C)



(D)



3-6 Discussion and conclusions

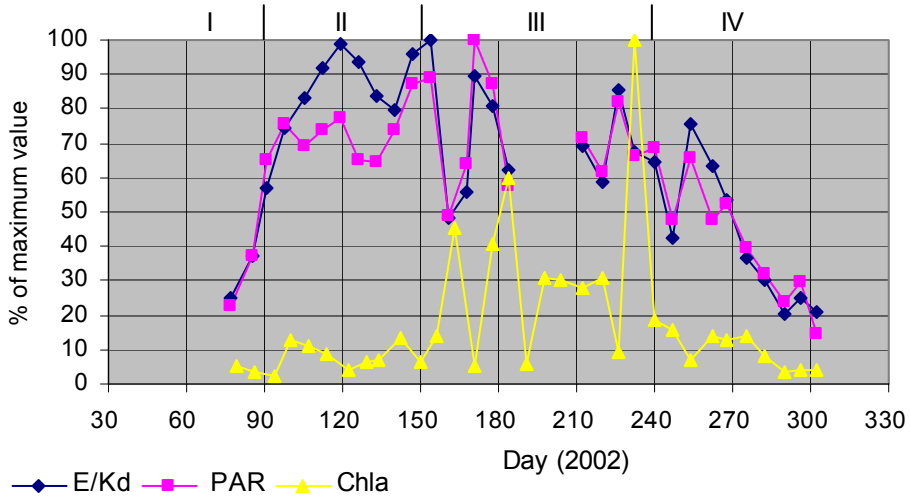
In this chapter, the distribution of biological parameters (phytoplankton pigments, species abundance, and estimated species/taxon biomass), physical parameters (irradiance, temperature, tides and salinity) and chemical parameters (inorganic nutrients N, P, Si) at the Empress Dock station in 2002 were described. Multivariate analysis was used to establish relationships between biological and environmental parameters.

The seasonal distribution of phytoplankton at the Empress Dock is similar to that in other temperate estuaries. The biomass of chl is relatively low in autumn and winter (when irradiance is low) and high in spring and summer. Earlier studies have also shown that chl increases as irradiance increases (Iriarte and Purdie, 2004). Likewise, photosynthesis also increases proportionally to light intensity, up to some maximal value (Lalli and Timothy, 1997). This is because primary production can be described as a linear function of biomass, usually expressed as chl, with some index of light availability (Cole and Cloern, 1987).

Figure 3-30 shows the distribution (scaled as % of maximum values) of chl, PAR and E/K_d, the latter representing a relative measure of water column irradiance. Note that fluctuations in PAR and E/K_d are similar, but there is not always a 1:1 correspondence between these two parameters.

The distributions of PAR and chl can be divided into 4 phases. In phases I and IV, before Day 90 and after Day 240 respectively, both chl and PAR were low. Phase II (Days 90-150) was characterised by high irradiance and persistently low chlorophyll. Finally, in phase III (Days 150-240), both Chl and PAR were generally high, although low chl values were observed between each of the chl peaks (events). The first part of phase IV (Days 240-270), when PAR was still quite high, is comparable to phase-II and, in both periods of time, chlorophyll values were somewhat higher than the lowest values recorded in the winter.

Figure 3-30: Comparison of relative changes in weekly mean PAR, and in E/K_d and chl



The low chl levels in phase I are attributed to low winter PAR and E/K_d in addition to low temperature. The river input of freshwater showed a sharp rise in the last 60 days of this phase. This could result in higher turbidity, which in terms of light availability to phytoplankton, augments the effect of low winter PAR. The dominant group of phytoplankton during this phase was flagellates, a proportion of which were heterotrophic. Therefore, their contribution to total chl is not as significant as that of other exclusively photosynthetic groups and may account for the low winter chl values which have characterised this study.

Phase 1 is also characterised by a depletion of Si. Changes in the pigment composition of the population, in particular as indicated by the fucoxanthin-to-chlorophyll ratio, suggest an increase in diatom abundance. However, the cell counts did not indicate any significant change in diatom density. A possible explanation is the growth of large diatoms at densities below the limit detectable by the procedure for microscopic counts (based on 10ml samples) but relatively well sampled for pigment analysis (500 ml samples). At this time of the year, *Coscinodiscus* and *Odontella* spp. are present at cell densities in the order of 0.1 cell ml^{-1}. Also silicate uptake by diatoms is known to be largely independent of irradiance (Martin-Jezequel *et al.*, 2000; Claquin *et al.*, 2002). Another possibility is that diatom numbers remained low due to predation, while ingested diatom pigments inside predators

could still appear in pigment extracts. In this scenario, Si could be temporarily lost through incorporation into fecal pellets that sink to the bottom.

In phase II, chl levels remained mostly below 10%, relative to the annual maximum, up until Day 150 (end of May). Such low chl levels in relation to the increase in PAR is rather surprising. Both the HPLC pigment data and microscopic counts indicated a difference in the taxonomic composition of the phytoplankton population compared with phase I, with numbers of the euglenoid *Eutreptiella* reaching a maximum for the year. Despite the small increase of biomass in relation to the increase in PAR, all nutrients decreased significantly before Day 120: N and P to about 33% of maximum values, and Si to below 10% (Figures 3-7 and 3-31). A possible explanation is that strong spring tides (tidal range > 4 m) diluted and later flushed out the nutrients, preventing any significant accumulation of phytoplankton apart from flagellates. If flushing were an important controlling factor, similar hydrographic and biological conditions would be expected in the open estuary. Between Days 120 and 150 nutrient levels decreased, then increased to a relatively high level, due to increased river flow.

Phase III is characterised by rapid changes in phytoplankton biomass. Four periods of high phytoplankton biomass were identified, each characterised by different combinations of diatom and dinoflagellate species and separated by sampling days, with chl concentrations as low or lower than those observed in phase II. The main difference in environmental conditions was the reduced tidal range in summer. However, no precise relationship could be established between changes in chl and the tidal range. For example, peaks in chlorophyll tended to occur some days before spring tides. Spring tides were associated with higher K_d, indicating increasing turbidity when the water column is more mixed and homogeneous. A stable water column appears to induce elevated chlorophyll levels.

Thus, a picture emerges in which it appears that tides play an important role in controlling phytoplankton biomass throughout the year. However, in mid- to late summer a combination of high PAR and reduced mixing/flushing allows blooms to

develop with a periodicity of 10-20 days. An alternative explanation for rapid changes in phytoplankton biomass is mortality brought on by predation (grazing) and/or pathogens. A system in which predators are the main controlling factor on phytoplankton abundance would not necessarily show any relationship to tidal strength.

In phase IV, conditions started to revert to those of phase I. There are decreases in PAR and chl levels, with dominance by flagellates, while nutrient concentrations and tidal range increase. So, possibilities described in phase I probably all apply to this phase.

Parallel changes in inorganic nutrients are shown in Figure 3-31. During phases I and II, Si fell to <20% of the maximum observed value on Day 72 and approached the minimum values for the year by Day 156. By contrast, N (and P) remained high during phase I, and declined in phase II but with large fluctuations, which could be attributed in part to changes in salinity (Figure 3-4). The concentration of N fell again during the first part of phase III when the first phytoplankton blooms were observed, and reached a consistently low value (~20% of maximum) by about Day 184. Changes in P were similar, although the minimum value of ~10% on Day 232 was lower. Finally, during phase IV, when chlorophyll levels had fallen, a slight increase in concentrations of all nutrients occurred, especially after Day 270, probably as a result of regeneration exceeding net uptake by phytoplankton.

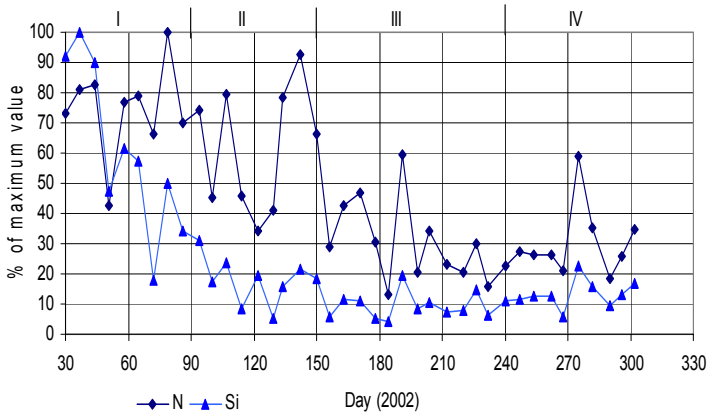


Figure 3-31: Comparison of relative changes in N and Si concentrations.

[Note that the distribution of P showed a pattern similar to that of N, See Figure 3-7.]

The changes in nutrient and chlorophyll levels are summarised in Table 3-8, along with information on tidal ranges and dominant phytoplankton types. Additional information is provided by the results of the multivariate analysis of the whole data set which suggest that PAR is the major environmental factor determining seasonal changes in phytoplankton biomass (chl), and that the seasonal decreases in inorganic nutrients are linked to taxonomic changes in the phytoplankton populations; e.g. Diatoms and SI. Finally, there is some evidence that low phosphate concentrations in summer favour the survival of small (<5um diameter) cells.

Table 3-8: Four phases of phytoplankton distribution at Dock 2002

Samples Period	Relative chl %	Relative PAR %	Nutrients	Spring tidal range (m)	Phytoplankton type
Phase I (before D 90)	<30	<50	Nutrients high, Si decreasing	>4	Flagellates
Phase II (D 90 -150)	<30	>50	All nutrients decreasing	>4	Flagellates Eutreptiella
Phase III (D 150-240)	>30	>50	Nutrients at lowest levels	<4	Dino /Diatom blooms
Phase IV (after D 240)	<30	<50	Nutrients begin to increase	>4	Flagellates

Chapter 4- ESTUARINE OBSERVATIONS IN 2002

Monthly sampling was carried out at three estuarine stations, NW Netley (NWN), Calshot and Horse Elbow (HE) (Figure 2-1), at high tide, between March and November 2002. The observations of physical parameters, inorganic nutrients, Tchl, Fchl, and phytoplankton pigments are presented in this chapter. The monthly contemporary data for Empress Dock, which represent a subset of the observations described in Chapter 3, are shown for comparison.

4-1 Physical parameters

Surface water salinity values are shown in Figure 4-1 A. The surface salinity ranged between 27.5 at Empress Dock in April and 34.6 at HE in September. Salinity generally increased from winter to summer. Dock showed the highest variability of salinity, from 27.5 to 32.2, while HE had the narrowest range, from 33.8 to 34.6. Values for NWN were generally similar to those at Dock, while Calshot values were intermediate between those of NWN and HE.

Salinity at 4m depth is shown in Figure 4-1B. It ranged from ~30 at Dock to ~34 at HE. Sub-surface salinity reached a maximum in summer at the mid–estuarine station of NWN and in autumn at Dock and HE.

Surface water temperatures at the different locations in the estuary, shown in Figure 4-2, followed the normal seasonal pattern. The maximum temperature was in August (Day 232) at Dock (20.6^{o}C) and the minimum temperature was in March (Day 86) at HE (9.4^{o}C). The temperatures at all stations were similar, apart from September (Day 262), when the Dock and HE temperatures (~ 17^{o}C) at either end of the estuary were about 3^{o}C lower than the Calshot and NWN temperatures in the mid estuary. The reason for this difference is not known.

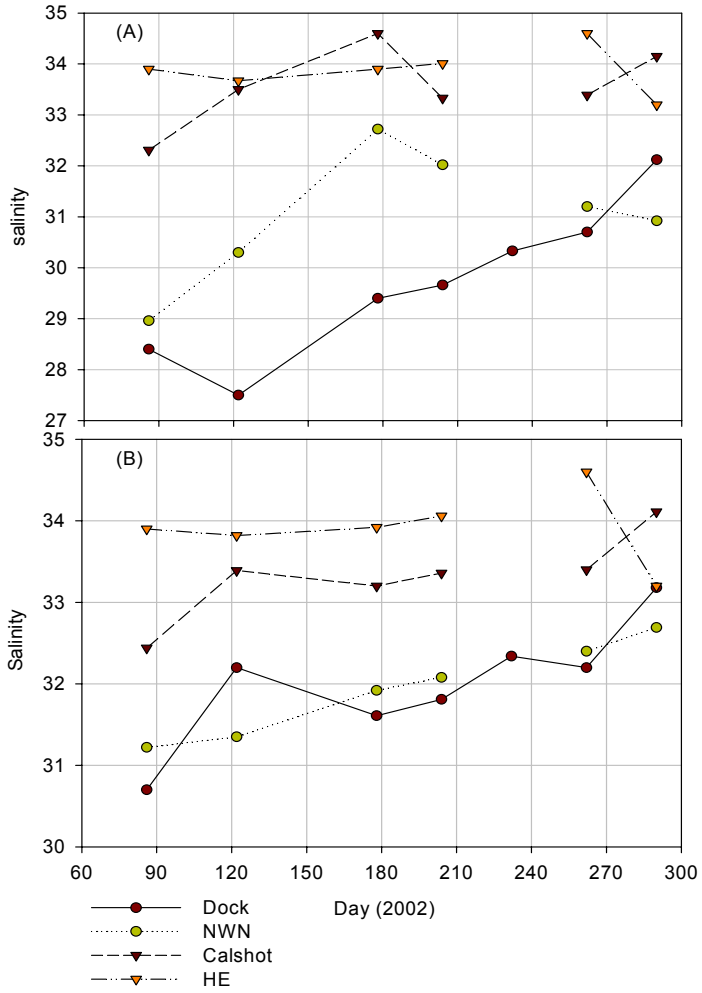


Figure 4-1: Water salinity at the four estuarine stations, 2002.

(A) The surface and (B) 4m depth. Note the difference in the salinity scale for A and B.

[Data are missing for May at all stations and for August at NWN, Calshot and HE].

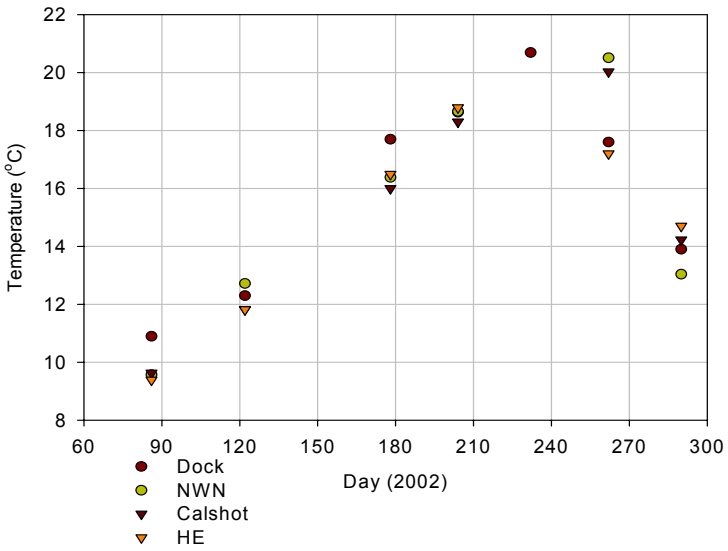
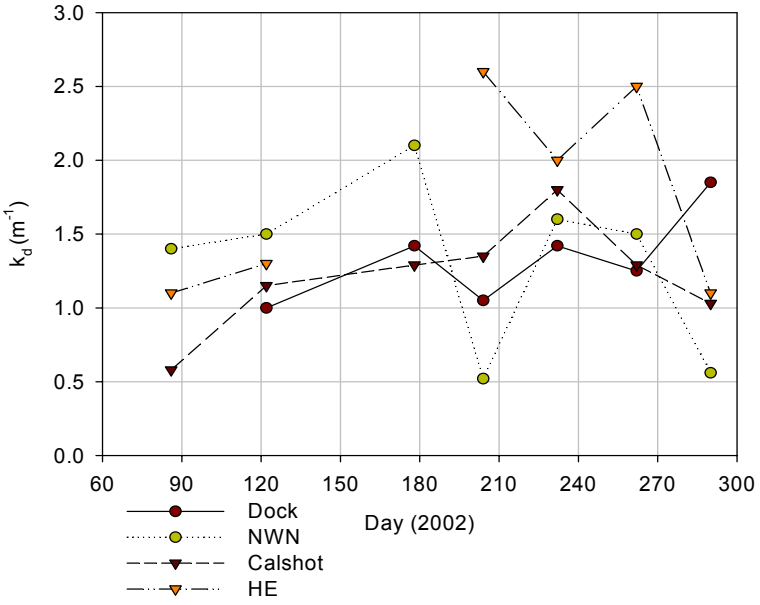


Figure 4-2: Surface water temperature, at the four estuarine stations, 2002.

[Data are missing for May at all stations, and for August at NWN, Calshot and HE. The 4m depth distributions (not shown) were similar to the surface distributions]

The light attenuation, K_d, values are shown in Figure 4-3. They ranged between 0.5 m^{-1}, the minimum value at NWN Station, and 2.7 m^{-1} the maximum at HE station.

Figure 4-3: The light attenuation coefficient (K_d), at the four stations, 2002.
[Data are missing for March at Dock, May at all stations, and for June at HE]



4-2 Chemical parameters

4-2-1 Inorganic nutrients

In general, the distribution of inorganic nutrients showed a consistent seasonal pattern (Figure 4-4); concentrations decreased from high values in spring to low ones in summer, and increased again in the autumn. The Dock and NWN (inner) stations had the highest concentrations of inorganic nutrients and HE the lowest. The lowest concentrations were in July for the outer (Calshot and HE) stations and in August for the inner stations.

Inorganic nutrients at the inner stations, Dock and NWN, showed a similar pattern, but at Dock fluctuated more than at NWN. This difference may relate to an increase in riverine input as indicated by lower surface salinity at Dock (see Figure 3-2).

N and P values at Dock station were higher and decreased gradually, P then showed significant depletion to reach the lowest value among all stations. The Si

concentrations at the outer stations were relatively low, perhaps because of significant depletion before the start of the sampling period.

The nitrate to phosphate, silicate to phosphate and nitrate to silicate ratios are shown in Figure 4-5. The N:P and Si:P ratios for all stations tended to increase gradually from winter to summer, and fluctuated most widely between Days 180 and 240, but nitrate to silicate ratio had the reverse pattern. The highest values of the nitrate to phosphate and the silicate to phosphate ratios were observed at Dock on Day 232, perhaps due to depletion of P, through phytoplankton uptake as suggested in Chapter 3 for Dock. A similar pattern emerged at the other stations, but HE had consistently lower ratios due to lower values of N and Si, and small relative increase in P in summer. The nitrate to silicate ratios were high at the outer stations early in the year, and tended to decrease toward summer, perhaps related to lower Si value in the early samples.

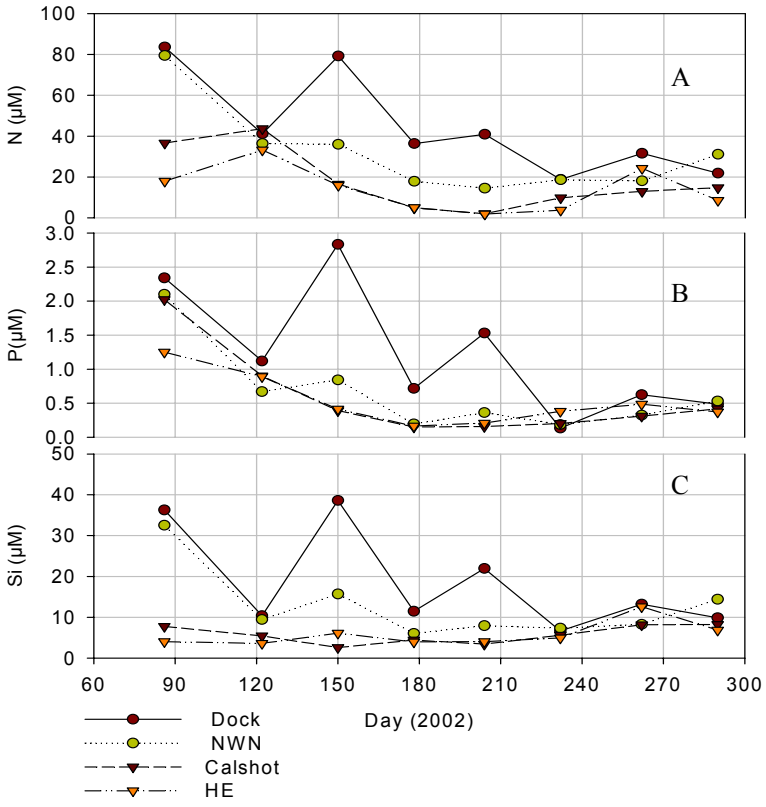


Figure 4-4: Surface nutrient distributions at the four estuarine stations, 2002

(A) N, (B) P, (C) Si

Nutrient ratios can indicate which particular nutrient might affect phytoplankton growth and succession. The nutrient ratios in the water column can be compared with the Redfield ratio for N: P of 16: 1 also for Si:P of 16:1. The high N/P and Si/P ratios observed by the end of the summer (Day 232) indicate that relatively low P may be the reason for high ratio at the inner stations, while relatively high N and Si could be the reason for low ratios at the outer stations. The N/Si ratio was > 1 at the inner stations and < 1 at the outer stations on the same day, perhaps due to higher levels of N at the inner station than the outer stations.

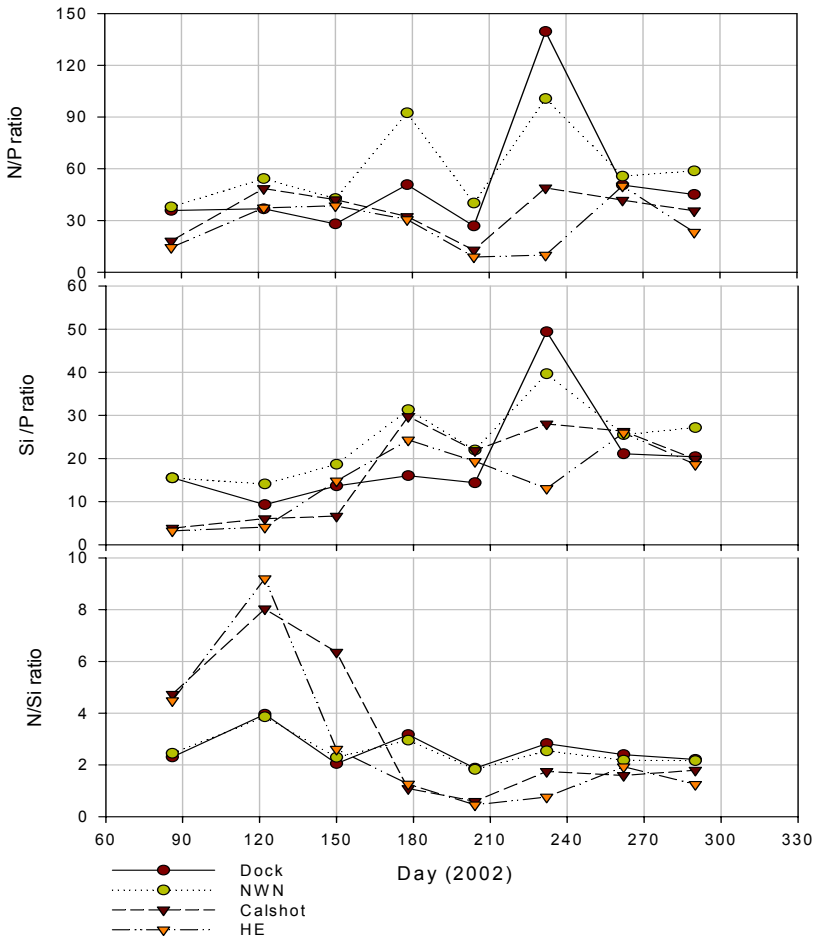


Figure 4-5: Changes in the N/P, Si/P and N/Si ratios distributions at the four estuarine stations, 2002.

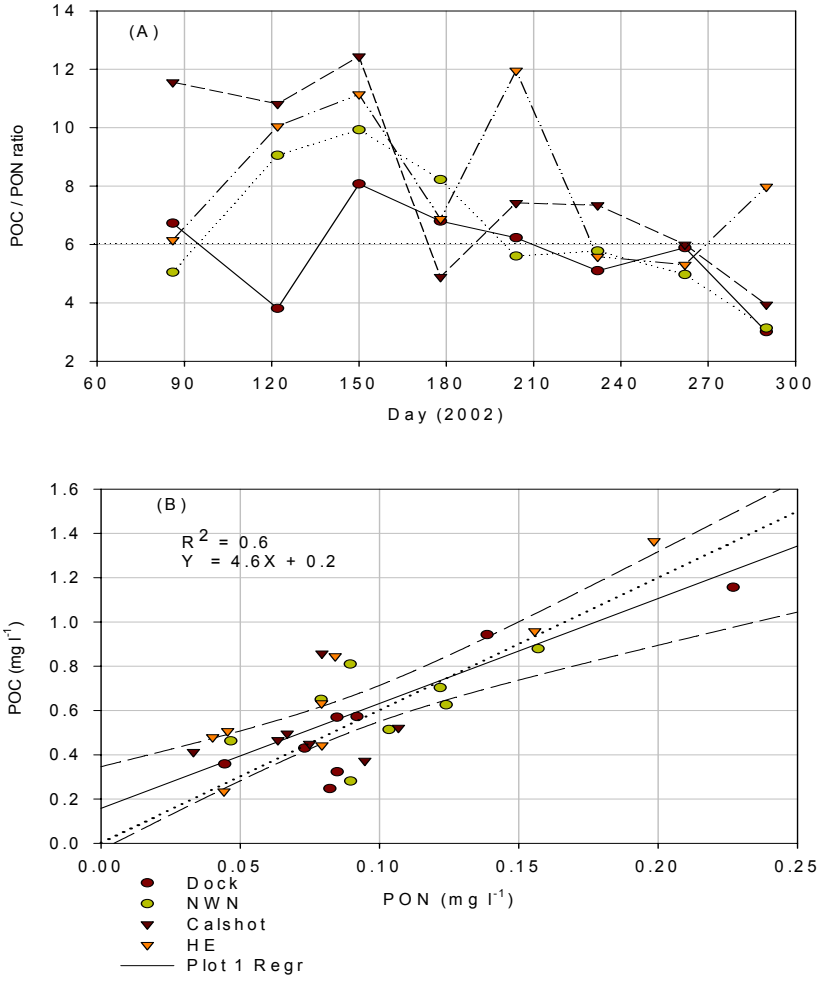
4-2-2 Particulate organic carbon and nitrogen distributions

Changes in the particulate organic carbon/nitrogen ratios (POC/N) for the four estuarine stations and the correlation between POC and PON are shown in Figure 4-6. The POC/N ratios were very variable, but they generally decreased during the

summer to be close to or below the Redfield ratio (6 by weight). Note that early water samples of Dock have POC/N values >15 (see chapter 3)

Figure 4-6: (A) Distributions of the particulate organic carbon/nitrogen (POC/N) ratio, and (B) the regression of POC to PON at the four estuarine stations,(2002).

[The dotted line in plot A and B is the Redfield ratio, the short dashed lines in plot B are the confidence interval 99%]



Regression of POC *vs* PON, gives a slope of 4.6, which is lower than Redfield ratio for C: N. This is probably due to concentration of carbon-rich, non-living material (detrital carbon), especially in the early samples, and nitrogen-rich samples with

abundant heterotrophic organisms (e.g. bacteria, zooplankton) which tend to conserve nitrogen.

4-3 Phytoplankton pigments

4-3-1 Total chlorophyll a and chlorophyll a size fractions

Total chlorophyll *a* (Tchl) distributions at the four stations are shown in Figure 4-7. They showed a similar pattern, with maximum values (up to 12mg m^{-3}) in June (Day178) for the open estuary and minimum values (<2 mg m^{-3}) during early spring and autumn. The Dock data were a subset of the weekly data set shown in the inset of Figure 4-7. Any rapid change in chlorophyll in the open estuary, as observed at dock, would not have been resolved by the monthly sampling.

However, there does appear to be a general pattern of relatively high (>5mg m^{-3}) chl between Days 160 and 240, with values greatest at NWN and least at HE corresponding to the gradient of decreasing nutrient levels (Figure 4-4) down the estuary.

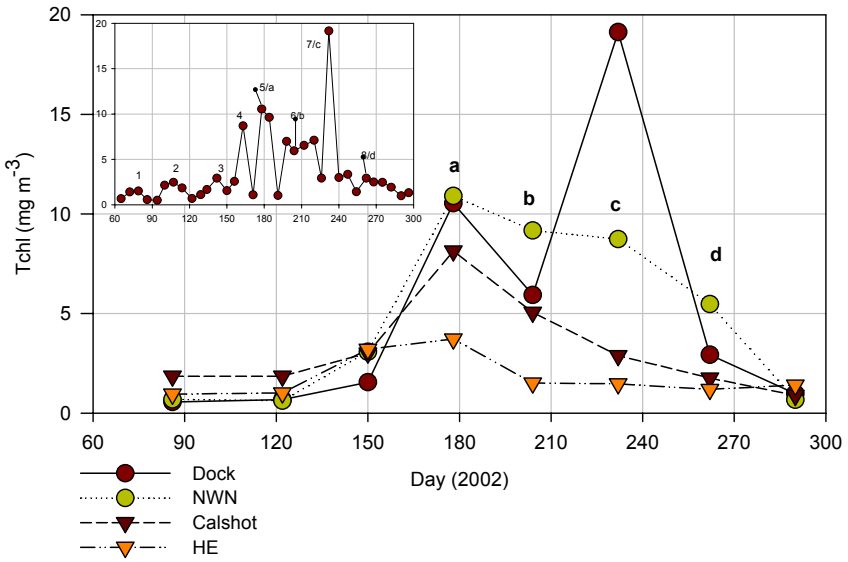


Figure 4-7: Seasonal distribution of surface Tchl at the four estuarine stations, 2002.

[The inset graph shows the weekly Dock observations. The letters a-d indicates the relation between chlorophyll "events" described for Dock (chapter 3).]

The distributions of the chlorophyll size fractions at the four stations are shown in Figure 4-8. A good agreement between Tchl and chlorophyll size fractions is shown.

The large cells were important when chl is high, especially Fchl >20µm, at all stations. Size fractionated data high and low chl samples (>2 and < 2 mg m^{-3}) are compared in Table 4-1. There are consistent shifts to larger cells when chl is high; the maximum value was at Calshot.

Figure 4-8: Seasonal distribution of chlorophyll size fractions at the four estuarine stations, 2002.

[There is a good correlation between total chl and the summation of Fchl, with R^2 0.99 and slope of 1.0]

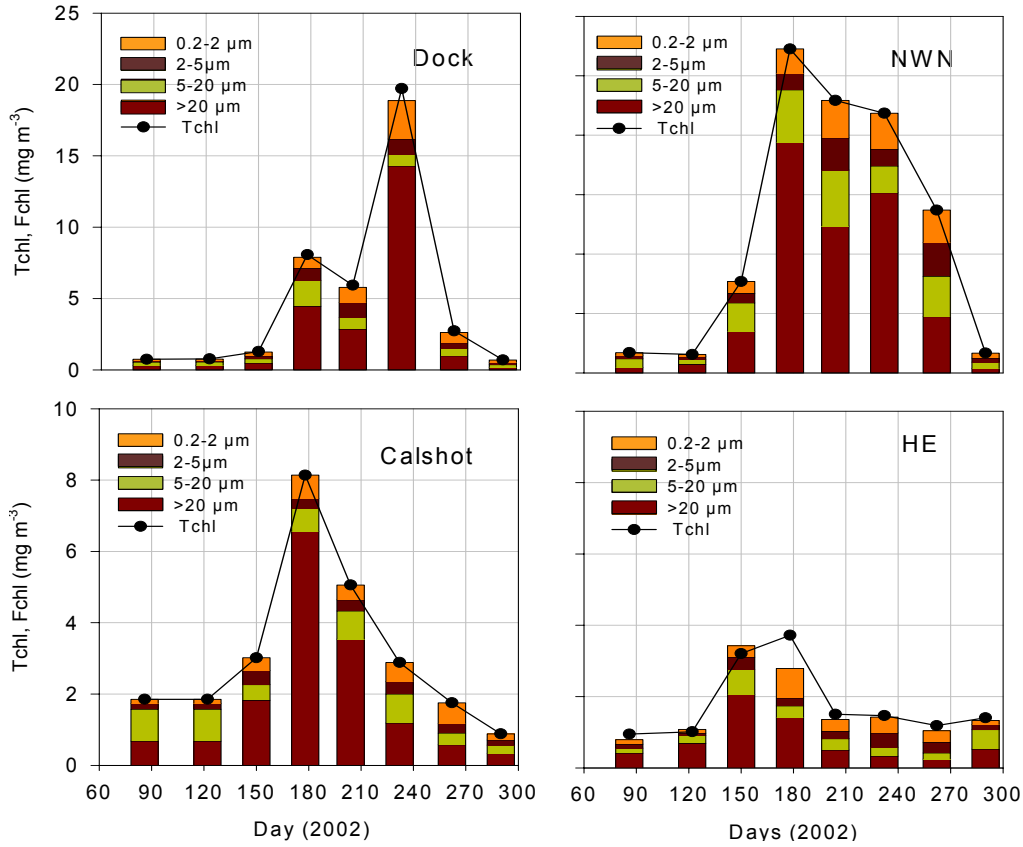


Table 4-1: The mean percentage of different chlorophyll fractions at high and low (>2< mg m^{-3}) chlorophyll values, at the four stations 2002.

Stations	High chl samples as mean Fchl%				Low chl samples as mean Fchl%			
	>20 µm	5-20 µm	2-5 µm	<2 µm	>20 µm	5-20 µm	2-5 µm	<2 µm
Dock	53	15	12	20	36	29	16	19
NWN	55	21	11	14	32	34	17	17
Calshot	63	17	9	12	38	38	13	16
HE	55	16	9	19	40	23	17	20
Mean	57	17	10	16	36	31	16	18

The general pattern in the distribution of small (<5µm diameter) phytoplankton cells is summarized in Figure 4-9 and Table 4-2. The relative importance of small cells tended to increase after mid summer, reaching a maximum percentage of 60% at HE in late September. The mean Fchl <5 µm percentages ranged between 25% and 35% of total chl, and were highest in the upper estuary at Dock and HE stations, where the absolute abundances of small cells was greatest (Figure 4-8)

Figure 4-9: Seasonal distribution of chlorophyll fraction <5 µm, percentages at the four estuarine stations, 2002.

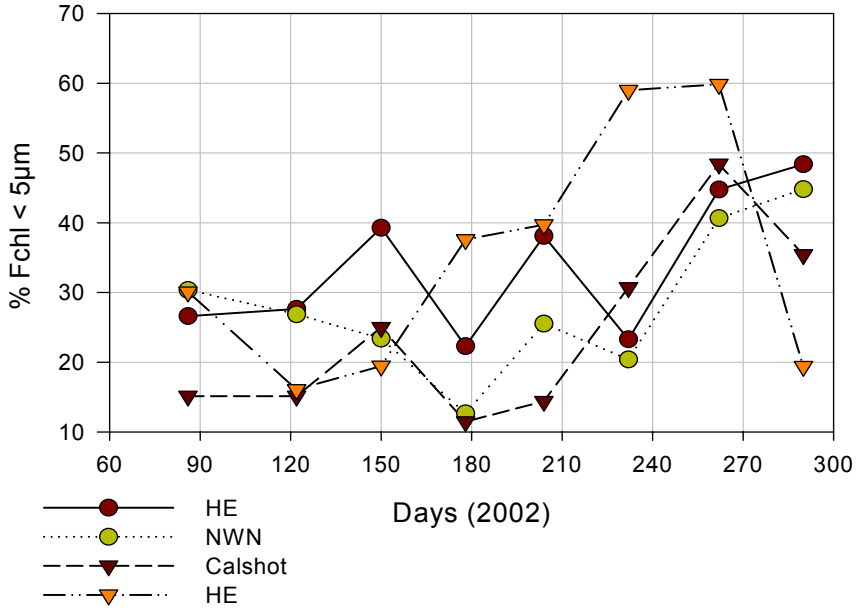


Table 4-2: Summary of chlorophyll size fractions (< 5 µm) mean percentages and range, during the sampling period at the four estuarine stations, 2002.

Station	%<5 µm	
	Mean	Range
Dock	34	22-48
NWN	28	13-45
Calshot	25	12-48
HE	35	16-60

4-3-2 Phytoplankton accessory pigments

Values of chl measured by fluorescence were higher than values of chl measured by HPLC, although there was a good correlation between the two measurements (Figure 4-10) with an R^2 value of 0.91 and a slope of 1.4, similar to that found at Dock station (Figure 3-15). Based on the HPLC data alone, chlorophyll *a* was highly correlated to both total pigment (Tpig) and total accessory pigment (Tac), giving R^2 values of 0.98 and 0.92 and slopes of 0.5 and 1.1 respectively (Figure 4-11), again similar to those observed at Dock station (Section 3-3-2).

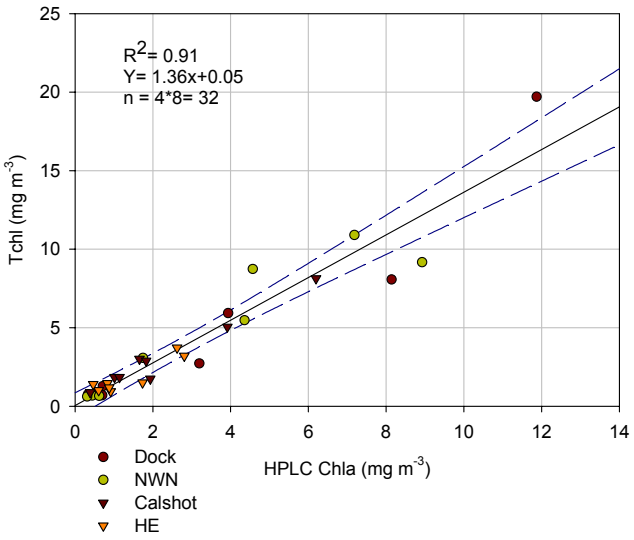
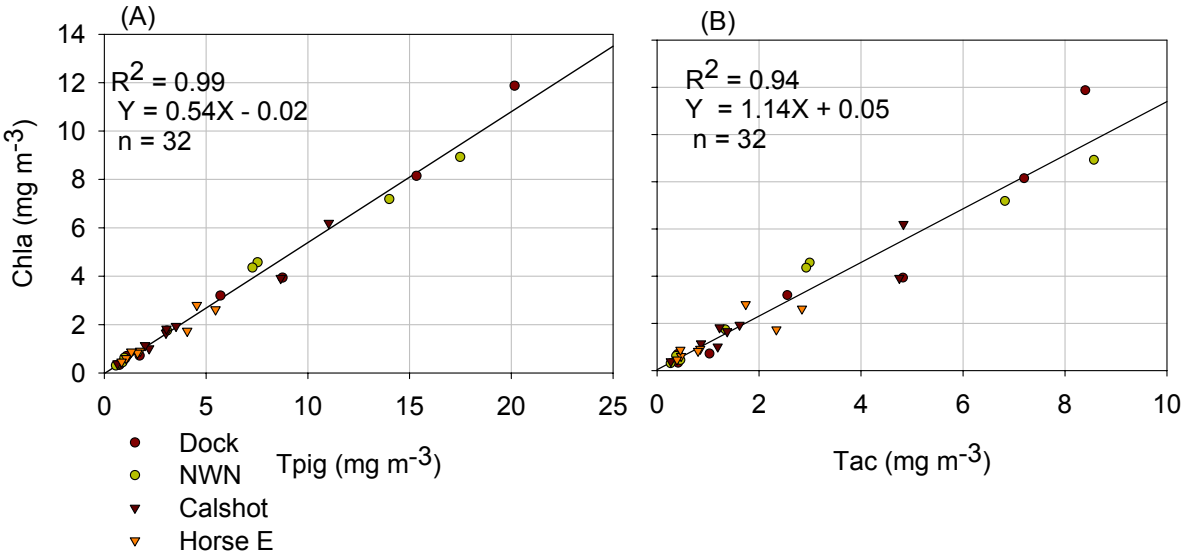


Figure 4-10: Comparison of chla measured by HPLC and fluorometer at the four estuarine stations, 2002.

[The dashed line indicates the confidence interval (99%)]

Figure 4-11: Relationships of chlorophyll a *to (a) total pigment (Tpig) and (b) total accessory pigments (Tac) at the four estuarine stations, 2002.*



The major phytoplankton pigments at the estuarine stations are compared in Figure 4-12, and variations in the pigment to chla ratios are summarised in Figure 4-13.

Figure 4-12: Temporal and spatial distributions of chla and accessory pigments at the four estuarine stations, 2002.

[The letters a-d indicates the samples with relative high chlorophyll (see Figure 4-7). The numbers indicate peak values in particular accessory pigment]

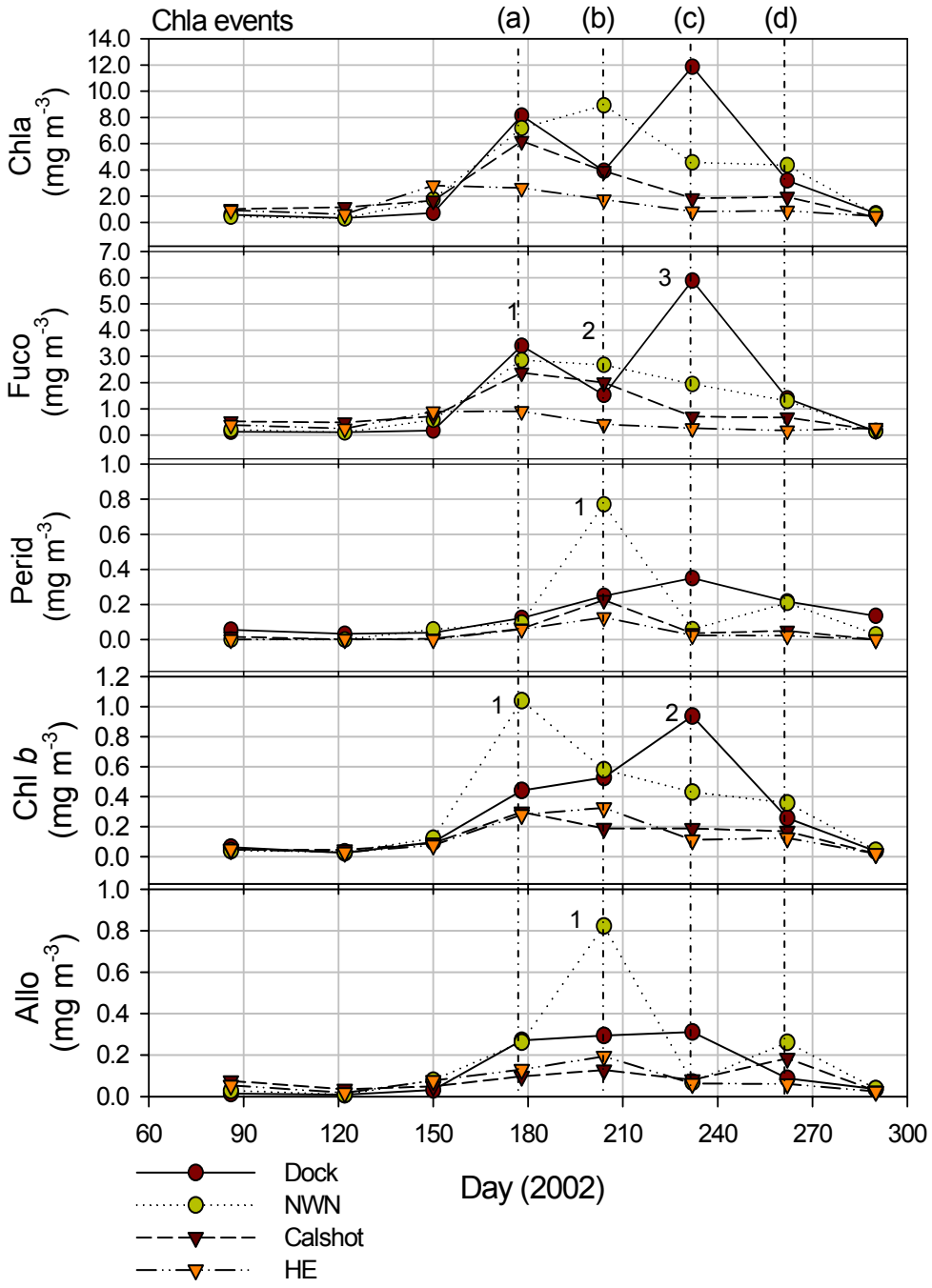
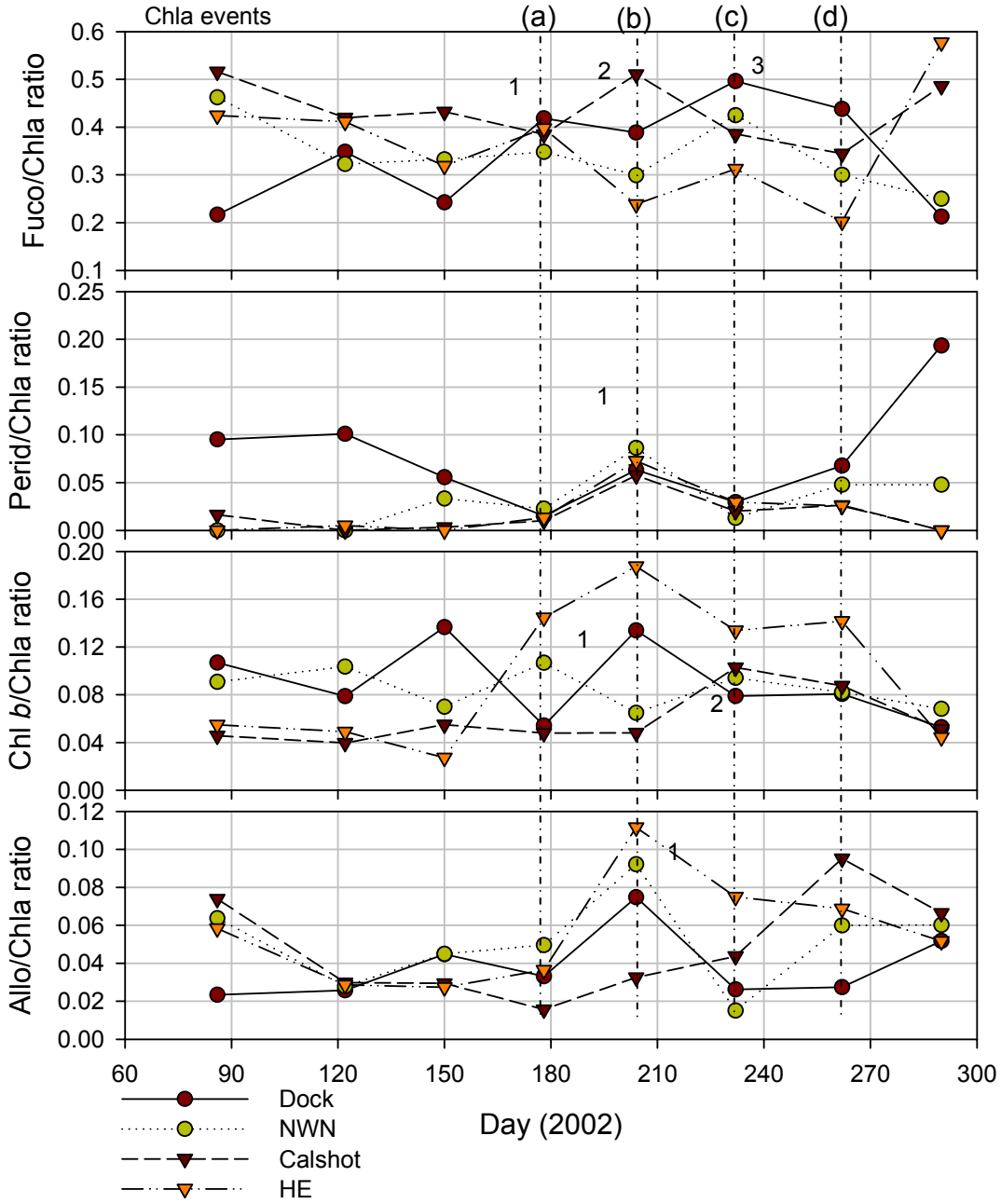


Figure 4-13: Temporal and spatial distributions of the accessory pigments to chla ratio at the four estuarine stations, 2002.

[The letters a-d indicate the samples with high chlorophyll (see Figure 4-7).The numbers indicate peak values in particular accessory pigment ratios relative to absolute value peaks]



Accessory pigments were higher when chla was also high. Fuco (diatoms) shows the highest peak at Dock; its distribution was similar to chla at all stations, suggesting that diatoms were consistently important through out the year. Distributions of other accessory pigments were low, suggesting that these components of the phytoplankton population were quite variable.

Variability of pigment ratios indicated similar variability in terms of absolute values. Some notable features were observed: the fuco ratios were the major and highest ratio during high chl values, but with low values at HE during summer, and high values at Calshot on Day 204. The perid/chl ratio was maximum at all stations in mid summer. The chlb/chl ratio had the highest ratio at HE in the summer; similarly the allo/chl ratio was high at HE. NWN also had a high allo ratio in summer. The pigment ratios showed different patterns at HE from other stations, apart from the perid ratio.

4-4 Phytoplankton taxonomic data

4-4-1 Phytoplankton cell abundance and carbon biomass.

The phytoplankton groups are shown in Figure 4-14 as percentage values for cell counts during the sampling period. The most numerous group was the <5µm flagellates, contributing between 63% at Dock and 81% at HE, of the total phytoplankton population, followed by >5µm flagellates contributing ~20 %, while the other groups together represented the remaining. Dock station had the highest diatom contribution.

Phytoplankton cell numbers were converted to carbon biomass and percentage distribution calculated for the major phytoplankton groups (Figure 4-15). The large flagellates (>5µm) gave the highest values, with percentages ranging from 35% at Dock to 41% at HE. Diatoms were important at all stations, with percentages between 31% at NWN to 39% at Calshot station. Other phytoplankton groups (dinoflagellates, euglenoids and *Cryptomonas* and *Mesodinium rubrum*) were relatively unimportant.

Figure 4-14: Cell count percentages for the main phytoplankton groups at the four estuarine stations, 2002.

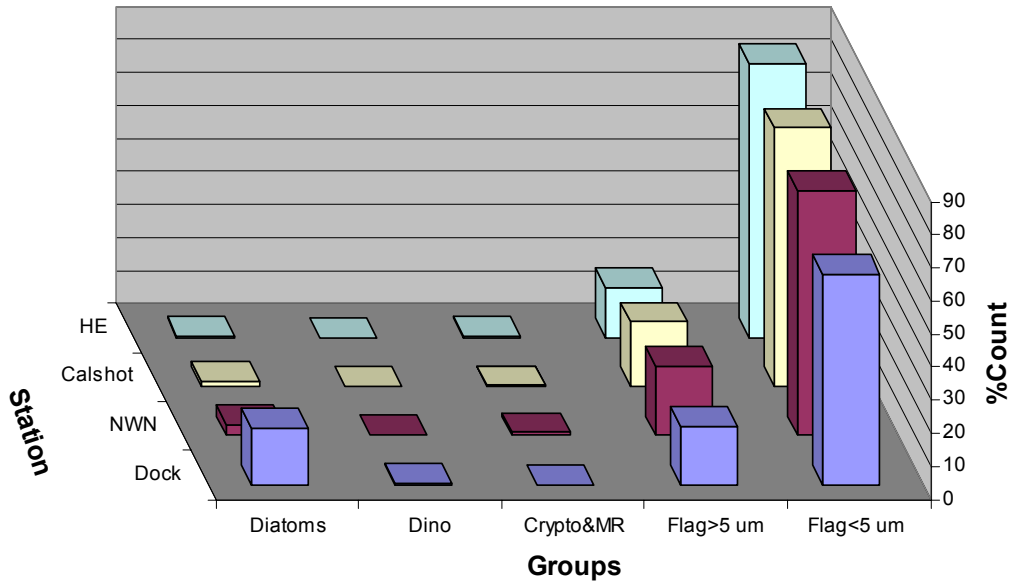
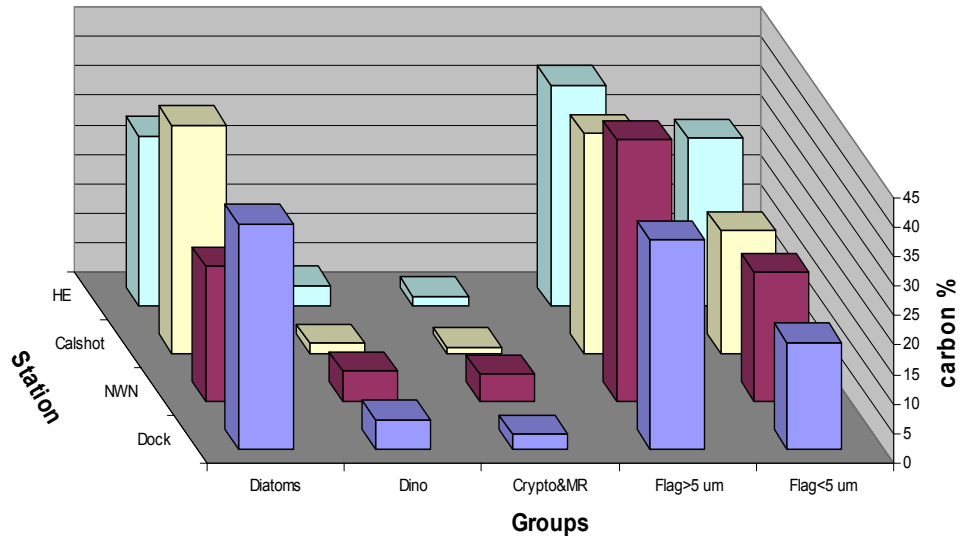


Figure 4-15: Biomass percentages for the main phytoplankton groups at the four estuarine stations, 2002.



The correlation between chl and carbon biomass at the estuarine stations is shown in Figure 4-16. The biomass was corrected by removing 50% of the flagellate biomass,

assuming that 50% were heterotrophic. The plot equation was (Y=14.2X +27.8) and R^2 value was 0.79. The carbon to chl ratio was ~14, which is lower than the value observed in the literature and comparable to observed at Dock Section 3-4. The C/ chl ratio for the high chl samples is similar (Figure 4-16 B).

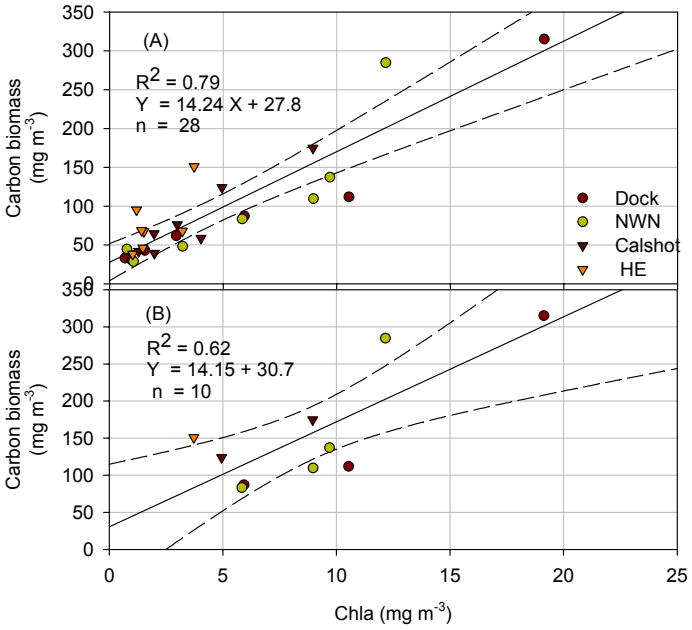


Figure 4-16: Correlation between phytoplankton biomass and chlorophyll for the four estuarine stations, 2002.

[(A) Assuming 50% of the flagellates were heterotrophic therefore subtracted from carbon biomass, slope when regression is forced through zero is 17.5 and (B) only the high chl samples, slope when regression is forced through zero is 16.9. The dashed line indicates the confidence interval (99%)].

4-4-2 Phytoplankton species composition

The phytoplankton species cell counts and biomass were expressed as mean percentages, as shown in Tables 4-3 and 4-4. Cell counts for the Dock samples were presented in Table 3-5 in more details.

In terms of species abundance, the stations are quite similar. Flagellates contributed higher percentages than other groups. In addition to that diatoms were most abundant at Dock and NWN, due to the high contribution of *Skeletonema costatum.* Dinoflagellates were less numerous at the outer stations, but *Cryptomonas* sp was common at all stations. The highest counts for *Mesodinium rubrum* were at NWN.

The phytoplankton biomass percentages give a different view of species contributions, particularly for diatoms, which represent a mean of ~30% of total biomass. A noteworthy species was *Cerataulina pelagica*, present at all stations, but with high contributions at NWN and Calshot. The highest biomass percentage for

Guinardia delicatula was at HE and Calshot stations. *Odentella* sp was found only at Calshot station. *Skeletonema costatum* contributed the highest percentages at Dock and NWN, the two inner stations.

Scrippsiella sp was the most abundant dinoflagellate species at all stations. The ciliate *Mesodinium rubrum* was significantly present at NWN station, while *Eutreptiella marina* appeared at Dock and NWN only.

Table 4-3: Mean phytoplankton cell count percentages at the four estuarine stations, 2002.

[Highlighted numbers are >1% of the total abundance at each station]

Species	Dock	NWN	Calshot	HE
Diatoms				
Asterionella glacialis	0.05	0.03	0.03	0.02
Asterionella kariana		0.01	0.02	
Bacillaria paxillifera			0.02	
Cerataulina pelagica	0.04	0.14	0.11	0.03
Chaetoceros sp	0.18	0.11	0.32	0.08
Coscinodiscus	0.01		0.01	
Cylindrotheca closterium	0.13	0.08	0.08	0.01
Guinardia delicatula	0.02		0.04	0.05
Lithodesmium	0.01	0.01		
Navicula	0.01	0.01	0.08	
Pennate	0.04	0.04	0.08	0.06
Pleurosigma	0.01			0.01
Pseudo-nitzschia			0.02	
Rhizosolenia setigera		0.02	0.02	0.02
Skeletonema costatum	**16.70**	**2.43**	0.60	0.15
Thalassionema frauenfeldii	0.05	0.01		0.01
Thalassionema nitzschioides			0.02	
Thalassiosira hyalina	0.05	0.14	0.08	0.04
Total diatoms	17.3	3.4	1.5	0.5
Dinoflagellates				
Gymnodinium sp1	0.01			0.01
Gymnodinium sp2	0.21	0.34	0.05	0.06
Prorocentrum micans	0.01		0.01	0.01
Scrippsiella trochoidea	0.04	0.08	0.02	0.03
Total dinoflagellates	0.28	0.31	0.07	0.17
Other groups				
Mesodinium rubrum	0.05	0.07	0.03	0.03
Cryptomonas	0.97	0.70	0.36	0.45
Eutreptiella sp	0.06	0.04	0.02	0.01
Flagellates<5	**63.71**	**76.16**	**78.31**	**81.32**
Flagellates>5	**17.64**	**19.58**	**19.67**	**17.17**

Table 4-4: Mean phytoplankton biomass percentages at four estuarine stations, 2002.

[Species contributing >1% of the total biomass were included]

Species	Dock	NWN	Calshot	HE
Diatoms				
Asterionella glacialis	1	1		
Cerataulina pelagica	6	17	13	4
Chaetoceros sp	2	1	4	1
Coscinodiscus	2		2	
Guinardia delicatula	5	1	8	14
Odontella mobiliensis			3	
Rhizosolenia setigera	2	4	3	5
Skeletonema costatum	18	3	1	
Thalassiosira hyalina	2	6	3	2
Total diatoms	**38**	**23**	**39**	**29**
Dinoflagellates				
Gymnodinium sp2	1	1		
Prorocentrum micans	2	1	1	1
Scrippsiella trochoidea	2	3	1	2
Total dinoflagellates	**5**	**5**	**2**	**3**
Other groups				
Mesodinium rubrum	1	4	1	1
Cryptomonas spp	1	1		1
Eutreptiella sp	1	1		
Flagellates<5	18	21	21	27
Flagellates>5	35	39	37	41

4-4-3 Seasonal succession of phytoplankton.

Phytoplankton seasonal succession is shown in Figure 4-17. It shows the absolute contributions of different phytoplankton groups, over the whole sampling period rather than percentage data, with the four chl events indicated by letters over vertical dashed lines and numbers indicating relative pigment signature.

The phytoplankton species counts are represented in the species data in Table 4-3. Higher diatom number was present at Calshot station at the second chl event and the highest diatom carbon biomass (*Skeletonema costatum*) was at Dock station at the third chl event. Dinoflagellate biomass followed the diatom peaks, associated with the second chl event.

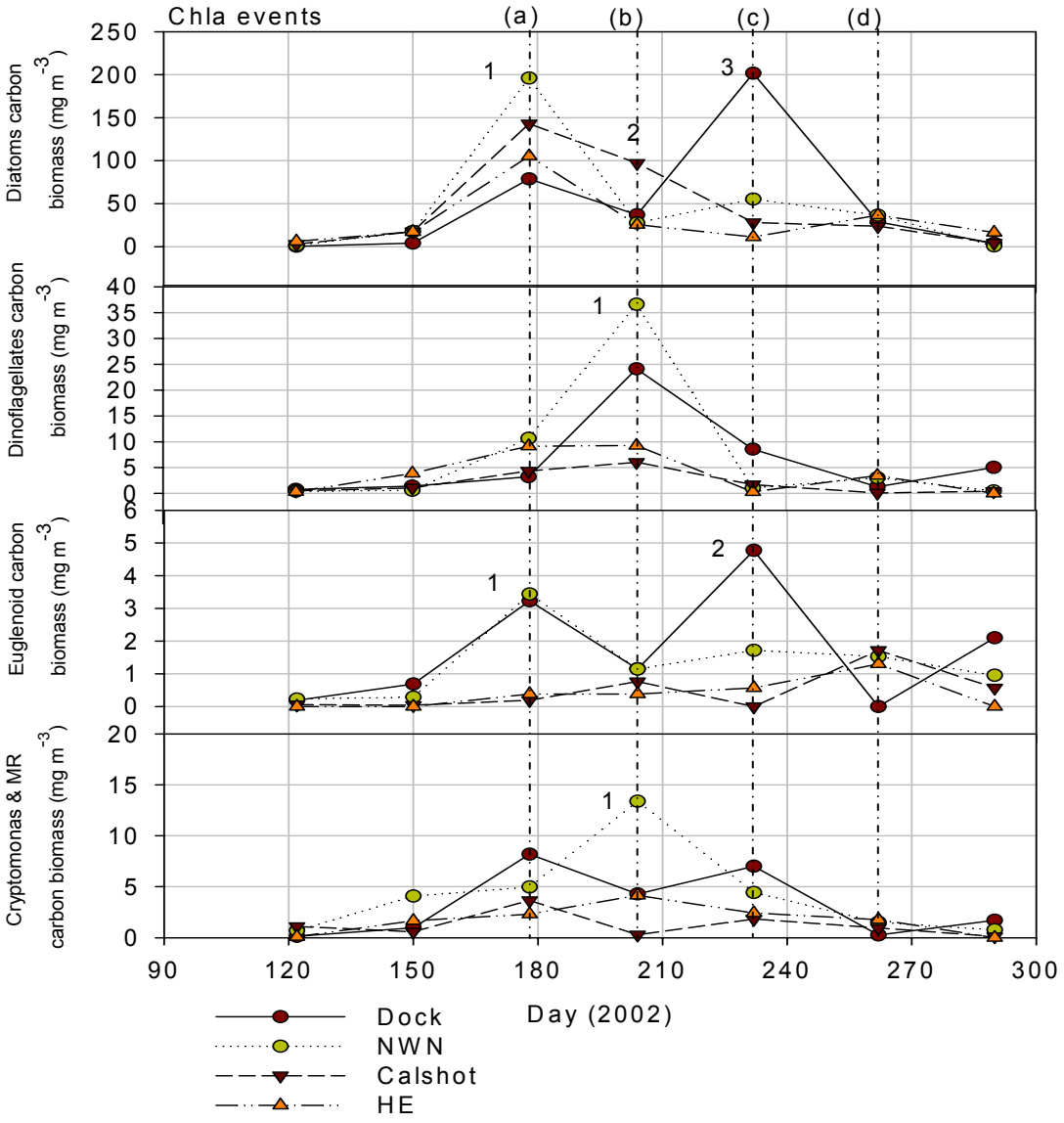
Those peaks of diatoms and dinoflagellates were associated with a peak of fuco and perid; the fuco to chla and perid to chla ratios are indicated with the peak numbers.

The peak of dinoflagellates at Dock station was not associated with the perid value, which indicated an over-estimated biomass.

For euglenoids and the group of *Cryptomonas* sp and ciliate *MR*, the biomass was higher at Dock and NWN stations. The Euglenoid (*Eutreptiella* sp) biomass at NWN did not show good agreement with the chlb value, which indicated that there are other chlb contuinning organisms, as microscopic analysis showed that there were many small flagellates. The chlb data fron the Dock station was in agreement.

Figure 4-17: The succession of the phytoplankton group biomass, related to the chl events at four estuarine stations, 2002.

[The letters a-d indicate summer/ autumn samples with relatively high chl levels. The numbers indicate samples with particular pigment signatures (see Figure 4-12)]



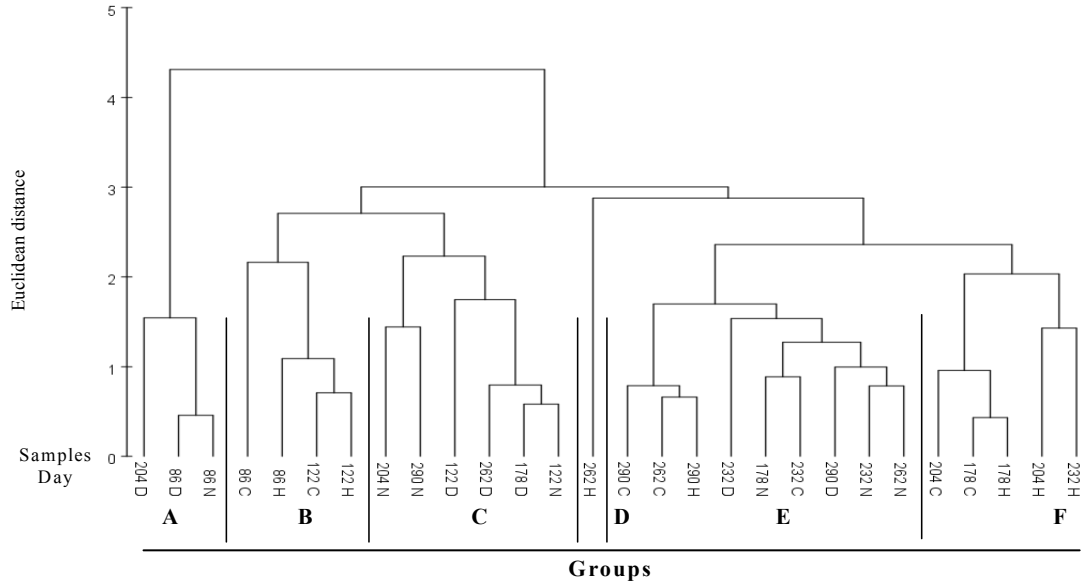
4-5 Multivariate analysis data and interpretation

Multivariate analyses similar to those described in the previous chapter were also carried out on the environmental and phytoplankton data from the four estuarine stations. The environmental parameters excluded meteorological and tidal data as they were assumed to be similar for the four stations. Water temperature was not considered for the reason given in Chapter 3.

4-5-1 Environmental data

The estuarine environmental data were normalised by log transformation to calculate the Normalised Euclidean distance. The environmental variables (water salinity, K_d, nutrients N, P, Si) were clustered to give the groups with the lowest distance between pairs of samples. The K_d data were not transformed. Six groups (A-F) were defined by the dendrogram for hierarchical clustering (Figure 4-18).

Figure 4-18: Dendrogram for hierarchical clustering of sample defined by environmental parameters, four estuarine stations, 2002.



The normalised Euclidean distance between groups ranged between 1.5% - 4.5%. Group E was the largest, with nine samples, while group D had only one sample and the remainder between 3-5 samples.

Groups A and C included samples from the inner estuarine stations (Dock, NWN) and groups B, D and F samples from the outer stations (Calshot and HE), while group E is a mixed sample. Groups A and B represented early samples, while the other groups represented late spring-summer.

Multi-Dimensional Scaling (MDS) analysis of the data provides a two-dimensional distance plot (Figure 4-19) which shows more clearly how the samples are grouped. The plot stress (0.09) indicated a very good two-dimensional representation of the data. The plot distinguishes the inner station groups A and C, from the outer station groups B, D and F, while the mixed group (E) is in the middle of the plot. Looking at the original data, it is found that the group positions reflect decrease surface salinity from low (group A) to high (group F). For nutrient levels, the distributions followed a reverse pattern. One sample in group B (86C) was separate from the other samples in the group, due to its relatively low salinity.

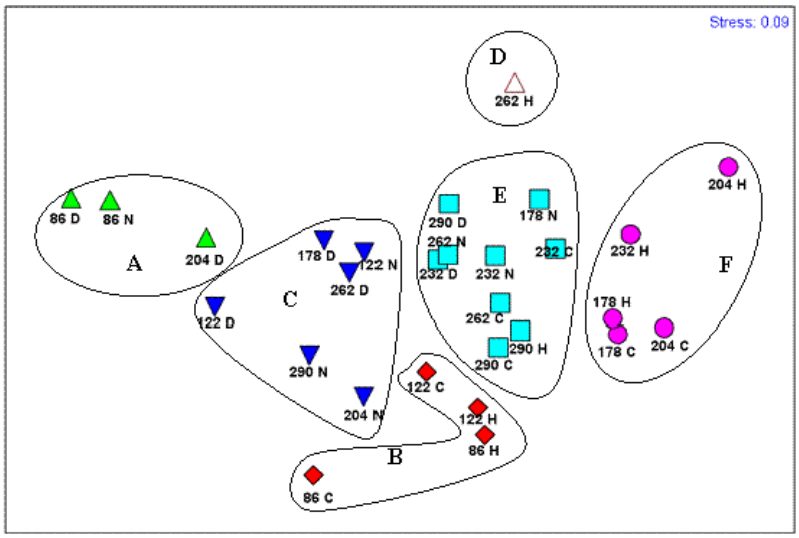


Figure 4-19: MDS plot of environmental parameter grouping, numbers and letters indicating the sample days and stations, 2002.

The group specifications are shown in Table 4-6. Nine samples with chl >5 mg m^{-3}, together with the highest chl sample for HE station, are indicated in bold. Salinity was the most important parameter in defining the groups, followed by nutrients (mainly N and Si).

Chl was high in most groups, but not in group B. Group A (inner stations) was the only group with N as the highest contributor

Table 4-5: Main characteristics of sample groups defined by environmental parameters.

[Letters indicate the station and numbers indicate sampling date; bold represents higher chl samples]

Group	Sample	Parameters (%) contribution to group	% of similarity
A	86 D, N; **204 D**	N (31), Salinity (27), Si (26)	95
B	86C,H; 122,C,H	Salinity (35), N (33), Si (17)	94
C	122 D N;**178 D**; **204 N**, 262 D; 290 N	Salinity (33), Si (23), N(21)	95
D	262H	K_d high	
E	**178N**, **232 D,N**;232C, **262 N**; 262C; 290, D,C,H	Salinity (36),N (27), Si (21), Kd (13)	95
F	**178 C,H**; **204 C**;204H; 232 H	Salinity (45), Si (20), Kd (18), N (16)	92

4-5-2 Phytoplankton data

Phytoplankton biomass

The phytoplankton data for the four stations were transformed to the fourth root before calculating the Bray-Curtis Similarity index. Only species/taxa representing >1% of total biomass were considered. The results from the hierarchical clustering analysis are illustrated in the dendrogram (Figure 4-20). There are eight groups (A-G), F being divided into two groups, F1 and F2. There were also 5 single samples (1-5). Within each group, the similarity level ranged from 62% to 82%.

The two-dimensional MDS plot (Figure 4-21) has a stress of 0.19, which is high, but could be interpreted using additional information from the three-dimensional plot (not shown) and from the similarity dendrogram. Groups A-D and 1 and 2 represent the inner stations (Dock and NWN). The other groups are from the outer stations (Calshot and HE).

Figure 4-20: Dendrogram for hierarchical clustering of samples defined by phytoplankton species/taxon biomass, four estuarine stations, 2002.

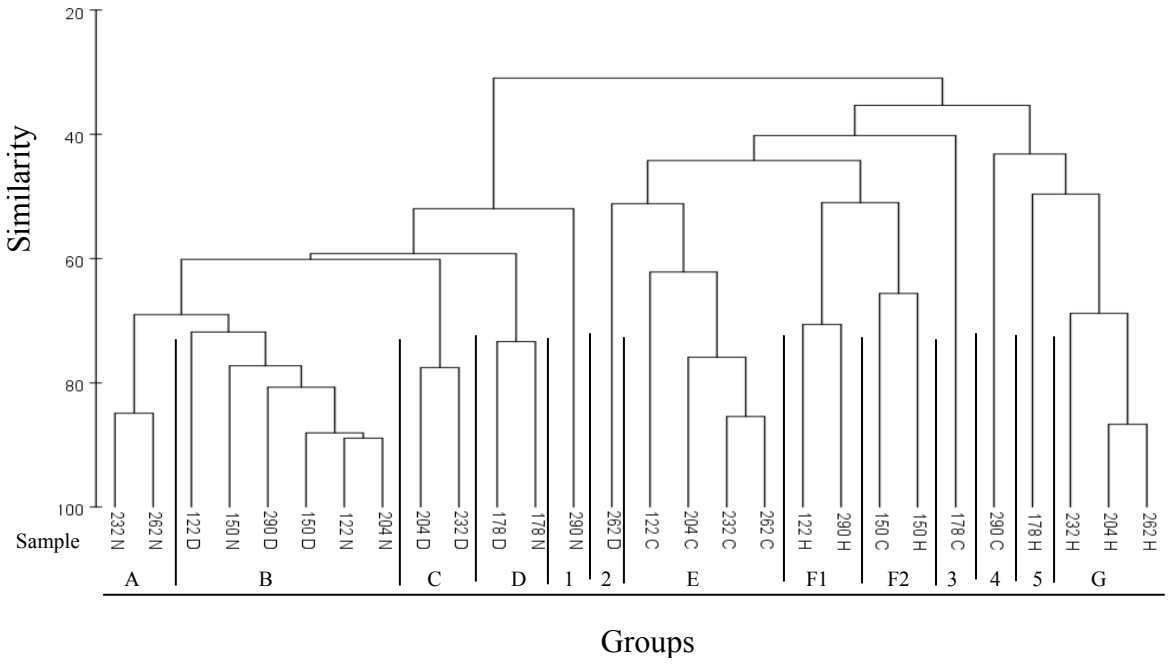
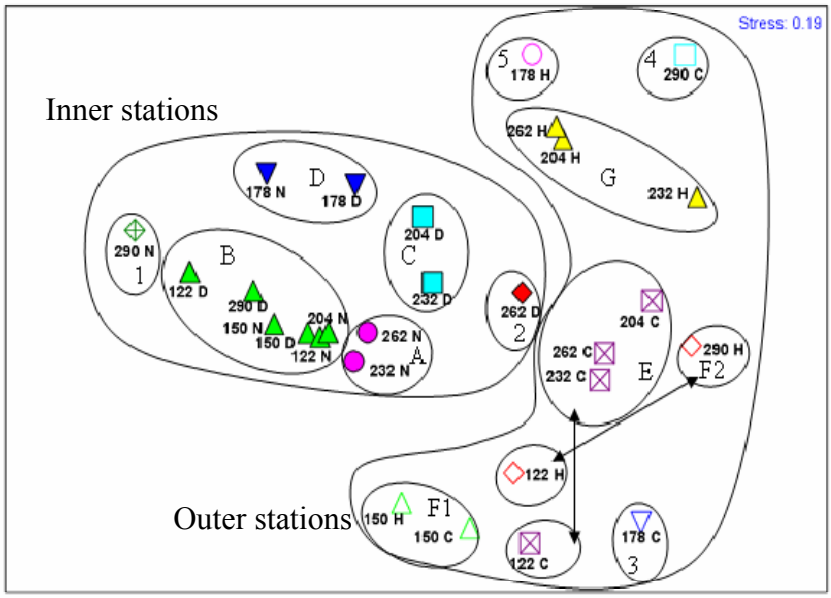


Figure 4-21: MDS plot of samples defined by phytoplankton species/taxon, four estuarine stations, 2002.



Further analysis of the groups was based on species carbon (SIMPER), which determines the dominant species of each group, using percentage contribution and average biomass. The results are summarised in Table 4-7.

Diatoms were the major contributors to most of the groups for both inner and outer stations. Dinoflagellates made a low contribution and were present only at the inner stations (groups B and D), as did other taxa (*Eutreptiella*, *Mesodinium rubrum* and *Cryptomonas)*.

Table 4-6: The main characteristics of sample groups defined by phytoplankton species/ taxa, four estuarine stations, 2002.

Group	**Sample (D station)**	**Species (%) contribution to group**	**% of similarity**
A	**232N, 262N**	*Thalassiosira hyalina* (33), *Skeletonema costatum* (17), *Rhizosolenia setigera (12)*	78
B	122D,N, 150D,N, 290D, **204N**	*Scrippsiella trochoidea* (24)*, Rhizosolenia setigera* (21), *Mesodinium rubrum* (17) *Eutreptiella marina* (14)*, Cryptomonas* (12),	69
C	**204D, 232D**	*Skeletonema costatum* (21), *Guinardia delicatula* (14), *Coscinodiscus* (12), *Chaetoceros sp* (10)	60
D	**178D,N**	*Cerataulina pelagica* (37), *Scrippsiella trochoidea* (20), *Mesodinium rubrum (16), Eutreptiella marina* (10),	68
1	290N	Low biomass for all phytoplankton	
2	262D	*Thalassiosira hyalina*	
E	122C, **204C**, 232C, 262C	*Odontella mobiliensis* (31), *Rhizosolenia setigera* (21), *Guinardia delicatula* (17), *Skeletonema costatum* (12)), *Thalassiosira hyalina* (10)	62
F1	150H,C	*Rhizosolenia setigera* (81), *Thalassiosira hyalina* (19)	65
F2	122H, 290H	*Coscinodiscus* (48), *Thalassiosira hyalina* (40)*, Rhizosolenia setigera* (12)	67
G	232H, 204H, 262H,	*Guinardia delicatula* (48), *Rhizosolenia setigera* (41)	75
3	**178C**	*Cerataulina pelagica* and *Chaetoceros sp*	
4	290C	Only diatoms with low biomass	
5	**178H**	*Cerataulina pelagica* and *Guinardia delicatula,*	

For the inner stations the most important diatoms were *Thalassiosira hyalina*, *Skeletonema costatum*, *Rhizosolenia setigera* and *Guinardia delicatula.*

At the outer station, the most important diatoms were *Coscinodiscus* and *Guinardia delicatula*, and *Odontella mobiliensis* (only at Calshot station). *Cerataulina pelagica* (diatoms) contributed in both inner and outer station groups (groups D, 3 and 5). Groups 1 and 4 were single samples because of low biomass contribution, and were separate from other sample groups.

The inner groups were distinguished from the other groups by the presence of *Eutreptiella marina*, *Mesodinium rubrum*, *Cryptomonas,* the dinoflagellate *Scrippsiella trochoidea* and the diatom *Skeletonema costatum*.

The distribution of species in terms of seasonality was not clear, due to the low number of species, but seemed well distributed in both the inner and outer sample stations. However, *Cerataulina pelagica* (diatom) contributed in both inner and outer station groups and distinguished the early samples. The late species *Skeletonema costatum* was among the inner samples (group C). Group B consisted of early/late samples with mixed diatom species (*Rhizosolenia setigera* and dinoflagellates *Scrippsiella trochoidea*) shifting to the late sample group A, which contained the diatoms *Thalassiosira hyalina* and *Skeletonema costatum.*

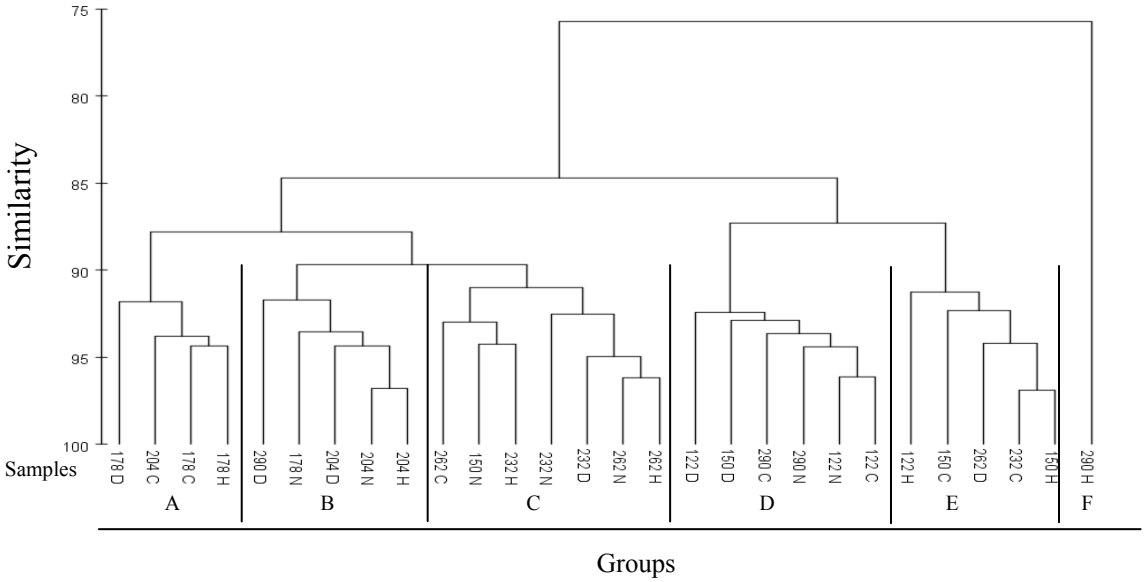
The outer sample groups did not show specific species in late samples apart from *Odontella* sp, which highly contributed to group E. Group E had a late high chl sample (204 C) that was distinguished by major species of diatoms *Guinardia delicatula* and *Rhizosolenia setigera*.

Phytoplankton taxa biomass

The data for taxon biomass were analysed using The Bray-Curtis Similarity measurement, after standardisation and 4^{th} root transformation. Six groups (A-F) were obtained from the hierarchical clustered dendrogram, with a level of similarity

of ~90% (Figure 4- 22). One was a single sample (F), and had the lowest similarity rank (~75%).

Figure 4-22: Dendrogram for hierarchical clustering of samples defined by biomass of phytoplankton taxa.



The phytoplankton groups detailed in Figure 4-22 can also be seen in the MDS ordination plot (Figure 4-23). The plot has a stress of 0.11, which is acceptable for a two-dimensional description of data distance.

The plot did not give a clear separation of inner or outer stations, or of early and late times, but groups A and B included all the early samples (Days 178, 204).

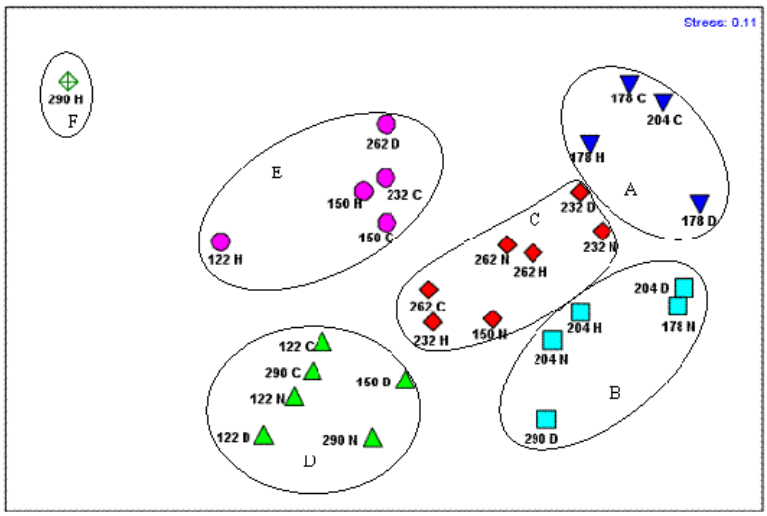


Figure 4-23: MDS plot of the sample defined by phytoplankton group (taxon, cell size), numbers and letters indicating sample days and the station.

The details of different phytoplankton taxon contributions are given in Table 4-7. The flagellates >5 µm and <5 µm biomass made the highest contributions to all groups, apart from groups A and F with diatoms as the most important taxan. Diatom, *Cryptomonas* and *Mesodinium rubrum* contributed to group B. Dinoflagellates had the highest percentage in group A. F is a single sample group due to low or non-contribution of one or more taxa, and is separated from the other groups.

Table 4-7: Main characteristics of samples defined in terms of phytoplankton group (taxon, cell size) biomass.

Group	Sample (D station)	Parameters (%) contribution to group	% of similarity
A	**178D,C,H; 204C**	Diatom (30), >5 µm (22),<5 µm (18), dinoflagellate (14)	93
B	**178N**, **204D,N**; 204H; 290D	>5 µm (22), <5 µm (20), diatom (19), *Cryptomonas* sp + *Mesodinium rubrum* (14)	93
C	**262N**;262C,H; **232D,N**;232H; 150N	>5 µm (26), diatom (23), <5 µm (21)	92
D	122D,N,C; 290N,C; 150D,	>5 µm (31), <5 µm (28), diatom (13), dinoflagellates (10)	93
E	122H; 150C,H; 232C; 262D	>5µm (31), <5µm (26), diatom (23), dinoflagellates (11)	91
F	290H	Diatoms and <5 µm > only	

Accessory pigment analyses

Data for the main HPLC accessory pigments were normalised by square root transformation to calculate the Bray Curtis Similarity Index, and hierarchical clustering (not shown) used to define groups with similarities in the range of 88-92%. Each group had >3 samples, except groups 1 and 2, which had just one sample. The group specifications are summarised in Table 4-9.

Groups A and 1 represent the inner stations, groups C and 2 represent the outer stations, and the rest are mixed in terms of group location. In terms of time, groups A, B and C are the late spring and summer samples, and groups D, E, 1, and 2 are the low chl early spring and autumn samples.

Table 4-8: Main characteristics of sample groups defined in terms of photosynthetic pigment composition.

[The high chlorophyll samples are identified by sample days in bold].

Group	Samples (Day station.)	Pigment (% contribution)	% of similarity
A	**178D,N; 232D; 204N**	fucoxanthin (37),), chlb (24), alloxanthin (20) peridinin (17)	92
B	**178C**; **204C; 204D**; 262D; **232N**; **N262**	fucoxanthin (38), chlb (24), peridinin (19), alloxanthin (19)	94
C	232C; 262C; **178H**; 204H	fucoxanthin (34), chlb (24), alloxanthin (13)	94
D	122C; 290C,H; 86H,N;	fucoxanthin (45), chlb (25), alloxanthin (22) peridinin (17),	94
E	86D,C;122D;290D,N; 232H;262H	fucoxanthin (33), chlb (24), peridinin (21), alloxanthin (20)	90
1	122N	fucoxanthin, chlb,	84
2	122H	fucoxanthin, chlb	85

Groups A, B, and C include all the high chl samples. They are very similar and cover the period of the diatom bloom, with fuco as the main accessory pigment. Group B is characterised by more perid than allo, which indicates that dinoflagellates were higher; in contrast group C has no perid present.

Groups D, E, 1 and 2 include almost all the early (Day 122 and before) and late (Day 282 and later) samples which are characterised by fuco and chlb in varying proportions. Those samples characterised by low chl and relatively high fuco, compared with other pigments, in early-late samples.

Generally, accessory pigment clustering agreed with the phytoplankton taxa biomass clustering (groups A, B and C agreed with groups A, B, and C in terms of taxa biomass)

4-5-3 Relation of environmental parameters and biological parameters

Further analysis to link environmental parameters with biotic data was performed using the BIOENV routine of PRIME, as described in the previous chapter (Section 3-4-3). BIOENV calculates the highest degree of correlation (ρ) (best matching)

between environmental parameters and biotic data, species carbon biomass, taxon carbon biomass and chlorophyll *a*.

The BIOENV analysis attributed best correlation values of 0.21, 0.24 and 0.17 for species biomass, for taxonomic group biomass and for chlorophyll *a* size fraction data, respectively, as summarized in Table 4-9.

Table 4-9: BIOENV analysis of the correlation factors (best variables) for different biotic data

Group	**Single variable (ρ) value**	**Best variable correlation (ρ)**
Species carbon biomass	N (0.18), Salinity (0.17)	N and salinity (0.21)
Taxon carbon biomass	P(0.24), Si (0.18)	One variable P (0.24) Two variables P and Si (0.23)
chl size fraction	P (0.11)	One variable P (0.17)

The previous results (Table 3-8) indicated that seasonal changes in total phytoplankton biomass are equally affected by light and nutrient availability, whereas nutrient levels, in particular silicate, can change the taxonomic biomass. Also, changes in the size fractions of chlorophyll were correlated, albeit weakly, with varying phosphate concentrations, and suggest that low phosphate in summer may restrict the growth of larger cells (i.e. diatoms and dinoflagellates).

The variables in table 4-9 show similar seasonal patterns at all station. The temporal distribution of salinity increased from winter towards summer, while nutrients decrease. Light (PAR) and ambient temperature are not covered in this chapter. They are assumed to be similar at all stations, and affect the phytoplankton distributions, as observed previously in Chapter 3.

The spatial distributions of those parameters reflect the gradient patterns of salinity (from lower to upper estuary). In contrast, nutrients concentration decreased from upper estuary towards the estuary mouth. As a result of the environmental analysis (PRIMER), the estuarine stations were separated into inner stations (Dock and NWN), and outer stations (Calshot, and HE). The inner stations have higher nutrients

and lower salinity than the outer stations, perhaps due to the riverine input increasing nutrient levels, and decreasing salinity.

However, spatial and temporal changes in nitrate and salinity gradient affected the phytoplankton biomass. High salinity was found to be associated with the highest chl, specifically on Day 178, particularly at NWN and Calshot stations.

Changes in taxonomic biomass are best explained by varying nutrient levels, in particular P instead of Si, showing strong depletion over time at all stations (Figure 4-3). Si distribution did not show any significant change with time, apart from at Dock and NWN stations, also perhaps because there are missing readings for Si. However, the data do not show whether the level was already high, before sampling as shown by another study (Iriarte and Purdie, 2004), although they show an effect on taxa biomass. Moreover, the difference in chlorophyll size fraction had a weak relationship with P, similar to what had been observed for Dock (Chapter3).

4-6 Discussion and conclusions

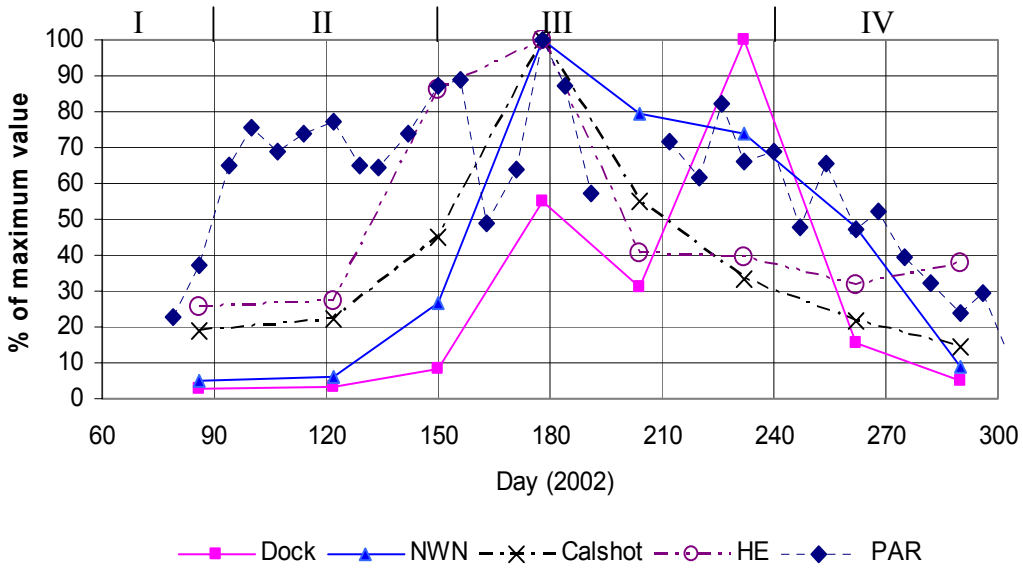
In this chapter the distributions of biological parameters (Tchl, phytoplankton species abundance, carbon biomass, taxon group biomass, phytoplankton biomarker pigments), physical parameters (temperature and salinity) and chemical parameters (inorganic nutrients N, P, Si) were compared for four sampling stations (Empress Dock, NW Netley, Calshot and Horse Elbow) in Southampton Water. The statistical multivariate programme (PRIMER) was used to establish relationships between the biological and environmental parameters.

The distributions of normalized (percentage of maximum) chl and PAR values for the four stations are shown in Figure 4-24. The phytoplankton biomass distributions showed similar midsummer maxima, except at Empress Dock where there was a second, stronger peak in late summer. The seasonal changes in phytoplankton biomass, both the increase after Day 120 and the decrease after Day 180, appeared to develop in sequence from the outer to the inner stations.

The same four phases of PAR and chl distributions as defined for Dock (Chapter 3, Figure 3-30) can also be distinguished for the estuarine stations (Figure 4-24):

Phase 1 (before Day 90) was characterised by low light and low chl. Nutrient concentrations were higher at the inner than at the outer stations (Figure 4–4). The contribution of the <5µm cells to chl was below 30%, the lowest value being ~15% at Calshot. Si was lowest at Calshot, approximately 5µM, a value appreciably lower than that reported for Southampton Water in late winter (Iriarte and Purdie, 2004). The depletion of Si is presumably due to removal by diatoms, and the highest biomass percentage of diatoms at this time of year was recorded at Calshot (Figure 4–15). Also, K_d values were lowest at Calshot, and it appears that the better light conditions early in the year at this station favoured the growth of larger phytoplankton.

Figure 4-24: Comparison of relative changes in weekly mean PAR and chl, at four estuarine stations.



Phase II (between Day 90 to 150): PAR increased to 90%, and chl started to increase, first at the outer stations and later at the inner stations, to reach 90% by Day 150 at HE, 45% at Calshot, ~ 25% at NWN, and ~10% at Dock. The < 5µm Chl fraction

was less than 30% of total, except at Dock (Day 150), where it was somewhat higher (Figure 4–9).

Phase III (between Day 150 to 240): In this phase PAR was maximal around Day 180 when the highest chl was also recorded at all stations except Dock, where it peaked on Day 240. All nutrients decreased to their lowest levels associated with the chl peak. Silicate showed lower values at the outer stations than the inner stations, with the highest Si fluctuation in the Dock sampling location. The <5µm chl size fraction contribution to Tchl fluctuated below 40%, except at HE, the outermost sampling station, where this size fraction contributed 60% during August (continuing in the next period). This is probably due to the low contribution of large cells.

Phase IV (Day 240 onward): This phase was characterized by decreasing light and chl at all stations. Nutrients started to rise, with Si reaching its highest value in the outer stations (Figure 4–4). The < 5µm chl fraction increased at all stations to > 40%, except at HE, where it decreased from 60% to 20%.

The results of the multi-dimensional scaling analysis of data (PRIMER) divided the stations into two groups, the inner estuary (Dock and NWN) and the outer estuary (Calshot and HE). This division was determined mainly by salinity and nutrient distributions (Figure 4-19) but was also shown by the phytoplankton analysis (Figure 4- 21)

The earlier increase in chl in the outer estuary is linked to lower K_d values and a high contribution to phytoplankton biomass by diatoms. At the outer stations, chl started to increase in April, reaching a maximum in June, and declined thereafter. In the inner stations, the peak started in May, with a more or less extended peak during June – August. The maximum chl concentrations at the outer stations were about 3.5mg m^{-3} compared to an average of ~ 9.0 mg m^{-3} at the inner stations, where nutrient levels were higher. The above inner-to-outer pattern of decrease in chl levels is well known for Southampton Water (Lauria, 1998).

At the inner stations, the rates of removal of N and P were rather variable with respect to changes in chl. By contrast, N and P utilization at the outer stations was consistent with chl events and both nutrients were depleted by Day 180. The same picture is true for Si utilization. For this reason, nutrient removal at the outer stations seems largely to reflect phytoplankton growth, while nutrient removal at the inner stations appears to be affected significantly by continuing riverine inputs. Overall the N/P ratio was noticeably higher than the Redfield value of 16, indicating more input of N than P, with the potential for P to be limiting for phytoplankton growth. Kocum *et al.* (2002a) also observed higher N:P ratios at the seaward end of the Colne Estuary and suggest that P limits algal biomass formation. The reasons for low P availability in estuaries remain uncertain, but include low inputs (relative to N), abiotic removal by absorption onto particles, and growth of organisms with high P content. The role of P in Southampton Water warrants more detailed investigation.

The percentage contribution of < 5μm-size phytoplankton to Tchl averaged ~30%, with the lowest values in the outer estuary, perhaps due to selective predation pressure on this size group in this region. The range of values for this fraction contribution in the outer stations is very wide, with lower contribution probably being attributable to the physical instability of the water column (see Kiorboe, 1993). The water column in the inner stations is obviously less turbulent.

The ever changing input and flushing of nutrients make it difficult to rely on nutrient ratios to explain variations in chl biomass. Boynton *et al* (1982) noted that, in a number of estuaries, phytoplankton biomass is most closely related to nitrogen availability. However, they could not define a general pattern to explain temporal variations in primary productivity or the occurrence of blooms, because of diversity in estuarine properties. As stated earlier, in Southampton Water, at least in 2002, P seemed to play a role in determining the pattern of chl events when sufficient N was available.

The succession of phytoplankton species did not provide a clear explanation for the temporal changes in chlorophyll. The chl event on Day 180 was dominated by

diatoms at all stations. The peak at Dock on Day 240 was dominated by diatoms and *Eutreptiella*. On Day 210, chl peaks at the inner stations were dominated by dinoflagellates, together with *Cryptomonas* and *Mesodinium rubrum* (Figure 4-17). This succession is not as distinct as that described by Kifle and Purdie (1993) and Ali (2003) for Southampton Water. The present results are similar to those reported by Iriarte and Purdie (2004) for 2000, the most likely reason being the particular climatic conditions for that year. The distribution and abundance of phytoplankton is known to respond to climate variability on a decadal time scale (Reid *et al.*, 1998).

From the above discussion, the following points can be highlighted. The sampling stations can be divided into two groups one comprising the inner stations and the other comprising the outer stations. The inner stations are characterized by higher chl levels and higher nutrient concentrations than the outer stations, which are also particularly deficient in silicate. The importance of nutrient ratios is not clear, but P availability possibly limits the growth of phytoplankton for short periods in the summer. The flagellates *Eutreptiella*, *Cryptomonas* and *Mesodinium rubrum*, are more abundant at the inner stations, but plankton biomass is higher at the outer than at the inner stations. Tides and K_d are two important physical factors affecting primary production. Some uncertainties might be resolved by increasing the sampling frequency. This will be addressed in the next Chapter (Chapter 5).

The next chapter concentrates on NWN station. This sampling area is intermediate between the inner and outer estuary, and was sampled during 2003, which was climatically different from 2002.

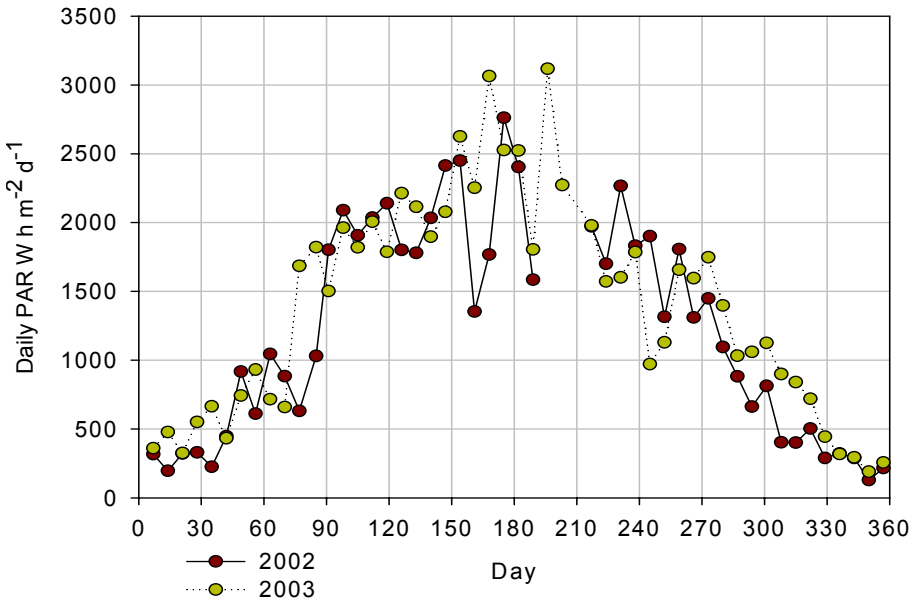
Chapter 5- NW NETLEY OBSERVATIONS 2003

5-1 Physical parameters

Meteorological and tidal data

Variations in the weekly means of daily solar irradiance (Photosynthetically Active Radiation (PAR)) during 2003 compared with 2002 are shown in Figure 3-1. In 2003, the weekly mean PAR values were in the range 221-3117 W h m^{-2} d^{-1}, with the lowest values recorded towards the end of December and the highest values at the end of July.

Figure 5-1: Weekly mean values for daily Photosynthetically Active Radiation (PAR) in 2002 and 2003.



The distributions of PAR in 2002 and 2003 (Figure 5-1) followed a similar seasonal pattern. PAR values reached 1500 W h $m^{-2}d^{-1}$ about two weeks earlier in 2003 (early March) than in 2002. The maximum PAR values recorded in mid June to mid July

were higher than any values observed in 2002. In both years, the summer period was characterised by wide fluctuations in PAR.

River flow rates in 2003 are compared with those in 2002 in Figure 5-2. The flow rates of 2003 were higher than those of 2002 in the first quarter of the year and they were lower than 2002 in the rest of the year. The 2003 flow rates ranged from 25-30 m s^{-1} in winter to ~5 m s^{-1} in summer- autumn.

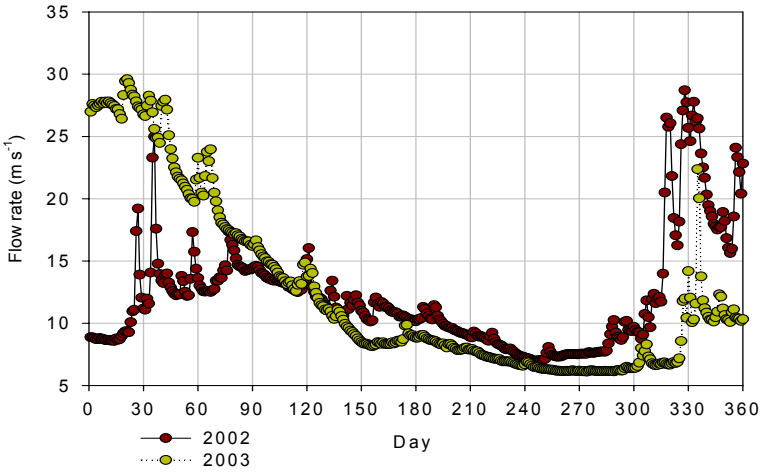


Figure 5-2: The Test, daily River flow rates in 2002 and 2003.

[From Broadland station, as measured by the HYDROLOG Data Management System, Winchester]

Change in tidal range (spring/neap cycle) in 2003, as recorded in the Southampton tide table (Admiralty charts and publication, Tidal tables V1 2003) (Figure 5-3), were similar to those in 2002. A maximum tidal range of <4m was observed between Days 150 and 230.

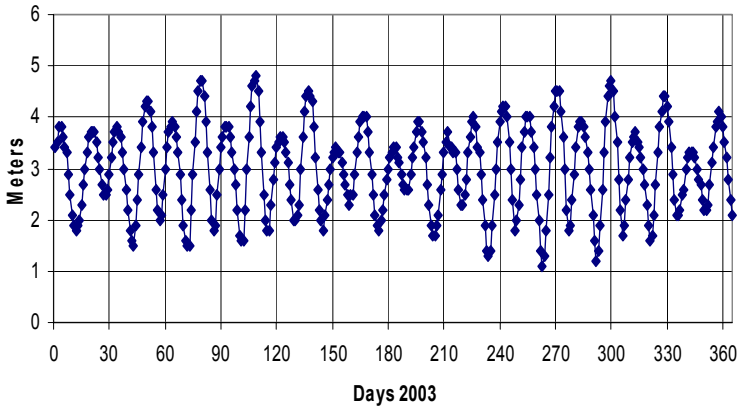


Figure 5-3: Tidal range for Southampton Water 2003.

Surface water salinity and temperature

The surface water salinity and temperature for 2002 and 2003 are shown in Figure 5-4. Surface water salinity in 2003 ranged between 29 and 33, apart from Days 216 and 223 when salinity was higher, and Day 104, when salinity was lower. In 2002, salinity had a similar pattern but from fewer observations.

The surface water temperature in 2003 ranged between 11 and 25°C, increasing gradually from the lowest value observed in mid-April to a maximum value in mid-July. A similar pattern had been observed during 2002, but in 2003 temperatures were generally higher during summer.

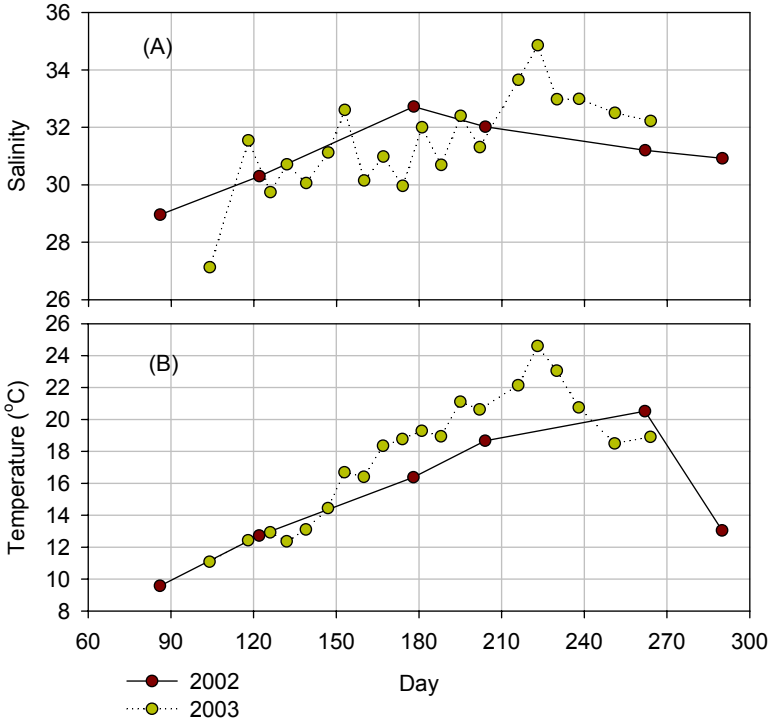


Figure 5-4: Surface water (A) salinity and (B) temperature, at NWN, 2002 and 2003.

[Note there are missing data on Days 91 and 210 in 2003 for both salinity and temperature]

Water column light attenuation

The water column light attenuation coefficient (K_d) data for 2002 and 2003 are shown in Figure 5-5. In 2003, K_d values decreased from winter to summer, and were mostly in the range 1.0 to 1.8 m^{-1} between June and August. In 2002, K_d values at

NWN were generally of a similar range, but on two occasions (Days 193 and 283) relatively clear water values of K_d values of ~0.5 m^{-1} were observed.

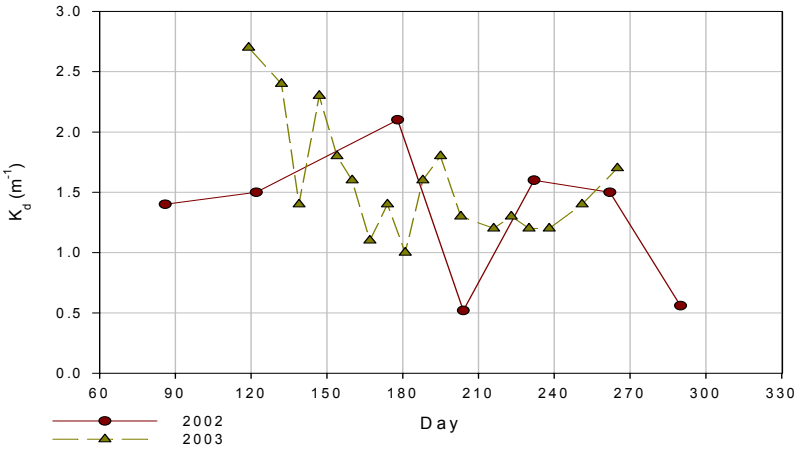


Figure 5-5: The light attenuation coefficient (K_d), at NWN, 2002 and 2003.

[Note, no data were obtained before Day 120 for 2003]

5-2 Chemical parameters

5-2-1 Inorganic nutrients

The distributions of inorganic nutrients (P, N, Si) at NWN for 2003 are compared with those for 2002 in Figure 5-6. The higher sampling frequency (weekly) in 2003 indicated short term fluctuations in the level of all three nutrients, which could not be discerned in the 2002 data (monthly samples). Values were minimal in summer for both years. However, in 2003, significant increases in Si and P concentrations were observed from the end of June onwards.

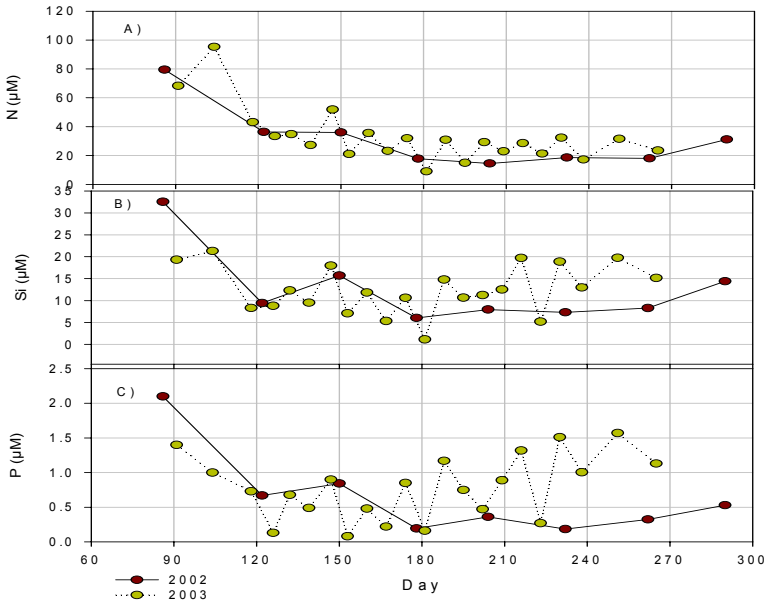


Figure 5-6: Surface nutrient distributions at NWN, 2002 and 2003: (A) N, (B) Si, and (C) P

[Note different scales of Y axis]

The nutrient ratios show a pattern of relatively high values in spring and low ones in summer (Figure 5-7). Nutrient ratios were significantly greater than the Redfield ratios of 16, 16 and 1 for N/P, Si/P and N/Si for marine waters, respectively. The N/P and Si/P ratios showed two peaks in the first part of the year, due to depletion of P (Figure 5-5 C). The N/Si ratio had one high peak on Day181, due to low Si concentration, perhaps indicative of diatom uptake of Si. However the N/P and Si/P ratios towards the end of the sampling time were <20; the N/Si ratio was <2, apart from two small peaks, which again could have been related to Si uptake by phytoplankton.

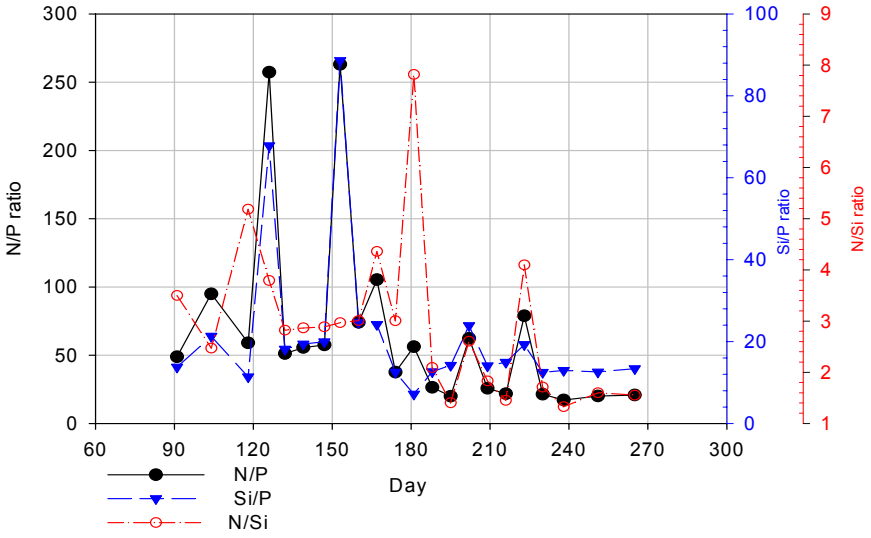


Figure 5-7: Changes in the N/P, N/Si and Si/P ratios, at NWN, 2003.

5-2-2 Particulate organic carbon and nitrogen (POC and PON)

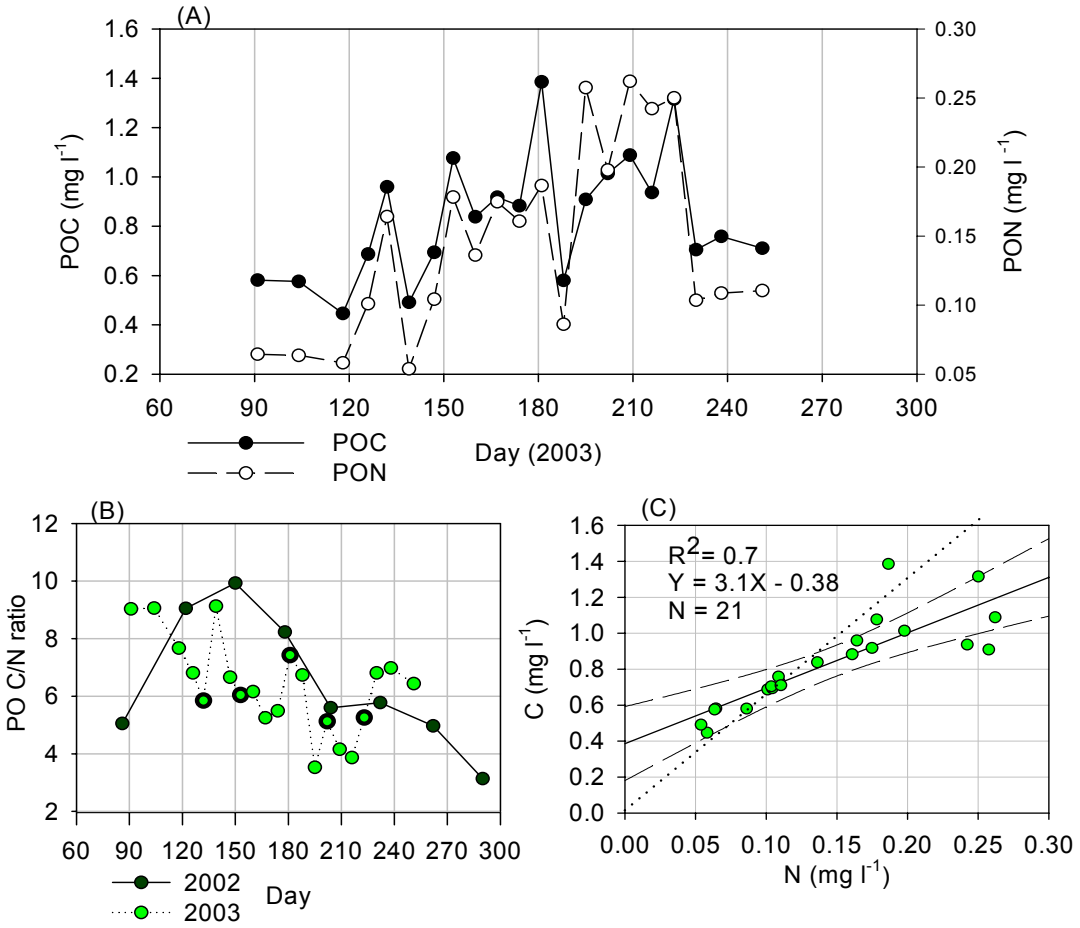
Particulate organic carbon and nitrogen (POC, PON) concentrations, particulate organic carbon/nitrogen ratios (POC/N) and the correlation between POC and PON are shown in Figure 5-8. During 2003, POC and PON increased at the end of April, reached maxima during summer and decreased thereafter.

The POC/N ratio showed a pattern of decreasing from winter to summer. In general, the C/N ratio for particulate organic matter was in the range 9.1 to 3.5, spanning the Redfield ratio. The high chl samples showed POC/N ratio close to the Redfield ratio.

The regression between POC and PON gave a slope of 3.1 (R^2=0.7), lower than the Redfield ratio. This low value is perhaps related to, firstly, high carbon component (probably detritus) in spring samples, and secondly high nitrogen content in summer samples (probably bacteria and other heterotrophic organisms).

Figure 5-8: (A) Distributions of particulate organic carbon and nitrogen; (B) particulate organic carbon/nitrogen (POC/N) ratios (2002 and 2003), and (C) the regression of POC to PON, at NWN, 2003.

[The thick edge green circles in plot B represent high chl values. The short dashed lines in plot C represent the confidence interval of 99%. The dotted line in plot C represents the Redfield ratio]



5-3 Phytoplankton pigments

5-3-1 Total chlorophyll a and chlorophyll a size fractions

Total surface chlorophyll *a* concentrations (Tchl) (Figure 5-9) ranged from 1.1mg m^{-3} in May (Day 139) to an estimated value of 24.2 mg m^{-3} in June (Day 154) (see note to Figure 5-9). Tchl fluctuated widely throughout the sampling period, with five

peaks >10 mg m^{-3} on Days 132, 154, 181, 202 and 223 (hereafter referred to as chlorophyll events 1-5).

However, the frequency of samples in 2003 was higher than in 2002, and revealed wide fluctuations in surface chlorophyll levels from week to week at NWN, as was also observed at Dock in 2002. The highest chl values observed in 2002 were lower than the highest chl values in 2003; the chl peak (>10 mg m^{-3}) was earlier in 2003 than that in 2002.

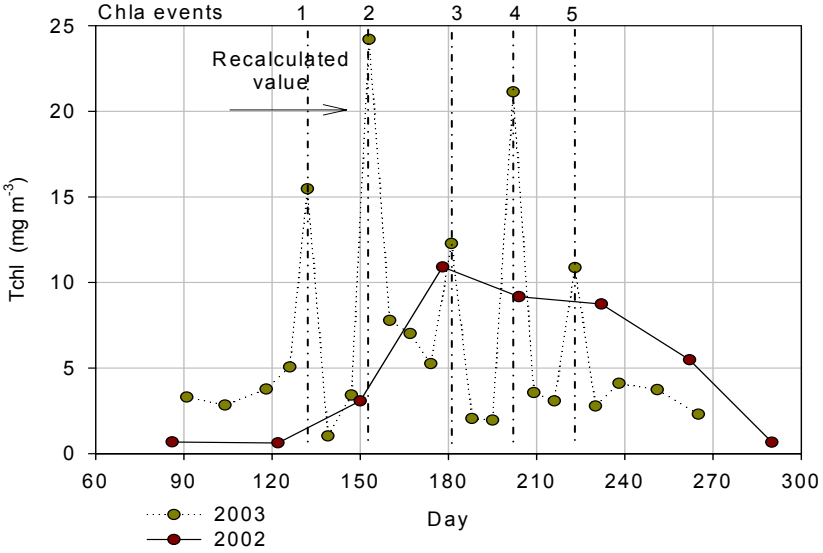


Figure 5-9: Seasonal distribution of Tchl, at NWN, 2002 and 2003.

[An anomalous Tchl value for Day 160 was recalculated from Fchl using the slope of the regression of Fchl and Tchl (see Figure 5-10) to give an estimated concentration of 24.2 mg m^{-3}.]

The correlation between Tchl and sum Fchl is shown in Figure 5-10. The overall correlation between Tchl and sum Fchl gives a slope of 1.3 (R^2 = 0.89), largely due to relatively low Fchl value at high chlorophyll concentrations (>10 mgm^{-3}).

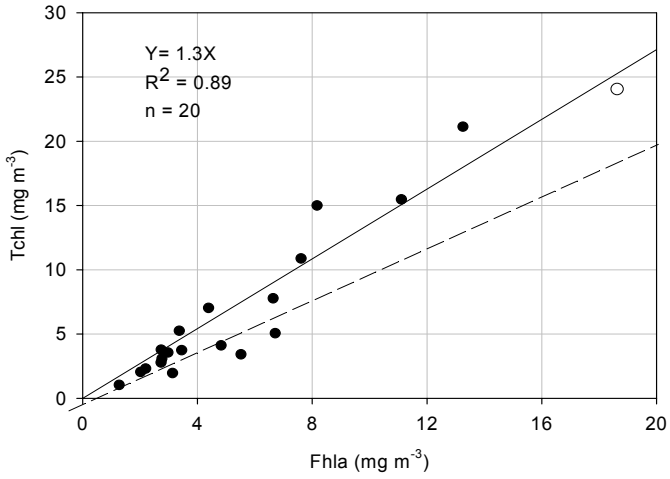


Figure 5-10: Correlation between Tchl and Fchl, at NWN, 2003.

[The open circle is the recalculated value of 24.2 mg m^{-3}, which is not included in the regression. The dotted line is the 1:1 slope for Tchl : Fchl]

The seasonal distributions of chl size fractions are shown in Figure 5-11, and expressed as percentages in Figure 5-12. The Fchl <5 percentages and Tchl distribution are compared in Figure 5-13. The <5µm fraction contributed a mean of 36% to the total chl, and was proportionally more important when chl was low.

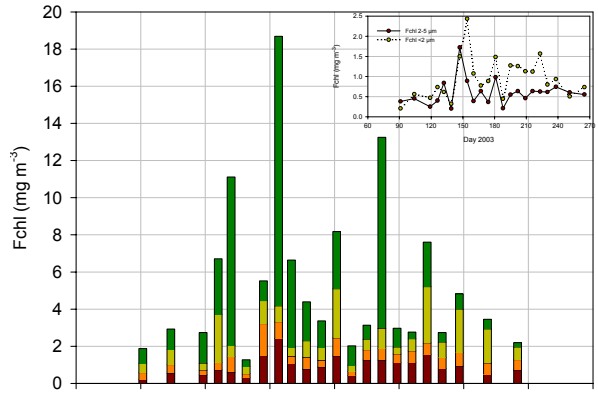


Figure 5-11: Seasonal distribution of chlorophyll size fractions at NWN, 2003.

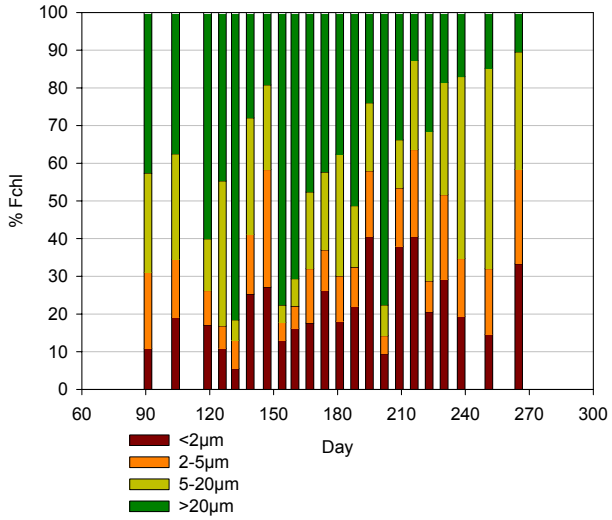


Figure 5-12: Seasonal distribution of chlorophyll size fractions, expressed as percentages, at NWN, 2003.

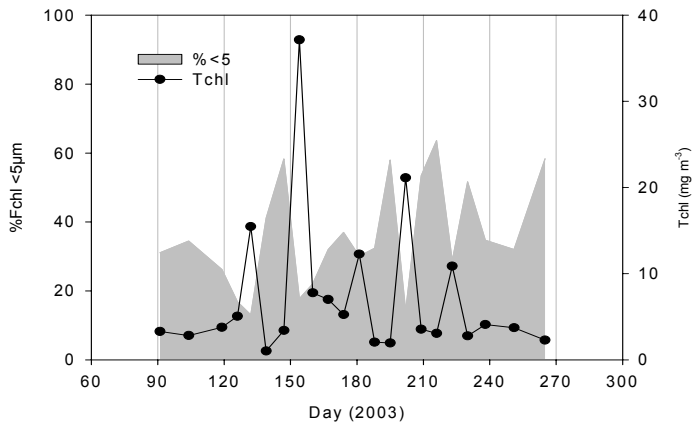


Figure 5-13: Seasonal distribution of Tchl and percentage of <5 µm chlorophyll a fractions. At NWN, 2003.

The mean percentages of the different chlorophyll

fractions at high and low chl concentrations (>5 and 5< mg chl m^{-3}) are summarized in Table 5-1 for 2002 and 2003. In both years, the >20µm fraction was generally the major size fraction, especially at high chl values. Conversely, the smaller fractions (2-5µm and <2µm) generally contributed least, and were relatively more important when chl was low.

Comparing data for the two years suggests that there was no clear difference between mean chl values. However, the percentage range for each size fraction was wider in 2003 than in 2002.

Table 5-1: The mean percentages of different chlorophyll size fractions at high and low (>5< mg m^{-3}) chlorophyll values, at NWN in 2002 and 2003.

Size fractions	2002				2003			
	Low chl		High chl		Low chl		High chl	
	Mean %	Range %	Mean %	Range %	Mean %	Range %	Mean %	Range %
>20 µm	32	22-47	55	43-71	28	11-60	57	32-82
5-20 µm	34	27-43	21	10-31	27	13-53	20	5-39
2-5 µm	17	15-20	11	5-21	18	9-31	8	5-12
<2 µm	17	12-25	14	8-21	26	4-15	15	6-26

5-3-2 Phytoplankton accessory pigments

The two methods (HPLC and fluorometric determinations) for determining chl showed good agreement as illustrated in Figure 3-15 although the slope of the relationship was higher than for data sets.

Based on the HPLC data alone, chlorophyll-a was highly correlated to both total pigment (Tpig) and total accessory pigment (Tac), with regression slopes of 0.5 (R^2 = 0.96) and 1.1 (R^2=0.83), respectively (data not shown). These correlations are similar to those obtained for the 2002 estuarine data (Chapter 4).

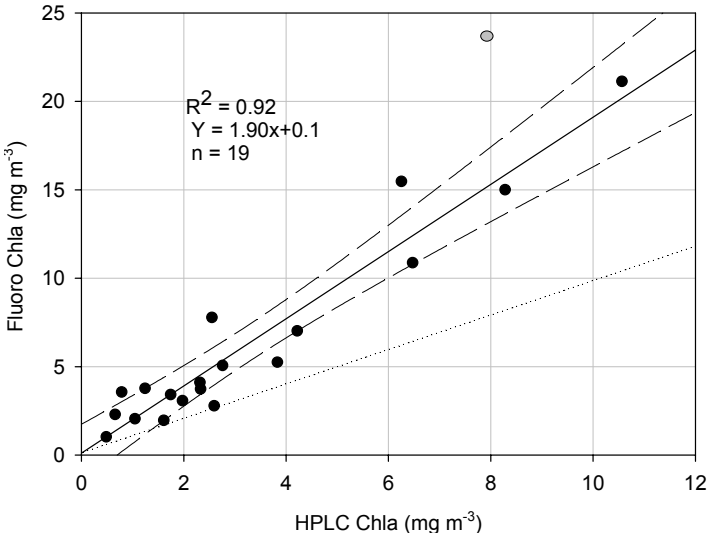


Figure 5-14: Comparison of chla measurement by HPLC and fluorometer, at NWN, 2003.

[The dashed lines indicate the 99% confidence interval. The grey colour point is the anomalous value excluded from the regression. The dotted line indicates the 1: 1 ratio]

The distribution of major pigments, namely chlorophyll a (chla), fucoxanthin (fuco), peridinin (perid), chlorophyll b (chlb) and alloxanthin (allo) are presented in Figure 5-15, and the pigment to chla ratios in Figure 5-16. chl events 1-5 (see also Figure 5-9) are identified on each of these figures.

The distribution of fuco was mostly similar to chla (Figure 5-15); peaks in fuco corresponded with the high chl events, except event 3 which has a low fuco/chla ratio Figure 5-16.

Perid concentrations were generally low, but a relatively high value on Day 210 (Figure 5-15), is linked with a peak in the perid/chla ratio (Figure 5-16). However, this peak does not correspond to any of the high chl events.

Chlb concentrations showed two early peaks (Figure 5-15). The chlb/chla ratio has one peak, which is related to a low chla sample. Allo concentrations were low and variable. Similarly, the allo/chla ratios were low, throughout the high chl events.

None of the major pigments and their ratios with chla explains the third chl event, suggesting that other phytoplankton groups contributed to this event (perhaps small flagellates belonging to Chlorophyceae, Prasinophyceae).

Figure 5-15: Temporal distributions of phytoplankton chla and accessory pigments, at NWN, 2003.

[The numbers indicate high Chl events. No HPLC samples were collected before Day 120]

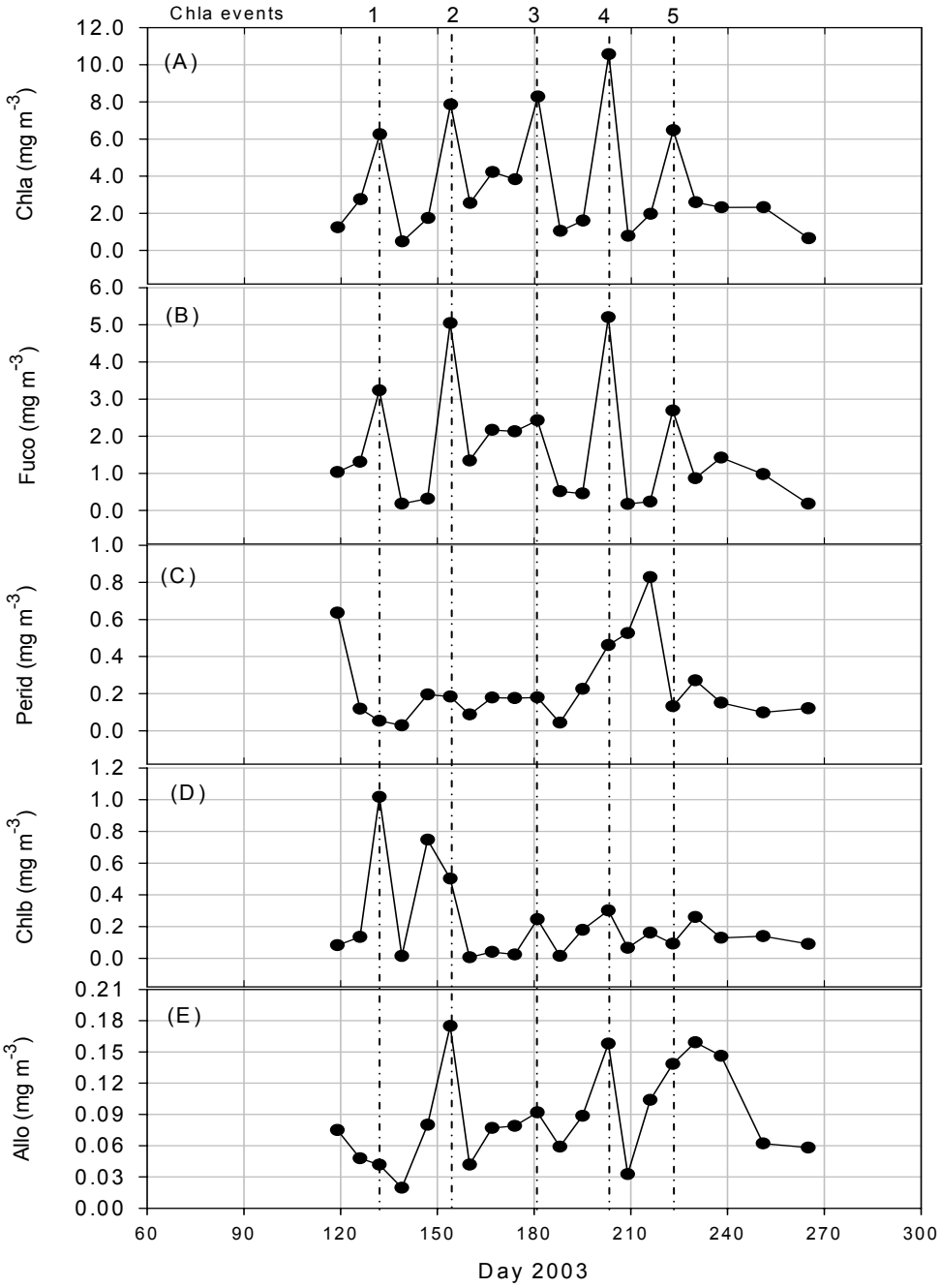
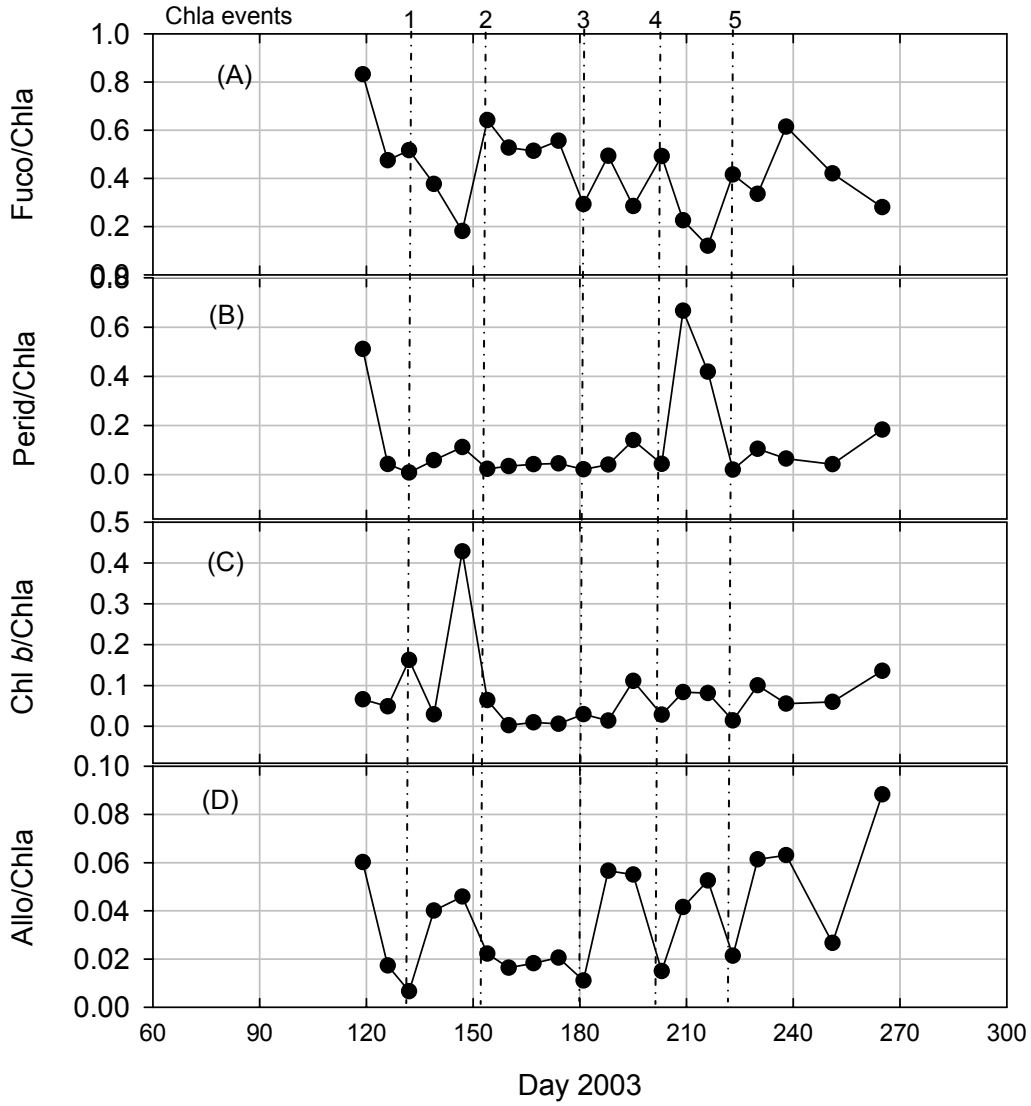


Figure 5-16: Temporal distributions of accessory pigment to chlorophyll a *ratios, at NWN, 2003.*

[The numbers indicate high chl events. No HPLC samples were counted before Day 120]



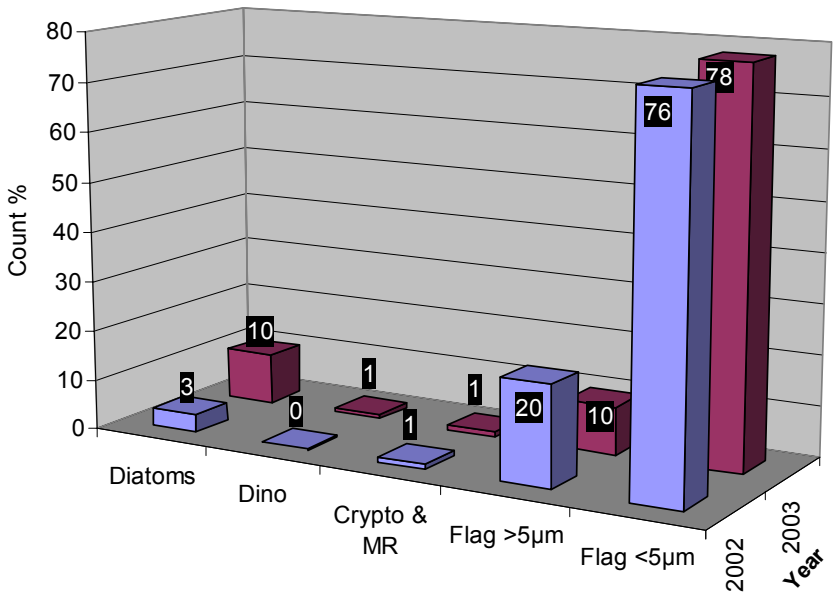
5-4 Phytoplankton taxonomic data

5-4-1 Phytoplankton cell abundance and carbon biomass

The cell count percentages for the main phytoplankton groups at NWN during 2002 and 2003 are shown in Figure 5-17. The dominant species during 2003 and 2002 were the <5µm flagellates, which represented about 78% and 76% of total counts for

the two years, respectively. Larger cells accounted for the remaining percentages and in 2003, they consisted mainly of diatoms and >5µm flagellates.

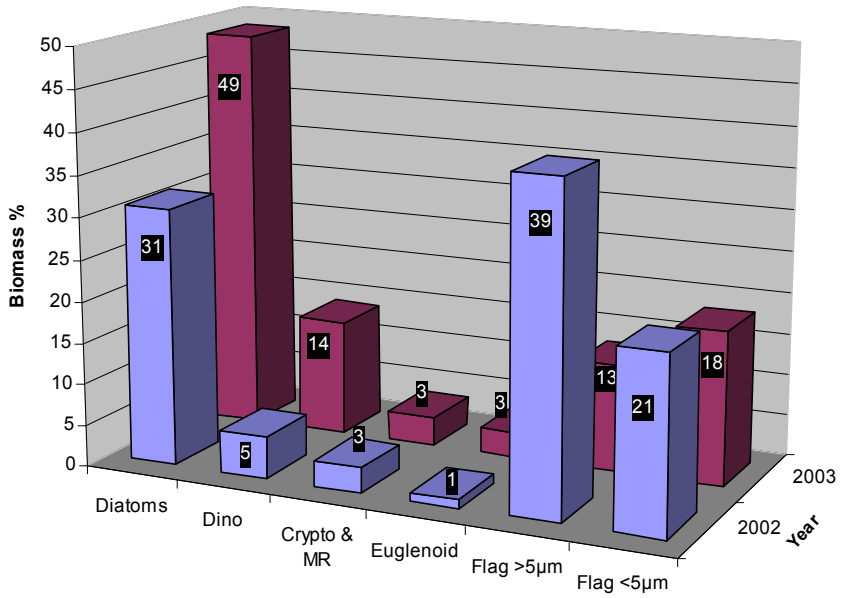
Figure 5-17: Cell count percentages of main phytoplankton groups, at NWN, 2002 and 2003.



When the cell counts are transformed into carbon biomass, the small (<5µm) flagellates are shown to be much less important compared to other groups (Figure 5-18).

In 2002, the >5µm flagellates and diatoms were the larger components of biomass, but as in 2003, diatoms represented the dominant group. The greater importance of diatoms in 2003 was accompanied by increases in other large cell (dinoflagellates, eugelonoids, Cryptophytes) and also in higher total biomass, as measured by chlorophyll.

Figure 5-18: Biomass percentages of the main phytoplankton groups, at NWN, 2002 and 2003.



As expected, measured chlorophyll concentrations and estimated phytoplankton carbon were positively correlated (Figure 5-19), and the slope (C/chl ratio) was ~15.0 The best fit between chlorophyll and phytoplankton carbon is found when it is assumed that 50% of each group of flagellates is heterotrophic, similar to what was observed for Dock in Chapter 3.

Several samples show high biomass values that lie outside the 99% confidence interval. Those with low chl values contained relatively abundant dinoflagellates, suggesting that autotrophic biomass was overestimated, due to the presence of heterotrophic cells. Other samples were dominated by diatoms, which change in size during their life cycle. If the dimensions of these cells were smaller than the values used for biomass calculations (see Methods), again carbon biomass would tend to be overestimated.

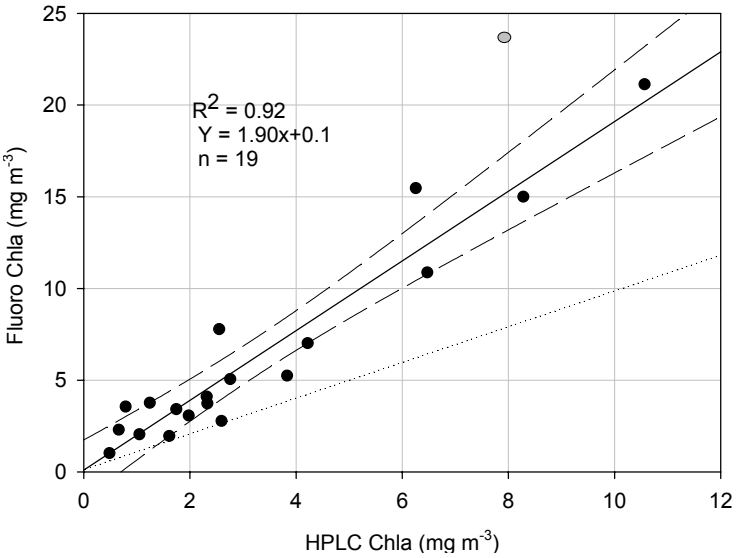


Figure 5-19: Correlation between phytoplankton biomass and chl, at NWN, 2003.

[The dashed lines are the boundary for the 99% confidence intervals. The white colour point is recalculated from Tchl to sum Fchl data and not included in the regression. The slope when regression is forced through zero is 20.9 while it force to zero]

5-4-3 Phytoplankton species composition

The phytoplankton cell counts and biomass values for the five chl events, identified in Figure 5-9, are summarised in Tables 5-2 and 5-3, respectively. Apart from the flagellates, by far the most numerous cell type was diatoms. The diatom populations were dominated by one or two species (e.g. Days 132, 181, 202, 223) but on one occasion (Day 153), many species were present in appreciable numbers. The diatom cell numbers tended to increase from early samples to late ones. By contrast, numbers of flagellates (>5µm and <5µm) were very variable with no clear seasonal trend.

Table 5-2: Phytoplankton cell counts (cells ml^{-1}) for the five high chlorophyll a events at NWN, 2003.

[The table includes all species contributing >1 cell ml^{-1}]

chl events	**1**	**2**	**3**	**4**	**5**
Day	**132**	**153**	**181**	**202**	**223**
chl mg m^{-3}	**15.5**	**24.2**	**12.3**	**21.1**	**10.9**
Diatoms					
Chaetoceros spp		145	1539	20208	604
Ditylum brightwelli			11		
Eucampia spp		60			
Guinardia delicatula	562	11			9
Guinardia flaccida	6	2			
Guinardia striata		3			
Lauderia		334			
Lithodesmium			9		
Nitzschia longissima		3			
Odontella mobiliensis					4
Pennate	3	3			
Pseudo -nitzschia sp	2	35			
Rhizosolenia imbricata		3			
Skeletonema costatum		103	26		1257
Thalassionema frauenfeldii		13			9
Thalassiosira rotula		127	4	23	
Thalassiosira hyalina		50		23	14
Total diatoms	**575**	**892**	**1589**	**20246**	**1897**
Dinoflagellates					
Gymnodinium sp1	5				8
Gymnodinium sp2			69	75	31
Prorocentrum micans				4	52
Scrippsiella trochoidea		44	27	36	33
Total dinoflagellates	**5**	**44**	**96**	**115**	**123**
Other groups					
Mesodinium rubrum	2	30	4	13	
Cryptomonas spp		70	78	155	32
Eutreptiella sp			11	4	5
Flagellates<5 µm	10112	23500	8074	8100	21000
Flagellates>5 µm	476	1600	6703	620	4500

Table 5-3: The phytoplankton biomass (mg C m^{-3}) of the dominant species, for the five high chl events, at NWN, 2003

[The table includes all species contributing >1 mg C m^{-3}]

chl events	**1**	**2**	**3**	**4**	**5**
Day	**132**	**153**	**181**	**202**	**223**
chl (mg m^{-3})	**15.5**	**24.2**	**12.3**	**21.1**	**10.9**
Total carbon (mg m^{-3})	**230.0**	**380.0**	**214.0**	**386.6**	**347.5**
Diatoms					
Chaetoceros spp		4	46	303	53
Coscinodiscus					
Ditylum brightwelli			6	1	
Eucampia spp		16			
Guinardia delicatula	169	3			3
Guinardia flaccida	17	6			
Guinardia striata	2	4			
Lauderia		156			
Lithodesmium			2		
Odontella mobiliensis					26
Odontella sinensis				2	
Pleurosigma				1	
Rhizosolenia imbricata	1	9			
Rhizosolenia setigera	1		2		
Skeletonema costatum		1			15
Thalassionema frauenfeldii		1			1
Thalassiosira rotula		41	1	8	
Thalassiosira hyalina		11		5	3
Total diatom	**190**	**253**	**57**	**319**	**101**
Dinoflagellates					
Gymnodinium sp1	1				1
Gymnodinium sp2			2	3	1
Prorocentrum micans				7	88
Scrippsiella trochoidea		20	12	17	15
Total dinoflagellates	**1**	**20**	**14**	**27**	**105**
Other groups					
Mesodinium rubrum	1	8	1	4	
Cryptomonas spp		1	1	2	
Eutreptiella sp			2	1	1
Flagellates<5 µm	30	70	24	24	63
Flagellates>5 µm	8	27	114	11	77

When cell numbers are converted to carbon biomass a different picture emerges (Table 5-3). Chl events 1, 2 and 4 were dominated by diatoms, the 3rd chl event by

large flagellates >5µm, and the 5th chl event by a mixture of diatoms, dinoflagellates and both size groups (>5µm and <5µm) of flagellates.

The most important species were the diatoms *Guinardia delicatula, Lauderia and Chaetoceros* spp., and the dinoflagellate *Prorocentrum micans*.

Other taxa (*Mesodinium, Cryptomonas and Eutreptiella*) showed low biomass values. The highest biomass estimates for total flagellates (<150 mg C m^{-3}) were considerably lower than the maximum values for diatoms (>300 mg C m^{-3}).

The lower chl samples (<10 mg m^{-3}, not presented in the Table) were normally associated with moderate densities of diatoms and dinoflagellates.

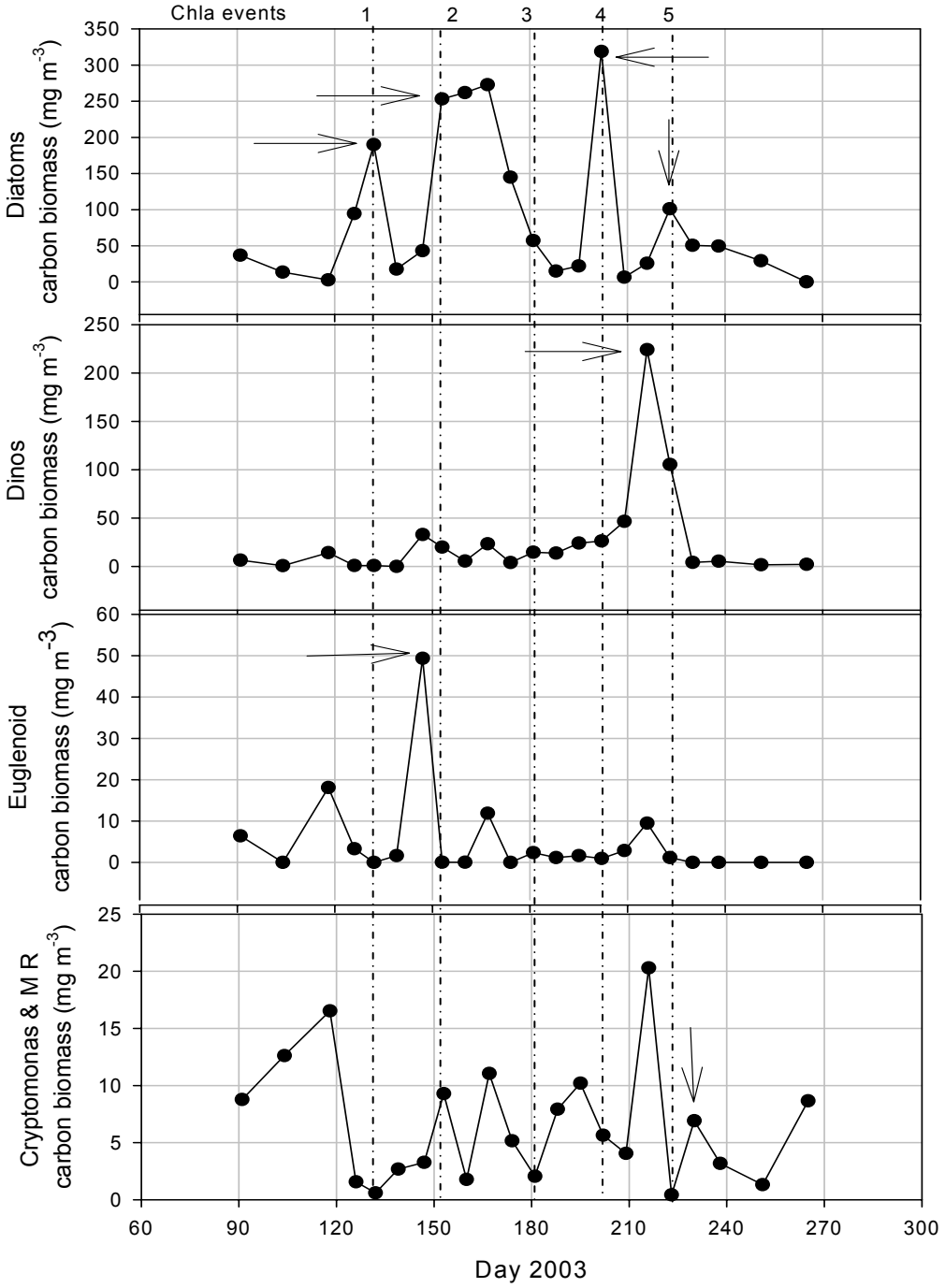
5-4-4 Seasonal succession of phytoplankton

Phytoplankton seasonal succession and the absolute contributions of different phytoplankton groups are shown in Figure 5-20, for the whole sampling period rather than selected days, with the five chl events indicated by dashed lines and arrows indicating taxa signature.

The diatom population is represented by the species data in Table 5-3. The second event (Day 153) appeared to present for more than one week. The diatom was similar mixed populations, with two species of *Thalassioiosira* sp. highly present on Day 160 and 167. The main biomass peaks for dinoflagellates (Day 216, *Scrippsiella trochoidea* and *Gymnodinium* sp2), euglenoid (Day 147, *Eutreptiella* sp) and *Cryptomonas* / *Mesodinium* (Day 216) occurred when the levels of chl was < 10 mg m^{-3}.

Figure 5-20: Succession of the main phytoplankton groups, at NWN, 2003.

[The numbers indicate the chl events. The arrows indicate peaks in ratios of accessory pigment to chlorophyll a (see Figure 5-15): >0.4 for fuco for (diatoms), >0.4 for perid (Dinoflagellates), ~0.4 for chlb (Euglenoid) and >0.06 for allo (*Cryptomonas* and *Mesodinium rubrum* (low ratio)].



5-4 Phytoplankton production

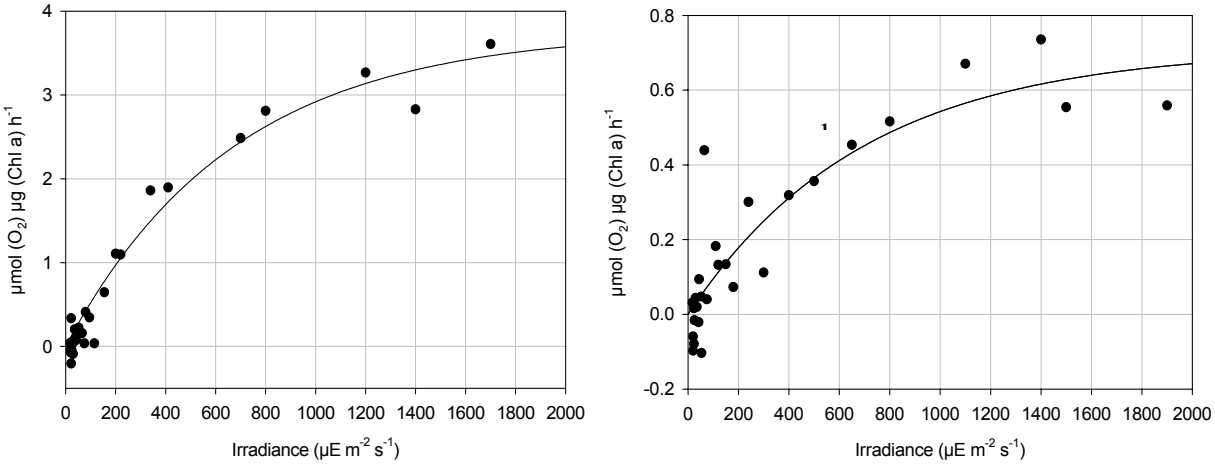
5-5-1 Oxygen production

The physiological activity of the phytoplankton was measured as gross oxygen production. Four sets of measurements were obtained for total phytoplankton (TP) and <5 µm fraction phytoplankton (FP). The results obtained from the experiment were normalised against chl and fitted with the Webb *et al*. (1974) equation, using a Sigma plot (V. 0.8) program:

$$P = P_m (1-\exp (-I^* \alpha / P_m))$$

Where P is oxygen production (µM (O_2) µg (chl) h^{-1}), P_m is the maximum O_2 production (µM (O_2) µg (chl) h^{-1}), E is the irradiance (µE m^{-2} s^{-1}) and α is the initial slope (µM (O_2) µg (chl) h^{-1} (µE m^{-2} s^{-1})$^{-1}$).

Figure 5-21: Phytoplankton oxygen production curve (P vs E) from NW Netley, a) total phytoplankton (16-June 2003) and b) <5 µm phytoplankton (16-July 2003).



P-E curves for TP and FP samples are shown in Figure 5-21. Photosynthetic characteristics determined from the oxygen measurements are summarised in Table 5-5. The P_m values ranged between 4.13 and 7.40 µM (O_2) µg (chl) h^{-1}. The set of TP 16-June had the highest E_k (the saturation parameter) (628 µE m^{-2} s^{-1}) and α [µM O_2 µg Chl h^{-1} (µE m^{-2} s^{-1})], with the highest P_m and relatively high chl. Increased P_m

values positively coupled with an increase in α and E_k value, apart from set TP 7-July which has the lowest R^2, and can be ignored.

Out of 4 sets of Fchl <5µm, only 1 set of Fchl <5µm attained obtained photosynthetic characteristics. The Fchl<5µm represented 80% of the Tchl. This fraction showed relatively low values of α and high E_k, while the P_m was lower than total phytoplankton. The oxygen production was correlated positively with chl values and the best correlation was associated with chl values of >3mg m^{-3}.

Table 5-4: Parameters of the oxygen production curve, for total (TP) and, <5µm Fchl(FP) (phytoplankton).

Series	P_m	slope (α)	E_k	R^2	Tchl/Fchl	%Fchl <5 µm
TP 16-June	3.8	0.006	628	0.96	8.1	32
TP 23- June	2.6	0.001	1600	0.95	6.0	37
TP 30- June	4.98	0.009	553	0.96	12.3	30
TP 7-July	1.7	0.009	189	0.65	2.1	32
FP 9- July	~1.1	Nd	Nd	Nd	0.5	34
FP 11- July	Nd	Nd	Nd	Nd	1.8	82
FP 14- July	~1.1	Nd	Nd	Nd	1.8	58
FP 16- July	0.7	0.001	710	0.91	4.6	80

P_m = µM O_2 µg chl h^{-1}, α = µM O_2 µg chl h^{-1} (µE m^{-2} s^{-1})$^{-1}$, E_k = µE m^{-2} s^{-1}, chl = µg l^{-1}, Respiration rate = µM O_2 µg chl h^{-1},Nd =no data

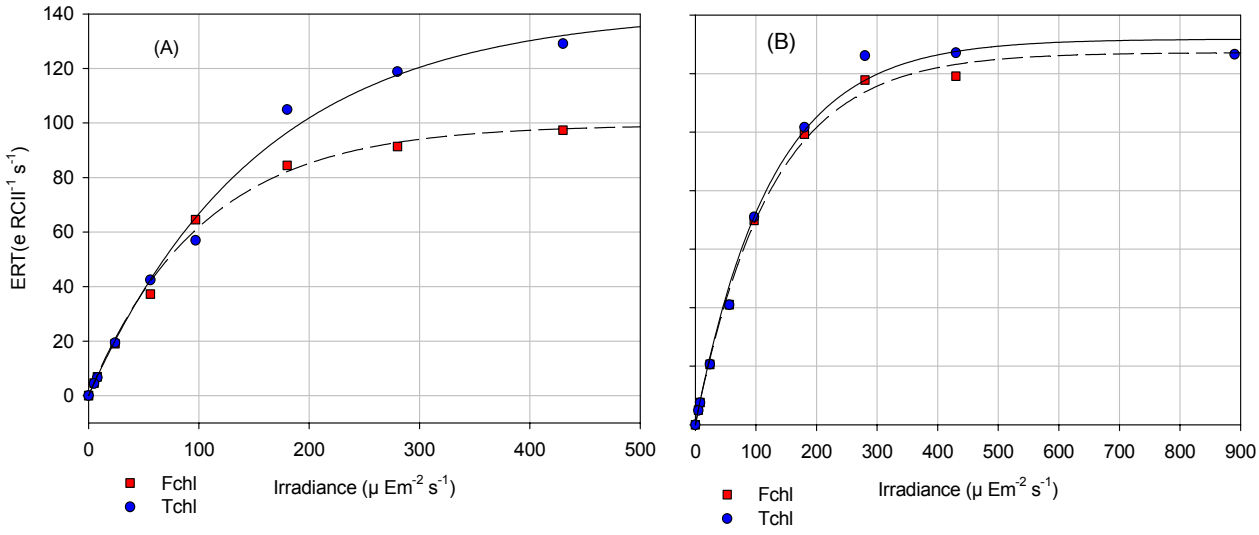
Comparison between the oxygen production of Tchl and Fchl could not be made because the sampling days were different. Also, there was insufficient data from the Fchl sets. However, the set of FP 16- July had 80% of the Tchl, although it had lower P_m and higher E_k than that observed for Tchl.

5-5-2 Fast Repetition Rate Fluorometer (FRRF)

The Fast Repetition Rate Fluorometer (FRRF) is an instrument that has recently been used to estimate phytoplankton production *in situ* in marine waters (Kolber *et al.*, 1998; Suggett *et al.*, 2001). Electron transfer rate (ETR) *vs* E curve was plotted using the FRRF parameters for Tchl and Fchl <5um (Figure 5-22). The FRRF measures fluorescence yield from the photosynthetic reaction centre PSII, which is a ratio of photons emitted to photons absorbed. As a result of reactions that occur in the PSII,

fluorescence and oxygen yields can be measured. The FRRF parameters are shown in Table 5-6. The FRRF provides the functional absorption cross-section of PSII (σ_{PSII}) (ETR) and variable fluorescence (F_v), which is calculated from maximum fluorescence (F_m) minus minimum fluorescence yield (F_0); photochemical efficiency is a ratio of F_v/F_m .

Figure 5-22: Phytoplankton production curve (ETR vs E) for Tchl and Fchl (<5 µm) samples from NWN, a) 11 July, 2003 and b) 16 July, 2003.



Out of 8 sets of measurements only two (11, 16 June) gave consistent results for <5µm Fchl and four for Tchl. However, Tchl on 16 June FRRF parameter measurements showed the highest value of ETR max ($eRCII^{-1}s^{-1}$), associated with high slope ($eRCII^{-1}s^{-1}$ (µM photons m^{-2} s^{-1})$^{-1}$), high photochemical efficiency (F_v/F_m), and low E_k (µM photons m^{-2} s^{-1}), while chl was at a high value compared with other Tchl data measured.

The FRRF parameters measured for Tchl and compared with two Fchl sets, 11 July and 16 July, are shown in Figure 5-22 and Table 5-6. The 11 July set has higher parameters than 16 July, which has relatively similar data for both Tchl and Fchl. The Tchl (11 July set) electron transfer rate ETR was higher than that of Fchl, as were the other parameters Ek, F_v/F_m and Sigma PSII.

Table 5-5: The FRRF parameters of different phytoplankton samples for Tchl and Fchl (<5 μm), from NWN.

Series	Fv/Fm	Sigma PSII	E_k	ETR max	slope	chl
T16June	0.50	477	128	179	1.7	8.1
T11July	0.36	440	182	141	0.9	2.2
F11July	0.39	405	126	99	1.0	1.8(82%)
T14July	0.40	428	115	112	1.2	3.2
T16July	0.38	463	160	131	1.1	5.7
F16July	0.37	466	151	127	1.0	4.6(80%)

E_K(μM photons m^{-2} s^{-1}), Sigma PSII (X10-20 m^2 $quanta^{-1}$), ETR ($eRCII^{-1}s^{-1}$), slope (($eRCII^{-1}s^{-1}$ (μM photons m^{-2} s^{-1})$^{-1}$) and chl (μg l^{-1})

The difference between the two fractions in the set of 11 July suggests that there is physiological difference between the Tchl and Fchl, which means that the phytoplankton content is different. The difference between the fractions is not clear in the set of 16 July since their parameters are relatively matching. This reflects similar phytoplankton content.

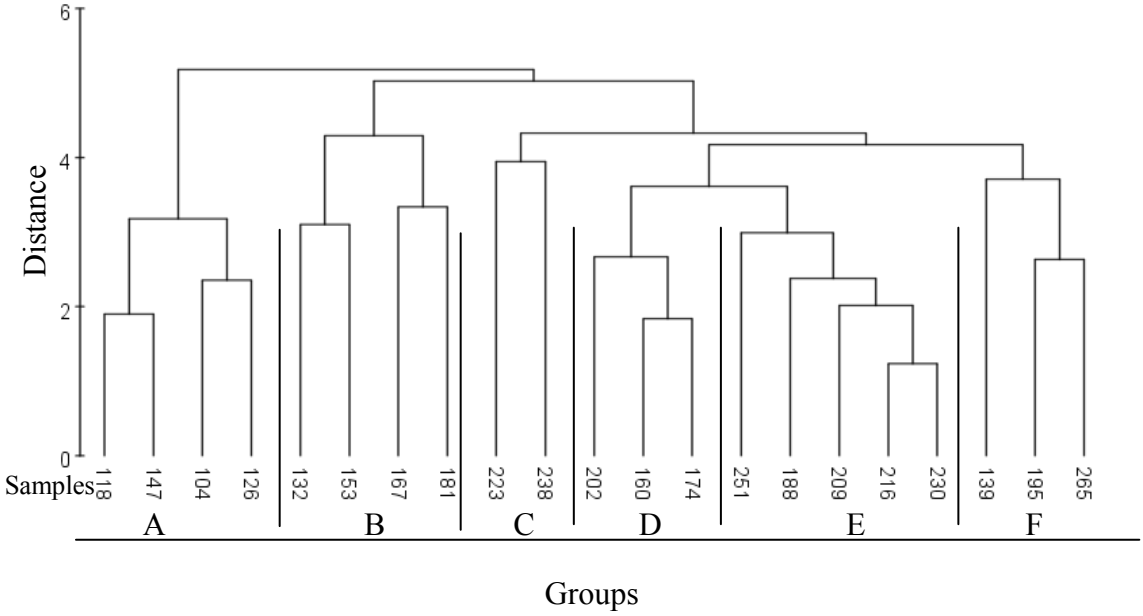
5-6 Data analysis and interpretation

5-6-1 Environmental data

Data for environmental parameters were normalised by log transformation to calculate the Euclidean distance. The environmental variables (air temperature, PAR, water salinity, water temperature, tide range, K_d, nutrients (N, P, Si) and chl) were clustered to give groups with the lowest distance between pairs of samples.

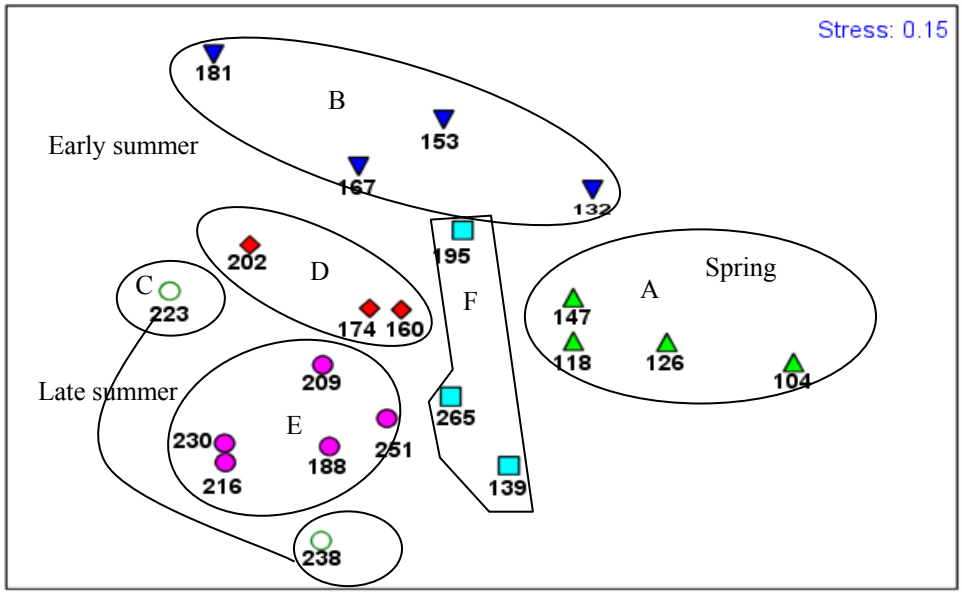
Groups A-F were defined by the dendrogram for hierarchical clustering (Figure 5-23). The Euclidean distance between most of the groups (B-F) was <4 %, while the distance between groups A and the other groups was ~5%. Each group had between 2 and 5 samples. The spring samples were all in group A, early summer samples in groups B, D and F, and the late summer samples in groups C and E. Few sampling dates fell in groups out of their season. The exceptions were samples 147 in group A, 188 in group E and 265 in group F.

Figure 5-23: Dendrogram for hierarchical clustering of samples defined by environmental parameters, at NWN, 2003.



The MDS plot of the data provides a two-dimensional distance plot (Figure 5-24), which shows more clearly how the samples were grouped. A stress of (0.15) indicated a good representation of the data.

Figure 5-24: MDS plot of environmental parameter groups, with each sample identified by day, at NWN, 2003.



Group specifications are summarised in Table 5-7. The main factors differentiating the groups were irradiance value (PAR), nutrient levels and salinity. PAR was more important for groups B, C and F, and represented periods of highest light levels. Groups A and E represented periods of relatively low light and high nutrients. Groups B and C had a higher level of salinity. Group D showed intermediate characteristics between the early summer groups and late summer groups.

Table 5-6: Main characteristics of sample groups defined by environmental parameters.

[Numbers indicate sampling date; bold represents higher chl samples]

Groups	Sample (Day)	Specification	Similarity percentage
A	104, 118, 126, 146	PAR (39), N(16), salinity (14), Si (14)	97
B	**132**, **153**, 167, **181**	PAR(43), salinity (15), N(13)	93
C	**223**, 238	PAR (41), salinity (16), N(13)	93
D	160, 174, **202**	PAR (40), salinity (14), N(14), Si (10)	97
E	188, 209, 216, 230, 251,	PAR (39), salinity (14), N(14), Si (12)	98
F	139, 265, 195	PAR (42), salinity (14), N(13), Si (11)	96

These groups included higher chl levels samples, characterised by relatively high PAR and relatively lower nutrient levels and the highest salinity value. This suggests a relationship between light irradiance, depletion of nutrient and salinity on the one hand, and on the other hand increasing chl (phytoplankton) values. By contrast, groups A and E have relatively low light irradiance and high nutrients associated with low chl.

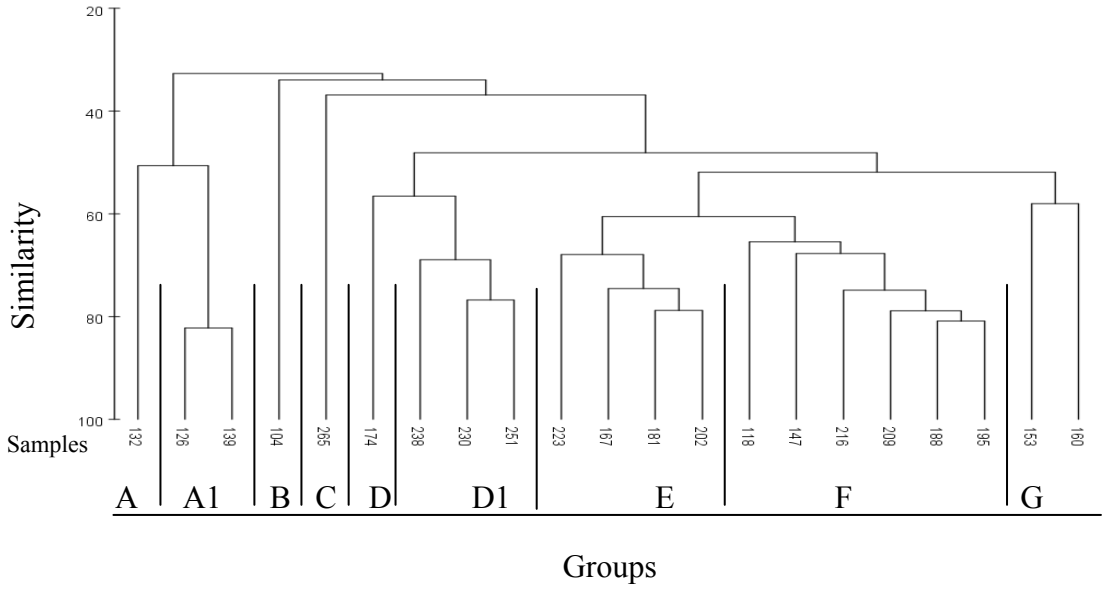
5-6-2 Phytoplankton data

Phytoplankton biomass data

The phytoplankton data were normalised and transformed to the 4^{th} root before calculating the Bray-Curtis Similarity index. The data were restricted to species representing >1% of total biomass content during the sampling period. The results

from Hierarchical clustered analysis are plotted as a dendrogram (Figure 5-25) to indicate the species similarity distribution. Seven groups were defined, 5 with a similarity level of <55 % (A and D-G) each group having ≥2 samples. The other two groups B and C contained a single sample with a similarity level of ~35%. The low similarity of single groups can be attributed to the presence of single species in each group. Group A was split into two subgroups, A and A1; similarly group D was split into subgroups D and D1 to increase the level of similarity.

Figure 5-25: Dendrogram for hierarchical clustering of samples defined by phytoplankton species/taxon biomass, at NWN 2003.



A two-dimensional MDS plot (Figure 5-26) with a stress of 0.16 gives an acceptable representation of the data. Group distribution follows seasonal patterns: groups A, A1, B and G are spring samples, C and D1 are late summer-autumn communities, D and E are summer season groups, the F group being mixed.

Further analysis of the groups was obtained from species biomass contributions (SIMPER). The results are summarised in Table 5-8.

The phytoplankton succession can be described as starting with a mixed sample (group B), shifting to spring diatom bloom (groups A, A1, D and G), with *Guinardia delicatula* and *Thalassiosira rotula* as the dominant species. The summer samples

(groups E and D1) contained small diatoms (*Chaetoceros* sp), together with dinoflagellates, such as *Scrippsiella* sp. In the mixed group F, dinoflagellate were present and they include *Eutreptiella* sp., *Mesodinium rubrum, Cryptomonas* sp. *Cryptomonas* sp. contributed especially highly to the late summer group C.

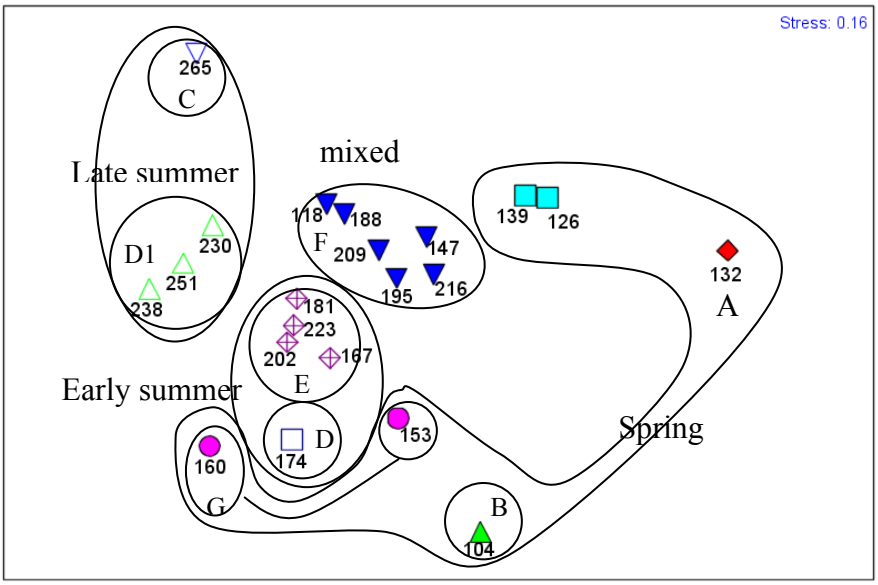


Figure 5-26: MDS plot of samples defined by phytoplankton species/taxa, at NWN, 2003.

Table 5-7: The main phytoplankton species/ taxa of sample groups, at NWN, 2003.

Groups	Samples (Day)	Species (%) contribution to group	% of similarity
A	**132**	*Guinardia delicatula*	
A1	126, 139	*Guinardia delicatula* (30), *Eutreptiella* (22), *Mesodinium rubrum* (19), *Cryptomonas* sp (17), *Gymnodinium* sp2 (13)	82
B	104	*Cryptomonas* sp (31), *Scrippsiella* (30), *Chaetoceros* sp (27).	60
C	265	One sample (*Cryptomonas sp*)	
D	174	*Rhizosolenia setigera*	
D1	238, 230, 251	*Chaetoceros* spp (45), *Scrippsiella* (23), *Cryptomonas* (20)	72
E	167, **181**, **202, 223**	*Chaetoceros* spp (25), *Scrippsiella* (19), *Gymnodinium* sp2 (11), *Eutreptiella* (10)	72
F	118, 147, 188, 195, 209, 216	Scrippsiella (19), *Gymnodinium* sp2 (17), *Mesodinium rubrum* (16), *Eutreptiella* (15), *Cryptomonas* sp (15), *Prorocentrum micans* (9)	71
G	**153**, 160	*Thalassiosira rotula* (30), *Thalassiosira hyalina* (21), *Scrippsiella* (18), *Chaetoceros* sp, (13), *Cryptomonas* sp (12)	58

Summer groups A, A1, D, D1, E and G were dominated by diatom taxa, and many of them have higher chl samples. Group F was dominated by the dinoflagellates, while groups B and C were dominated by the *Cryptomonas* sp. taxon.

Phytoplankton taxon data

The taxonomical distribution of carbon biomass (diatoms, dinoflagellates, *Eutreptiella*, *cryptomonas*, flagellates <5> µm) was analysed using the Bray-Curtis similarity measurement, after 4th root transformation. Seven groups, A-G, were obtained from the hierarchical clustered dendrogram, with a level of similarity of 80-95% (Figure 5-27). Groups A and F were single samples, while other groups contained more than two samples.

The phytoplankton groups obtained in Figure 5-27 can be shown on the MDS ordination for the same data, with distribution of the groups on a two-dimensional plot (Figure 5-28).

Figure 5-27: Dendrogram for hierarchical clustering of samples defined by biomass of phytoplankton taxa, at NWN, 2003.

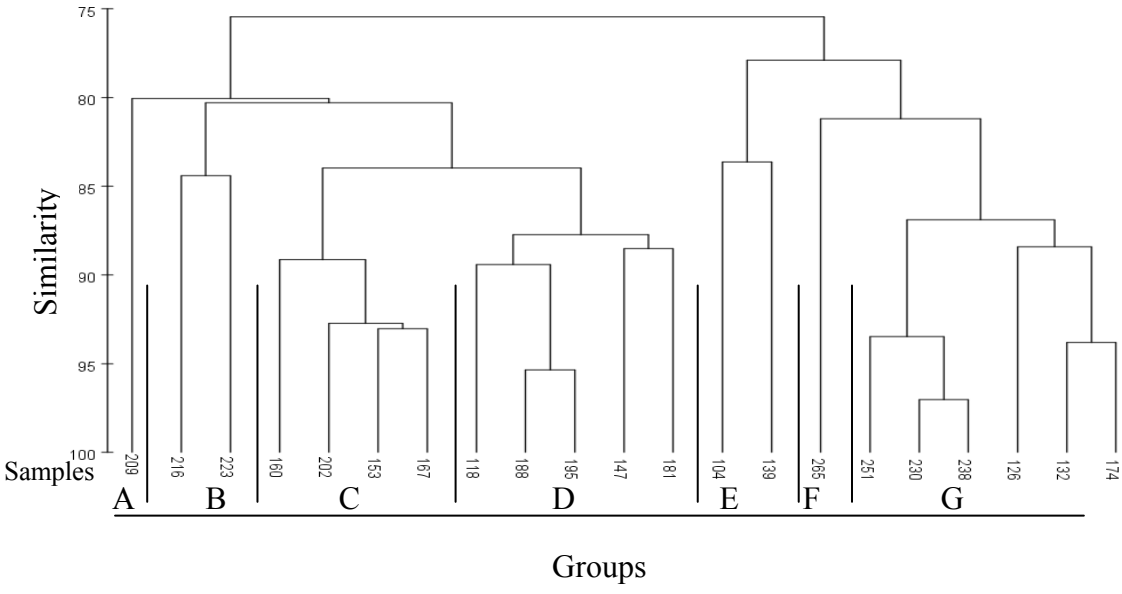
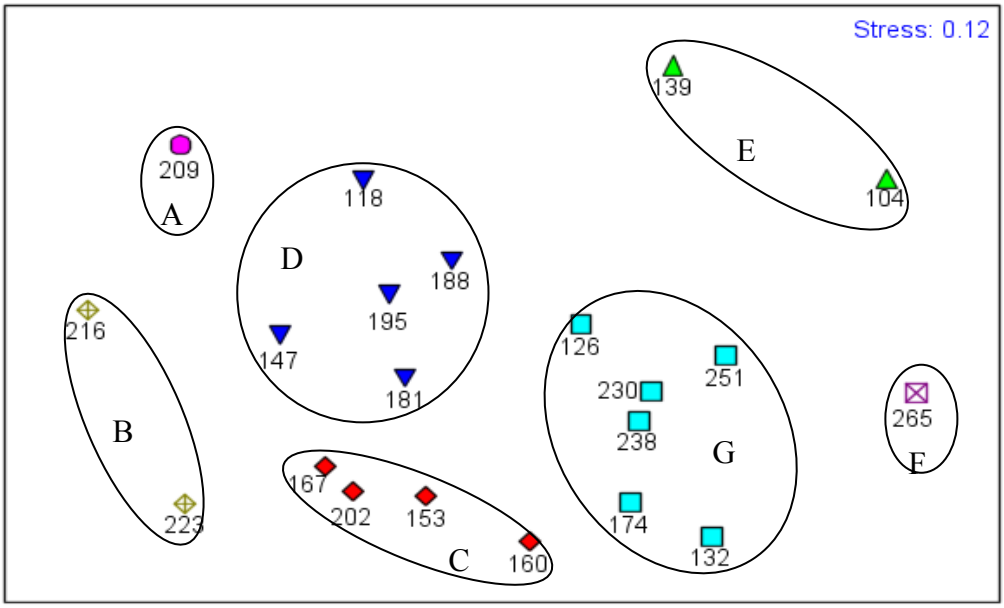


Figure 5-28: MDS plot of the sample defined by phytoplankton group (taxon, cell size), numbers indicating sample days, at NWN, 2003



The group specifications are summarised in Table 5-8. The dinoflagellate taxa were the most important component of group B (late summer group), and diatoms of group C (early summer sample). Group D had mixed taxa, representing spring/ early summer. The flagellates (<5µm taxa) highly contributed in the spring and late summer samples (groups D, E and G). *Cryptomonas* sp contributed to many groups with different values, but highly contributed ~~in~~ to group B. The single sample groups, such as group A, were due to a low contribution of >5, <5 µm flagellates, and group F had low diatom contribution (for example, *Cryptomonas* sp) in summer samples.

Table 5-8: Phytoplankton taxon groups, percentage of contribution to groups, and similarity percentages, at NWN 2003.

Groups	**Samples (Day)**	**(%) contribution to group**	**% of similarity**
A	209	One sample	
B	216, **223**	Dinoflagellates (23), *Cryptomonas* (23), Diatoms (16), Flagellates<5 (16), Flagellates>5(14)	84
C	**153**,160, 167, **202**	Diatoms (32), Flagellates<5 (17), Flagellates>5 (16), Dinoflagellates (15), *Cryptomonas* (15)	91
D	118, 147, **181**, 188, 195	Flagellates<5 (22), Dinoflagellates (17), Cryptomonas (17), Flagellates>5 (16), Diatoms (16), *Eutreptiella* (11)	89
E	104, 139	Flagellates< 5(32), Diatoms (30), Flagellates>5 (16), Dinoflagellates (11), *Cryptomonas* (11)	84
F	265	One sample	
G	126, **132**, 174, 230, 138, 251	Flagellates<5 (30), Diatoms (27), Flagellates>5 (20), Dinoflagellates (12) *Cryptomonas* (12)	89

Flagellates (<5µm) were the highest contributors to many groups, followed by diatoms and dinoflagellates. This gives a different picture of the phytoplankton community distribution in terms of taxon biomass. The results are also consistent with a previous section but with a different grouping.

Accessory pigment analyses

Data for the main HPLC pigments (excluding the ubiquitous chlorophyll *a*) were normalised by square root transformation to calculate the Bray Curtis Similarity Index, and hierarchical clustering (not shown) was used to define groups with similarities in the range of 85-93%. Each group had >3 samples, except group D, with one sample and group A, with two samples. The group specifications are summarised in Table 5-9.

Table 5-9: Main characteristics of sample groups defined in terms of photosynthetic pigment composition.

[The high chlorophyll samples are identified by sample days in bold].

Group	Samples (Day no.)	Pigment (% contribution)	%of similarity
A	139, 188	fucoxanthin (43), peridinin (27), chlb (23)	85
B	167, 174, **181**	fucoxanthin (51), peridinin (27), chlb (17),	91
C	**132**, **153**, **202**	fucoxanthin (43), chlb (24), peridinin (17), alloxanthin (16)	91
D	160	fucoxanthin higher than others	
E	209, 216, 265	peridinin (29), fucoxanthin (28), chlb (23), alloxanthin (19)	91
F	147, 195, 230	fucoxanthin (29), peridinin (25), chlb (25), alloxanthin (20)	93
G	119, 126, **223**,238, 251	fucoxanthin (38), peridinin (22), chlb (21), alloxanthin (18)	93

Fuco dominated most of the groups, while groups B and C included high chl samples in which diatoms (*Guinardia delicatula*, *Thalassiosira* sp., and *Rhizosolenia setigera*) were observed.

Perid characterised group E as the dominant accessory pigment associated with the mixed dinoflagellate species (*Scrippsiella* sp and *Gymnodinium* sp.2). Group C has a relatively higher chlb contribution than other groups (*Eutreptiella* sp).

This result is associated and consistent with earlier sections but with a different grouping.

5-6-3 Relation of environmental parameters and biological parameters

Further analysis to relate environmental parameters with biotic data (species carbon biomass, phytoplankton taxa, and chlorophyll size fractions) was carried out using the BIOENV routine of PRIMER (Table 5-10), which calculates the highest degree of correlation (ρ) (best matching). The best correlation factors for the three biotic groups were identified: species carbon biomass correlated with K_d, tide, N and P (ρ

= 0.24); taxon biomass correlated with tide, Si and N (ρ = 0.22) and chlorophyll *a* size fraction correlated with P (ρ = 0.14).

Table 5-10: BIOENV analyses of the correlation factors (best variables) of different biotic data

Group	Single variable (ρ) value	Best variable correlation (ρ)
Species carbon biomass	K_d (0.15), Tide (0.14), N (0.07) and P (0.06)	K_d Tide, N, P, (0.24)
Taxon	Tide (0.19), Si (0.11), N (0.06) and K_d (0.06)	Tide, Si, N (0.22)
chl size fraction	P (0.14)	P (0.14)

These factors could affect phytoplankton in different ways, directly or indirectly. For example, K_d indicates indirectly the importance of light through the water column, which is linked to PAR (incident light). It also indicates a clearer water column, which affects phytoplankton growth, due to the level of irradiance (photic zone). However, BIOENV analysis did not emphasize PAR directly as an outstanding correlation factor, but it was indirectly emphasized in the form of K_d. Other than this, PRIMER ignored the incident light as a determinative factor in the grouping. This is understandable, because sampling was carried out during periods of comparable PAR, while PAR was obtained as a mean factor controlling phytoplankton (Chapter 3).

Tidal range is another factor, introducing an energy input to the water column. It has an affect on phytoplankton accumulation or distribution in a similar way as stratification, water column mixing, flushing out, increasing the K_d (water turbidity) and nutrient level.

Inorganic nutrients were also a factor that could control phytoplankton growth, generally N and P, and Si particularly for diatoms. Si depletion could limit diatom accumulation and change the phytoplankton community to taxa not require Si for their growth as flagellates. In terms of chl fractions, P is detected as a feature related

to size fraction (a similar observation was recorded in earlier chapters), perhaps due to P being a limiting factor for large phytoplankton (diatoms and dinoflagellates).

This result in general was similar to that obtained from earlier chapters. However, here the K_d and tidal range was related to species carbon biomass, while PAR was also related to species carbon biomass at Dock and estuarine stations (2002).

5-7 Discussion

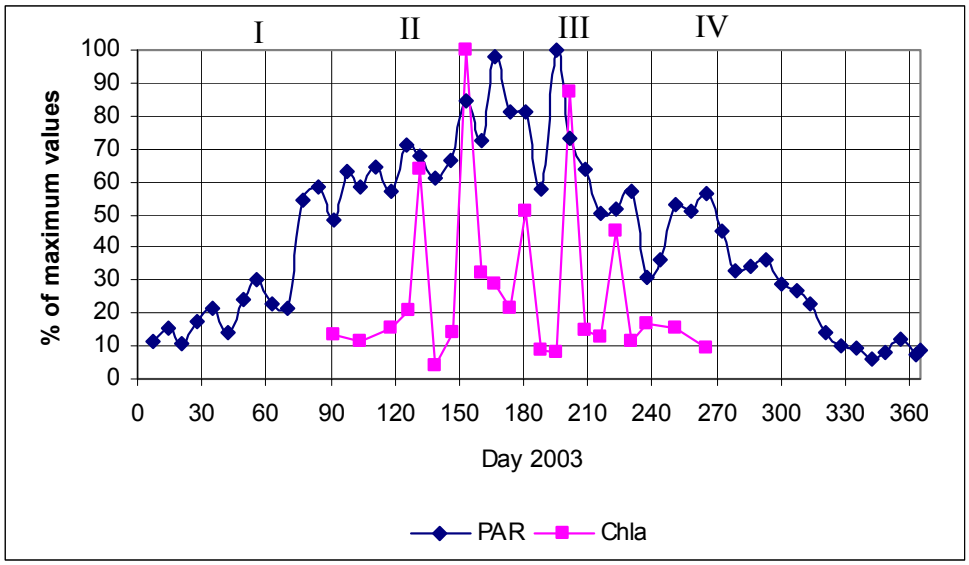
The distributions of chlorophyll and PAR at NWN in 2003 (Figure 5-29) are typical for Southampton Water (Kifle, 1992; Kifle and Purdie, 1993; Crawford *et al.*, 1997; Ali, 2003). As for 2002, the 2003 observations could be divided into distinct phases (phase I up to Day 90 was not sampled): Phase II (Days 90-120) was characterised by relatively low light (60-70% of maximum PAR attained) and low chl values, apart from one chl peak on Day 130, phase III (Days 120-240) was characterised by high irradiance (~ 80% of maximum on average) and intermittent high chl values, and phase IV (Days 240-270) was characterised by decreasing PAR associated with decreasing chl values.

The chl values in 2003 were consistent with those observed in 2002. However, chl peaked earlier in 2003 and exhibited wide fluctuations over a weekly time scale. Low values of chl, typical of winter conditions, were observed on Days 138, 185 and 195. Although, PAR was relatively low (60% of the maximum) at these times, it is likely that other factors, such as sinking to the bottom and/or high grazing rate and perhaps tidally-induced mixing and flushing, contributed to the reduction of phytoplankton biomass in surface water.

The phases that are described above for NWN in 2003 were comparable with those observed in 2002 at Dock station (Figure 3-30). Phase II in 2002 at Dock had a similar level of light, but the chl values were lower than in 2003. This suggests that other factors might control the increase of chl, such as water column turbidity, and flushing rate.

Figure 5-29: Comparison of relative changes in weekly mean PAR and in chl, at NWN 2003.

[The phases I- IV correspond to those defined in Figure 3-30]



The <5µm chlorophyll size fraction (the sum of the 2-5 and < 2µm fractions) contributed an average of 36% of the Tchl, and increased proportionally to the Tchl value. However, the Fchl <5µm percentages were highest when Tchl values were lowest. This is an indication that the smaller phytoplankton cells are relatively well adapted to changing physico-chemical conditions compared to the larger cells.

The main contributor to the chl peaks (events) during spring was diatoms, while dinoflagellates were most important in late summer. Phytoplankton succession was more evident in late spring and summer, with larger diatoms (*Guinardia delicatula* and *Rhizosolenia setigera*), followed by *Eutreptiella* sp. and then by smaller diatoms (*Chaetoceros* sp.) and dinoflagellates.

Similar seasonal chl and phytoplankton patterns have been observed in Southampton Water in the past. Chl peaks are usually found in May-June (Kifle and Purdie, 1993; Ali, 2003; Iriarte and Purdie, 2004). Phytoplankton succession starts in spring with populations of large diatoms, shifting to flagellates then to small diatoms and dinoflagellates (Kifle, 1993; Ali, 2003). However, the flagellate *Phaeocystis*, and the ciliate *Mesodinium rubrum,* which form blooms at Southampton Water in some years

(Iriarte, 1991; Kifle and Purdie, 1993; Crawford *et al.*, 1997), were only observed in small numbers during the present study.

Influence of light availability

For the 2003 NWN data, PRIMER distinguished K_d and tidal range as the factors most influencing species carbon biomass. For the 2002 Dock observations (Chapter 3), PRIMER had distinguished PAR as the factor most influencing carbon biomass. However, the effects of these three parameters on phytoplankton abundance are difficult to separate. PAR is linked to meteorological conditions, and determines total light availability, whereas K_d is a function of how light is distributed within the water column. Tides can affect K_d through the effects of bottom stirring on the suspension of particulate matter and also on the mixing depth of the water column. At neap tides, when a pyconcline may form, the mixing depth is most likely to be less than the critical depth (see Introduction). K_d is also affected by light absorbing matter of external origin, including particulate or dissolved organic matter (POM or DOM) and by chl transported within the estuary.

Generally, chl values were higher during late spring and summer and lower in autumn and winter. Such changes are associated with the seasonal distribution of irradiance (Figure 5-29). At shorter time scales, high chl values often occurred when PAR was high, but there was also times (e.g. around Day 165) when PAR was high but chl low. This observation suggests that factors other than light were affecting phytoplankton biomass.

The distribution of chl and tidal range at NWN 2003 are shown in Figure 5-30. There was no clear relationship between the tidal range and chl events. In general, large spring tides (>4m range) were associated with low chl values, and the combination of a weak tide and high PAR was associated with increasing chl values. On Days 188, 195, 238 and 247, when the tides were weak, low chl values could be attributed to relatively low PAR.

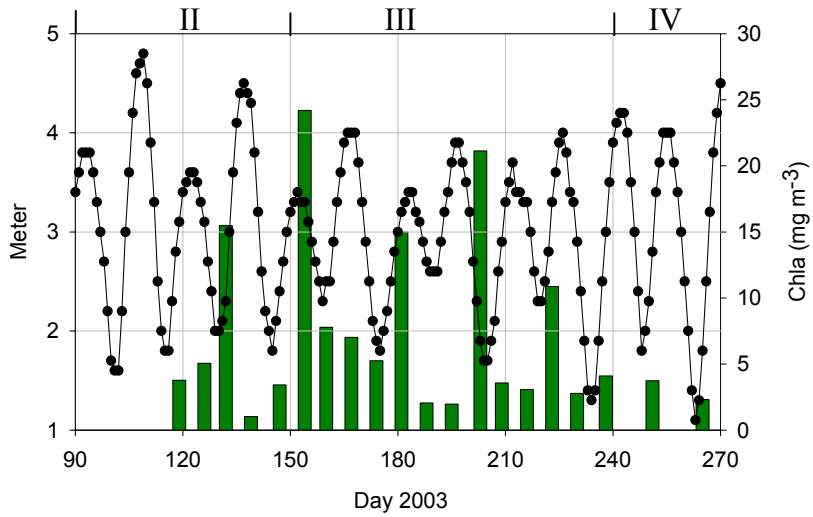


Figure 5-30: Chl and Tidal range distribution, NWN 2003.

K_d showed a weak relationship with chl values, probably because light scattering by non-organic particles and light absorption by dissolved materials were more important than light absorption by chlorophyll in determining K_d. However, the relatively low K_d value (Figure 5-5) during phase III, when the tidal range was also low, may have been an important factor contributing to increases in chl during this time.

An equally important question is what causes the rapid decreases in chlorophyll during summer months. It has been observed that factors other than surface incident irradiance limit phytoplankton growth in Southampton Water (Iriarte and Purdie, 2004). Those factors may be physical parameters, such as the tide cycle or the level of turbidity, chemical parameters, such as limitation of nutrient concentrations and /or biological factors such as grazing rate (Shi, 2000). Tidal strength, with high spring tides flushing phytoplankton out of the estuary, grazing rate and phytoplankton mortality and consequent sinking are all factors that could cause fluctuation in chl.

The relationship between these multiple interacting parameters and chl values (phytoplankton carbon biomass) can be summarised by the following relationship:

$$chl \sim PAR / K_d \, . \, f \text{ (tidal range)}$$

Where f is a function of tidal range.

Shi (2000) has developed a hydrodynamic model to simulate the tide, tidal current and water circulation of Southampton Water which is coupled with a water quality model, and predicts the spatial and temporal changes in oxygen, nutrients, chlorophyll, and planktonic respiration (Shi, 2000). However, the above-mentioned interrelationships between chl values and various factors may warrant the introduction of new models. This could be fulfilled in future work.

Influence of nutrient availability

PRIMER analyses also identified the inorganic nutrients N and P as related to phytoplankton carbon biomass, while Si and P were related to taxon carbon biomass and to size distribution.

N is an important factor for phytoplankton assimilation and growth. Phytoplankton biomass peaks were associated with depletion of N, although N is not normally thought of as a phytoplankton limiting factor in estuary. P followed N behaviour, and it could be a limiting phytoplankton growth factor, especially during bloom time (chl peaks) e.g. P depletion on Day 132.

Si is an essential element for diatoms. Si depletion was associated with diatom accumulation, which increased N/Si ratio. This could be a reason for community shifts from diatoms to dinoflagellates and flagellates during summer, the latter groups not requiring Si for growth. Similar observations were made by Kifle (1992), Kifle and Purdie (1993) and Ali (2003).

P was identified (PRIMER) as a variable factor related to chl size fraction. P depletion from the water column perhaps limits the growth of large cells, such as diatoms and dinoflagellates. Conversely, the small cells, with a high surface area to volume ratio, are more adapted to low P concentrations than larger ones. Therefore, Fchl percentages were low when Tchl values were high and the populations were dominated by diatoms and dinoflagellates.

In conclusion, phytoplankton biomass followed a seasonal pattern associated mainly with light irradiance. Other factors associated with summer fluctuations in chl values

are water column irradiance (K_d), tidal range, grazing rate, and phytoplankton mortality die-off and sinking. Favourable initial nutrient availability causes an increase in chl (phytoplankton carbon biomass) and subsequent nutrient depletion. In the end, nutrient depletion (mainly Si) limits phytoplankton growth and enhances taxa succession; from diatom to flagellates and dinoflagellates. Low P availability could limit the growth of larger phytoplankton for some periods in the summer.

Nanophytoplankton (<5μm) were an important component of the phytoplankton community. On average, they attribute approximately 1/3 of total chl during summer. Nanophytoplankton follows a similar seasonal pattern to that of larger phytoplankton, but their contribution to total phytoplankton biomass decreases as Tchl increases.

Chapter 6- CHARACTERISATION OF NANOPHYTOPLANKTON

6-1 Previous observations in Southampton Water

Nanoplankton (cells 2-20µm in diameter) and the smaller picoplankton (<2µm) are important constituents of the phytoplankton community in all aquatic environments. For example, in continental shelf waters such as the Celtic Sea, phytoplankton cells <5µm in diameter account for about 60% of annual primary production with larger phytoplankton (mainly diatoms) dominant only during the spring bloom (Joint et al., 1986). Similarly in the coastal waters of the western Gulf of St Lawrence, Canada, the <5µm phytoplankton contribute 80-90% of both biomass and production during the summer (Tamigneaux *et al*., 1999). The first investigations of the size structure of phytoplankton communities in Southampton Water were those of Savage (1969) who reported that nanophytoplankton passing through a 20µm net was the most important component of phytoplankton community (Savage, 1969; cited by Kifle, 1992).

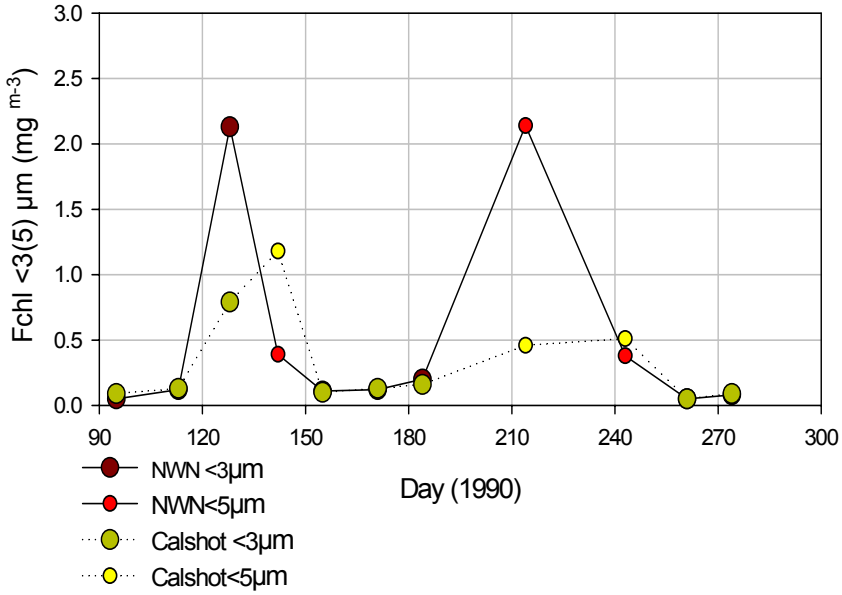
Subsequent studies in Southampton Water (Iriarte, 1991; Iriarte, 1993) using membrane filters with different pore sizes have shown the importance of particular size fractions relative to each other. Cells <5µm in diameter represent a lower proportion (up to 20%) of annual production and mean biomass than in more oligotrophic shelf waters (Iriarte & Purdie, 1994) but show strong maxima in abundance during the summer months, as illustrated in Fig 6.1, when they can constitute as much as 50% of total chlorophyll *a*.

No detailed taxonomic analysis of the small (<5µm) nanophytoplankton has been carried out in Southampton Water, although certain taxa (Cyanobacteria, Cryptophyceae and the prymnesiophyte, *Phaeocystis*) are known to be abundant at certain times of the year (Iriarte, 1991; Iriarte & Purdie, 1994; Kifle, 1993). It is also important to note that Brandt and Sleigh (2000) reported that nanoflagellate

community in Southampton Water was dominated by heterotrophic, bacteriverous cells (chrysomonads) as opposed to autotrophic forms.

Figure 6-1: *Nanophytoplankton in Southampton Water at NWN and Calshot during 1990 (data from Iriarte & Purdie, 1994).*

[Large and small circles represent chlorophyll retained by 3µm and 5µm filters, respectively.]



6-2 Chlorophyll size fractions at Dock and NWN 2002 and 2003

Data on the abundance of nanophytoplankton (defined as Fchl <5µm) at Dock and NWN in 2002 and 2003 are summarised in Figure 6-2 and Table 6-1. At Dock the observations for the two years were similar, with sharp changes in abundance from week to week, but with a higher mean Fchl in 2003. At NWN Fchl values were generally lower and in 2003, when weekly samples were collected, showed smaller fluctuations than at Dock especially after midsummer. Despite these differences between stations and between years, Fchl as a proportion of total chlorophyll (Tchl) was relatively consistent (~40%).

Comparison of the Fchl observations at NWN for the two years (Figure 6-2B) indicates that temporal changes in abundance of small nanophytoplankton cannot be described adequately by monthly sampling. The causes of such rapid changes remain

uncertain, there being no obvious link to environmental factors as discussed in previous chapters.

Figure 6-2: *Chlorophyll <5µm A) Dock and B) NWN in 2002 and 2003*.

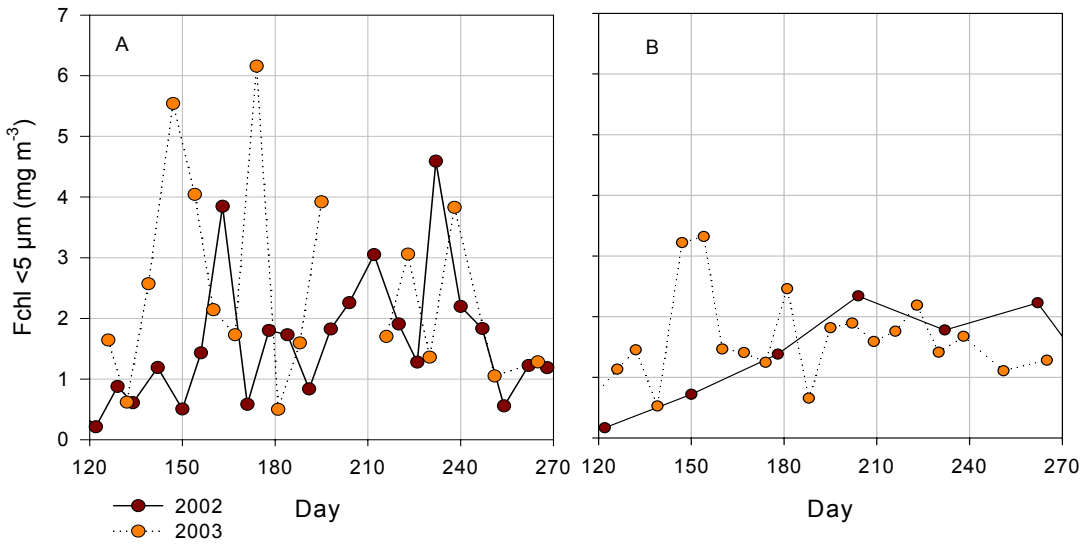


Table 6-1: The means and ranges for Fchl<5µm at Dock and NWN during 2002 and 2003. Percentages of Fchl <5µm to total Chl are given in brackets.

Stations	Dock		NWN
Years	2002	2003	2003
Mean Fchl <5µm (mg m^{-3})	1.6 (45%)	2.5 (41%)	1.7 (37%)
Range (mg m^{-3})	0.2-4.6	0.5-6.2	0.5-3.3

6-3 Flow cytometer data for NWN in 2003

Small eukaryotic phytoplankton cells distinguishable by flow cytometry were divided into four groups, i) picophytoplankton (<2µm in diameter, including both cyanobacteria and very small eukaryotes), ii) nanophytoplankton 2-5µm, iii) nanophytoplankton >5µm, and iv) *Cryptomonas*. These groups were defined by side scattering signals to distinguish cell size and by fluorescence signatures to distinguish *Cryptomonas* from other autotrophs (Tarran *et al.*, 2001). The results were expressed as absolute cell abundances, as percentages of total abundance and as percentages of carbon biomass (Figure 6-3).

The picophytoplankton (<2µm) was the dominant group in terms of cell numbers, accounting for a mean of 82% of total cell number (range 40-100%). In contrast, the 2-5µm nanophytoplankton was the more important component of the biomass with a mean of 51% (range 4-94%), compared to a mean of 40% (range 4-95%) for the picophytoplankton. Cell numbers and biomass for *Cryptomonas* were relatively low although on certain dates the biomass of *Cryptomonas* was equivalent to that of picophytoplankton. Relative cell numbers for the >5µm nanophytoplankton were highest (~20% of total) on Days 135 and 180 when this group also represented the major component of nanophytoplankton biomass (not shown in Figure 6-3C in order that the biomass and pigments of <5µm cells can be compared – see following section). A comparison of Figures 6-2B and 6-3C indicates that, in general, flagellates >2µm in diameter dominate nanophytoplankton biomass when the corresponding chlorophyll values are >1.5 mg m^{-3}.

Biomasses for the 2-5µm nanophytolankton and <2µm picophytoplankton at NWN in 2003 are compared to their corresponding chlorophyll size fractions in Figures 6-4. In general, the Fchl fluctuated proportional to carbon biomass (cell density), with the highest values for both parameters on Day 147 for the 2-5µm cells and on Day 154 for the <2µm cells.

However, for some samples, high carbon biomass was not associated with high chlorophyll values, examples being after Day 240 for the 2-5µm fraction and around day 210 for both fractions. At these times the cells appear to have an anomalously high pigment content although allowance should be made for any overestimation of biomass (for example, if cell size was overestimated).

Figure 6-3: Flow cytometer data for <2, 2-5,>5µm and Crypt size categories at NWN 2003.

[a) cell numbers, b) percentages of total cell number and c) carbon biomass. Note different right hand scale in a) for <2µm cells. For biomass estimates, cell diameters of 1.5, 3, 5µm were assumed for <2µm, 2-5µm and crypt groups respectively (see Methods)]

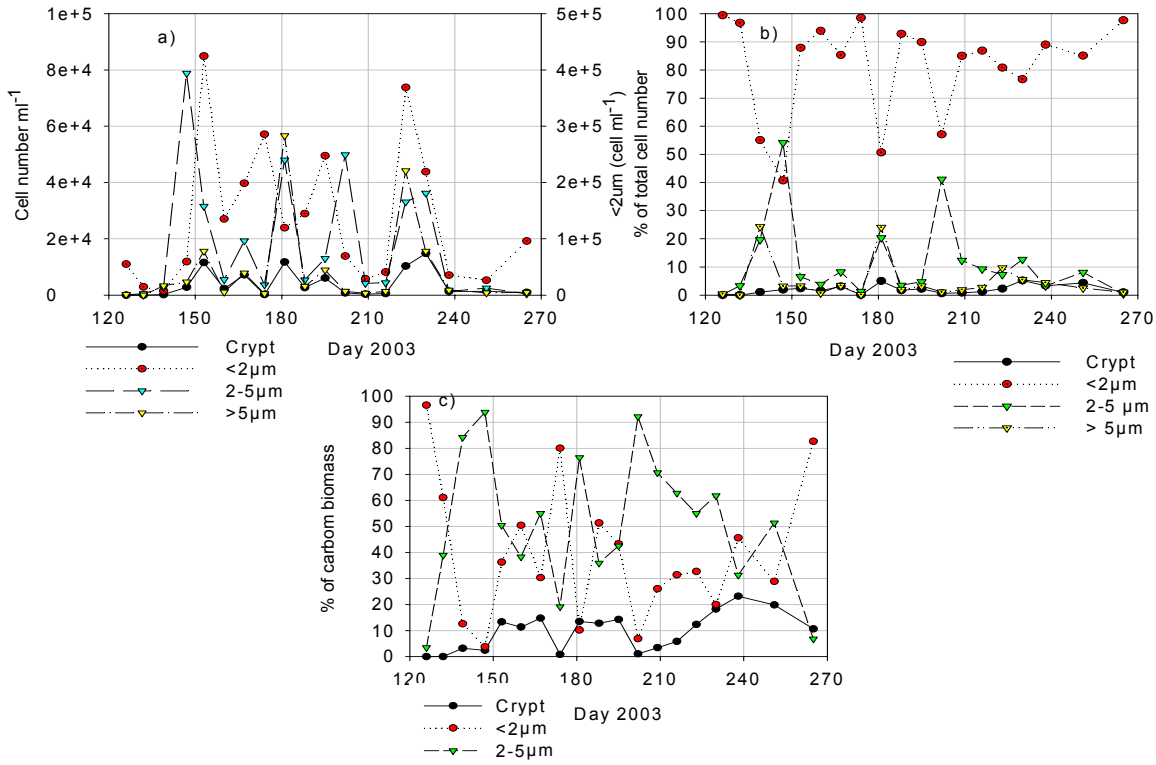
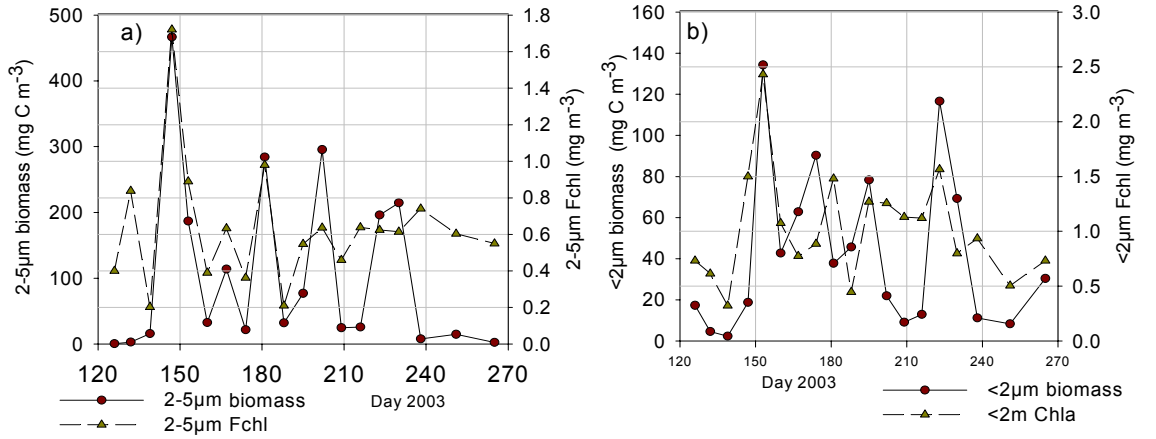


Figure 6-4: Changes in chlorophyll and cell biomass for a) the 2-5µm and b) <2µm fractions at NWN in 2003.

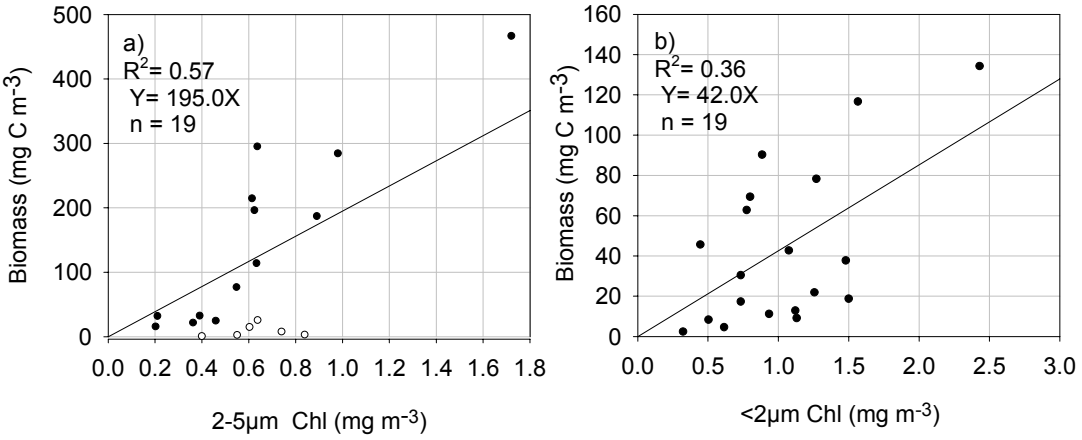


The correlation between carbon biomass and Fchl for each size fraction (Figure 6-5) was positive, but weak due to samples with a high pigment to biomass ratio. These low carbon samples are mainly from early (before Day 140) or late (after Day 235) during the sampling period as can be seen in Figure 6-4, suggesting that pigment per cell may be high when PAR is relatively low. The estimated mean C/chl ratios for the 2-5µm and <2µm chl fractions are 195 and 47, respectively.

However, it should be noted that the estimates of carbon biomass and, therefore, of C/chl ratios are very sensitive to cell size. For example the C/chl ratio for the 2-5µm fraction changes from 195 to 78.7 (R^2 =0.57), when the cell diameter reduced from 3 to 2.5 µm. Conversely, removing the low carbon biomass samples from the regression analysis increases the average C/chl ratio as indicated in the legend to Figure 6-5.

Figure 6-5: The correlation between chlorophyll and carbon biomass for the 2-5µm and <2µm fractions at NWN in 2003.

[Note that if the low biomass samples (open circles, Figure 6-4a) are ignored the C/Chl ratio increases from 195 (R^2=0.57) to 246 (R^2 =0.75)]



6-4 Size fractionated HPLC pigment analysis for NWN in 2003

In 2003 NWN <5µm samples were also analysed for pigments by HPLC. The regression between HPLC chla and fluorescence chl gives a slope of 0.81 (R^2=0.64) which is comparable to that given in previous chapters for total chlorophyll determinations. Values of chla and of chla/accessory pigment ratios for the <5µm fraction are shown in Figure 6-6. A comparison of changes in the ratios with changes

in biomass (Figure 6-4) provides some information about variations in the taxonomy of the nanophytoplankton.

On one or more dates high ratios relative to chla were observed for chlb, chlc, fuco and perid, suggesting that taxa containing these accessory pigments were important components of the population on these dates. By comparison, the maximum observed ratios for the other pigments (hex, prasino, zea and allo) were low throughout the sampling period although considerable variability was observed. If the high (>1.5 mg m^{-3}) chla samples only are considered high pigment ratios are found only for fuco (Days 130, 180) and chlb (Day 147). Possible sources of fucoxanthin are small (<5μm) diatoms and prymnesiophytes (including *Phaeocystis*) and of chlb are Chlorophyceae and euglenoids. Neither of the samples on Days 130 and 180 contains significant quantities of hex, which is also a marker for prymnesiophytes, but it should also be noted that for a particular species such as *Phaeocystis* the ratio between fuc and hex is very variable. The strong signal for perid on day 239 is attributable to small dinoflagellates which were present in most samples.

A comparison of the pigment ratios for the size-fractionated and total samples allows an enrichment index to be estimated, with values >1 indicating that a particular pigment is relatively abundant in the <5μm cells. As shown in Table 6-2, of the major pigments, the small cells appear to be poor in fuco and perid (as expected for the marker pigments of diatoms and dinoflagellates with cells generally >5μm in diameter) and rich in chlc, with little difference for chlb. Small cells with chlc are most likely to belong to the Prymnesiophyceae and Chrysophyceae.

Figure 6-6: HPLC chla values and pigment to chla ratios for the < 5 µm fraction at NWN, 2003.

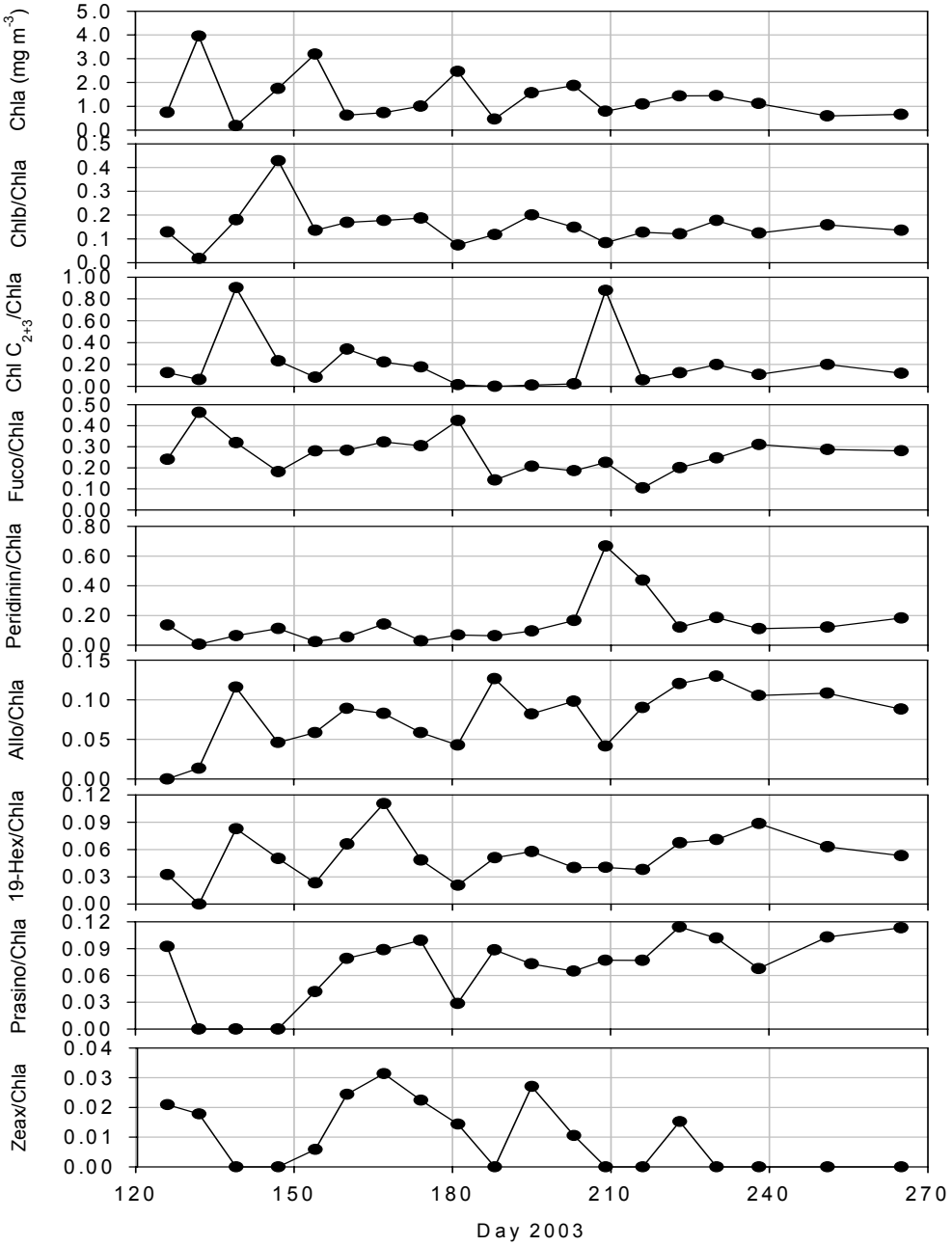
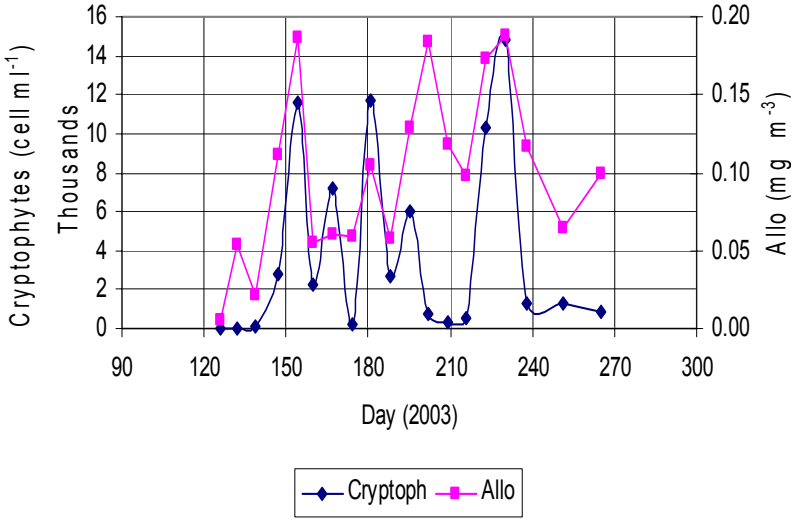


Table 6-2: The annual mean total phytoplankton pigment and pigment enrichment index values for< 5µm size fraction, at NWN 2003.

Pigment (averages)	chlb	chl c_2c_3	perid	fuco	allo	19'hex	prasino	zea
2003 (mg m^{-3})	0.27	0.47	0.26	1.53	0.09	0.06	0.06	0.01
Enrichment index	0.94	1.14	0.52	0.7	0.94	0.9	0.98	nd

The minor pigments show enrichment indices close to unity. Of these allo can be considered as a marker pigment for cryptophytes, and in Figure 6-7 the distribution of allo is compared to cryptophyte counts from the flow cytometer. In general higher counts are accompanied by elevated levels of allo, but at the beginning and end of the sampling period and around Day 210 there are samples with low cell counts and relatively high concentrations of allo. Several of these samples also showed anomalously low chla (see Figure 6-4).

Figure 6-7: Flow cytometer cryptophyte cell counts and <5µm alloxanthin distribution, NWN 2003.



6-5 Identification of nanophytoplankton by hybridisation

Small nano- (<5µm) and pico- (<2 µm) phytoplankton cannot be identified routinely by light microscopy because of their small size and simple cell morphology. Therefore, a fluorescent *in situ* hybridisation (FISH) technique was applied in an

attempt to identify these small cells. The results are summarised in Table 6-3. Although the recommended procedure was followed with care, counts for Chlorophyta and Prymnesiophyceae in some samples exceeded those for total eukaryotes. Furthermore, no significant relationships between the counts of a particular group and the abundance of the corresponding marker pigment (e.g. hex for prymnesiophytes) could be established. Despite these inconsistencies in the data they do indicate that chlorophytes and prymnesiophytes were the main types of small cells at NWN in 2003.

Table 6-3: Relative numbers (expressed as % of total eukaryote count) of particular phytoplankton types as identified by hybridisation method.

[Probe Chlo01 for Chlorophyta, Prym02 for Haptophyta, Pela01 for Pelagophyceae and Boli02 for Bolidophyceae.]

Days of (2003)	Chlo 01	Prym02	Pela01	Boli02
132	97	3	0	0
139	90	10	20	0
153	85	8	0	3
160	161	6	3	0
167	27	11	2	5
174	75	5	0	2
181	135	90	20	15
188	123	82	18	41
202	233	144	0	11
209	100	550	0	0
216	95	32	34	16
223	107	91	48	7
238	56	84	76	12
251	89	0	7	4
258	233	58	92	17

Additional samples were collected at NWN and Dock during 2004 with the hope of obtaining better results from the FISH technique. Surface water samples were collected at neap high tide in the summer as in previous years, and Fchl <5µm values were relatively similar at both sampling stations to those for 2003 but with maxima at end of July (Day 210).

The flow cytometer counts and estimated biomass values for the 2-5µm cells are shown in Figures 6-8 and 6-9 respectively, again showing similar distributional patterns at both stations to those for 2003. The maximum biomass values were also observed on Day 210.

Figure 6-8: Nanophytoplankton distributions at Dock (A) and NWN (B), 2004.

[Note the different right hand scale for the <2µm cells]

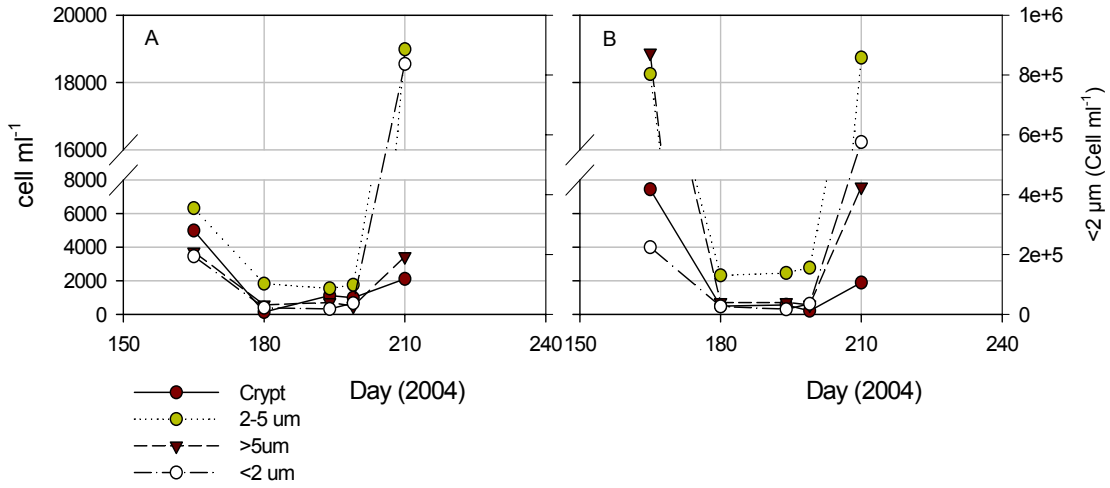
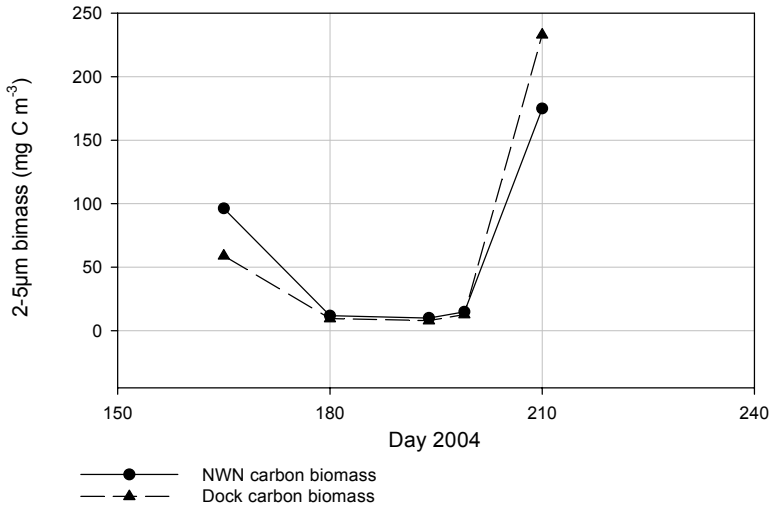


Figure 6-9: Carbon biomass at Dock and NWN, 2004 of small flagellates (2-5µm) counted by flow cytometry.



Once again the application of the FISH technique to identify small nano- and pico-phytoplankton gave very inconsistent results (data not shown).

6-6 Comparison of flow cytometer and microscope counts

Another uncertainty in evaluating the importance and taxonomic affiliation of estuarine nano- and pico-phytoplankton is the reliability and comparability of data on cell counts. Precision with the light microscope is poor due to lack of sufficient resolution for small (2-5µm) cells, the inability to distinguish autotrophic and heterotrophic forms unambiguously, and masking by detritus and other non-living material. By contrast, the flow cytometer depends on well defined criteria (side scatter, fluorescence) to detect autotrophic cells <1µm in diameter.

A comparison of the microscope and flow cytometer data for NWN in 2003 (see Figure 6-3 and data on flagellates presented in Chapter 5) shows general agreement between the two methods, with the highest and lowest counts for the same samples. However, for the high chlorophyll samples the flow cytometer gives numbers for the >5µm and 2-5µm cells that are up to 8x higher (up to 80 x 10^6 ml^{-1}) than the microscope counts for large and small flagellates. Conversely, for low chlorophyll samples which are easier to count, the microscope tends to give higher numbers, probably because both heterotrophic (see Brandt & Sleigh, 2000) as well as autotrophic forms are included. The flow cytometer also provides information on the picoplankton (<2µm) which cannot be detected with the microscope. In this study up to 400 x 10^6 cells ml^{-1} (prokaryotes + eukaryotes) were found in this category.

The earlier work of Iriarte & Purdie (1994), based on epifluorescence microscopy, reported maximum counts of 15 x 10^6 cells ml^{-1} for small (<3µm) eukaryotes and also for prokaryotes, with densities of eukaryotes as high as 290 x 10^6 cells ml^{-1} during blooms of *Phaeocystis*.

Although there is some degree of consistency in these numbers for small autotrophic cells, it is also clear that comparison of different data sets is only really possible when the same methods are used. When molecular techniques, such as FISH, which are used to identify and separate different taxonomic groups be fully applicable, it will be essential to intercalibrate counting methods if the composition of nanophytoplankton populations is to be quantitatively described.

6-7 Discussion

Previous work on the autotrophic small nanophytoplankton in Southampton Water has included size fractionated chlorophyll measurements (Iriarte and Purdie (1994) and others), cell counts of eukaryotes and prokaryotes by epifluorescence microscopy (Iriarte and Purdie, 1994), and a small number of observations using FISH (Hazeem, 2003). There is no published flow cytometry data, and size fractionated HPLC analyses of pigments have not previously been attempted.

In this study both at Dock (see Chapters 3 and 4) at NWN (see Chapter 5) light microscope counts showed the presence of high numbers of small and large flagellates but did not distinguish autotrophs from heterotrophs. The size categories could only be subjectively defined leading to much uncertainty in the conversion of counts to biomass, and the picoplankton (<2µm in diameter) was not resolved. Finally no taxonomic information could be obtained by light microscopy apart from recognising larger cryptophytes by their morphology. The application of a combination of flow cytometry, HPLC analysis of size fractionated samples, and FISH was undertaken with samples from NWN in 2003 and 2004 in an attempt to overcome these deficiencies in knowledge.

The main conclusions from the flow cytometry were that the picoplankton was always numerically the most abundant category of small autotrophs but contributed significantly to biomass only when chlorophyll was low (<1.5mg m^{-3}), and that small nanoplankton (2-5µm) and occasionally larger nanoplankton (>5µm) dominated the biomass when chlorophyll was relatively high. As shown by Iriarte & Purdie (1994) the picoplankton includes cyanobacteria (probably *Synechococcus*) which are most numerous in summer when the water temperature is high. Cryptophytes were present in almost all samples and made the largest contribution (~20%) to nanophytoplankton biomass in late summer. Comparison of autotrophic carbon biomass (as estimated from flow cytometer counts) with chlorophyll indicates that the nanophytoplankton had a variable C/chl ratio. Although much of this variability may be largely attributable to uncertainty in the carbon values it does appear that, at

certain times, the cells have a high chlorophyll content (see Figure 6-4). The most likely explanation is that the chlorophyll per cell increases when light is low due either to low surface irradiance (early and late in the year) and/or to some combination of absorption and scattering within the water column (around Day 210).

The HPLC data did not give any very clear indicators about the taxonomic composition of the nanophytoplankton. The cells have a relatively high content throughout most of the summer of fucoxanthin and, at particular times, of chlorophylls b and c and of peridinin. When filtered (<5µm) samples were compared with non-filtered ones only chlc was relatively more abundant compared to chla. From knowledge of the distribution of pigments in different classes of algae (Jeffery, 1997, Jeffery & Vesk, 1997) these results suggest that prymnesiophytes and chrysophytes (both containing chlc and fuco) were widely distributed in the samples, *Phaeocystis* was observed in mid July (Purdie unpublished data), with occasional populations of chlorophytes (chlb) and small dinoflagellates (perid). Cryptophytes were also generally present as indicated by the presence of allo and by the flow cytometer counts. Similarly the chlc and chlb was linked to flagellates in Southampton Water (Ali, 2003)

The results of the FISH analyses were somewhat ambiguous as counts for specific groups of eukaryotic algae either alone or together frequently exceeded the counts for total eukaryotes. Also significant numbers off groups that would not be expected in an estuary (e.g. pelagophytes that usually contain butanoylhexofucoxanthin) were detected. However, of the groups for which probes were available, the highest counts were obtained for prymnesiophytes and chlorophytes, thus giving some agreement both with the HPLC pigment analyses and with the independent studies of Hazeem (2003). A critical evaluation of this technique is required in order to gain consistency with the results of the flow cytometer and HPLC analyses (see Simon et al., 1995). In this instance hybridization did not give reliable results for a number of possible reasons. Back ground overlapped with probe signals, weakens probe signal, storage period, the content of and quality of samples, configuration and nature of the probe (Amann, 1995; Simon *et al.*, 1995).

Chapter 7- GENERAL DISCUSSION

7-1 Phytoplankton Distributions in Macrotidal Temperate Estuaries

Macrotidal (mean tidal range >2m) temperate estuaries can generally be divided into an inner region where turbidity and nutrient levels are high and chlorophyll is low throughout the year, a middle region where turbidity and nutrient levels are intermediate and chlorophyll is relatively high, and an outer region where turbidity and nutrients are relatively low and chlorophyll is intermediate (see reviews by Heip *et al.*, 1995; Underwood and Kromkamp, 1999). These regions represent a down-estuary transition from phytoplankton growth being light limited throughout the year to situations where growth is only limited by light in the winter (Cloern, 1987) and may become limited by nutrients in summer (Cloern, 1999). The interplay between light and nutrients in controlling the productivity of estuaries is complex (Cloern *et al.*, 1995; Cloern, 2001) but a comparison of different systems has shown that primary production increases with mean photic depth and with N loading (Cloern, 1999).

Within the middle region of such estuaries the timing of the annual maximum of chlorophyll varies from early spring to midsummer depending on local hydrological conditions, in particular the turbidity and depth of the surface mixed layer which effectively determine the ambient light environment for phytoplankton (Underwood and Kromkamp, 1999). At this period the dominant species are usually diatoms which are later succeeded by various types of nano- and pico-phytoplankton as levels of nutrients (in particular Si) fall and larger herbivores (mesozooplankton and suspension feeders) increase in abundance. Correlations between phytoplankton biomass, usually estimated as chlorophyll a, and environmental variables are often found to be rather weak, suggesting that the rates of biological processes, including grazing, are important in the control of biomass (Boynton *et al.*, 1982). However, it is well established that primary production is largely a function of phytoplankton

biomass and ambient irradiance, the latter determined by surface irradiance, depth of surface mixing and the attenuation coefficient (Cole and Cloern, 1987), and that in tidally-stirred waters photoadaptation does not have a strong influence on rates of carbon fixation (Pennock and Sharp, 1986; Heip *et al.*, 1995) as the time scale of vertical mixing is relatively short (order 1h).

The reduction of inorganic nutrients during the summer months can largely be attributed to assimilation by phytoplankton (and other types of autotrophs, depending on the ecological setting). However, in many estuaries, denitrification is a significant sink for nitrate and absorption onto particles a sink for phosphate (Nedwell *et al.*, 1999). For this reason, and also because nutrient sources to estuaries are inherently variable, any nutritional limitation of phytoplankton growth (if it occurs at all) can take different forms between the generally recognised patterns of P-limitation in fresh waters and N-limitation in marine waters.

Year-to-year variations in phytoplankton biomass reflect both climatic factors (freshwater run off, surface irradiance etc.) and biotic factors (species competition for resources, herbivory, infection by pathogens etc.) (Underwood & Kromkamp,1999). Thus control of primary production in macrotidal estuaries has been considered to be either 'bottom-up' (by light, nutrients) or 'top-down' (by herbivores and pathogens), and both situations are likely to occur within any particular macrotidal estuary depending on local conditions within the system (Underwood and Kromkamp, 1999).

In the first part of the year when incident light is increasing and nutrient levels are still relatively high blooms of diatoms, which have relatively low rates of respiration compared to other taxa (Underwood and Kromkamp, 1999), are regularly observed. The timing of such blooms depends largely on water column irradiance which is also affected by turbidity and the depth of mixing, and their duration depends on the balance between biomass production (growth) and biomass removal (predation, sinking etc.). The effect of grazing may be weak initially but becomes strong as the abundance of herbivores, mainly mesozooplankton and suspension feeders, increases

(Heip *et al.*, 1995). Blooms later in the year of other phytoplankton, including different types of flagellates, are less predictable, and their occurrence depends on rather more specific combinations of environmental conditions such as strong stratification, recycling of nutrients and inhibition of grazing (Cloern and Difford, 2005). During times of growth limitation by light (winter) and nutrients (summer), algal biomass is relatively low and generally dominated by small nano- and pico-phytoplankton.

These general features of phytoplankton distributions in temperate macrotidal estuaries are consistent with observational data for Southampton Water as presented in this thesis and in earlier work (e.g. Iriarte and Purdie, 1994; 2004). The Dock and NWN stations are representative of a mid-estuary region, where the highest levels of chl are found, and the Calshot and HE stations are representative of an outer region.

7-2 Environmental Effects on Phytoplankton Distribution in Southampton Water

7-2-1 Seasonal changes in chlorophyll

Any description of the temporal distribution of phytoplankton depends to some degree on the resolution of sampling, and for this reason care has to be taken in comparing data sets with different sampling frequencies. This problem also applies to spatial distributions which are affected by relatively small scale differences in physical parameters related to tidal mixing and advection (see Lucas *et al.*, 1999a; 1999b). For this study weekly sampling was considered to give an adequate description of changes in phytoplankton biomass with time although Ali (2003) noted marked changes in chl over periods of less than a week at the Dock entrance monitoring site.

A comparison of the inner (Dock and NWN in mid-estuary) and outer (Calshot and HE) stations shows that the seasonal increase in chl starts somewhat earlier at the outer stations, as found also by Iriarte & Purdie (2004), probably because of better penetration of light into the water column (lower turbidity). Chl levels are generally

highest around the middle of the year when PAR is highest, suggesting that light is the primary limiting factor on phytoplankton growth. This conclusion is supported by measurements of N and P which are rarely depleted to levels (<2μM and <0.2μM respectively) that might limit algal growth except briefly in mid to late summer.

Weekly observations at Dock (2002 and 2003) and NWN (2003) showed that seasonal changes in chl, irradiance and nutrient levels could be described consistently by four phases (Figures 3-30 and 5-29) as summarized in Table 3-8. The decrease in Si early in the year (up to Day 90), before there is any significant rise in chl, is attributable to large diatoms which show net growth under low light due to a relatively low rate of respiration. During spring and early summer (phase II, Days 90-150) there is still little increase in chl despite marked decreases in N and P as well as Si, and it appears that accumulation of phytoplankton biomass is restricted by some combination of losses through physical (flushing, sinking) and biological (grazing) processes. Thereafter in phase III (Days 150-240), chl increases sharply once surface light has reached a threshold value of ~2000 W h m^{-2} d^{-1} (see Iriarte and Purdie, 2004), but shows fluctuations over periods of 1-2 weeks between high and low values. Finally, after Day 240, chl decreases and nutrients increase as phytoplankton growth is restricted by low light and nutrient regeneration exceeds assimilation.

Statistical analysis (PRIMER) of the environmental observations indicated that light (surface PAR and to a lesser extent K_d) was the primary driver of these seasonal changes with nutrients (N and to some extent Si) of secondary importance. Salinity appeared to be a major factor in determining differences in chl between stations, probably as a result of associated down-estuary decreases in K_d which enhance water column irradiance and promote earlier growth of phytoplankton.

An explanation for the marked fluctuations of chl in midsummer is much less easy to establish. At this time the abundance of herbivores is likely to be maximal and regeneration processes are important for maintaining supply of nutrients for phytoplankton growth. Any temporal imbalance between these two processes,

together with fluctuations in PAR and tidal mixing (which affect both the surface stability of the water column and the sinking of cells), is likely to lead to rapid changes in phytoplankton biomass. The relationship between tidal range and chlorophyll concentration (Figure 5-30) suggests that high levels of chl are associated with reduced mixing of the water column around neap tides. This situation is likely to favour the growth of neutrally buoyant or motile cells, mainly larger flagellates since total and >5µm chl are positively correlated (Figures 3-14 and 5-13). Therefore, it appears that under conditions of high PAR and weak tides in midsummer the growth of larger flagellates can overcome losses due to grazing by mesozooplankton (Cloern, 1982), whereas the same is not true for the smaller autotrophic flagellates due to continuous control by microzooplankton with generation times similar to those of algal cells (Brandt and Sleigh, 2000).

7-2-2 Annual variability in chlorophyll

Large year-to-year differences in the abundance of phytoplankton in temperate estuaries are well documented (e.g. Boynton *et al*., 1982; Iriarte and Purdie, 2004), as are differences between estuaries in the same year (e. g. Fisher *et al.*, 1988; Lemaire *et al.*, 2002). The nature of such differences includes ones related to the timing of seasonal changes in biomass or to the maxima and duration of peaks in chlorophyll (including bloom formation). The former tend to be driven by variations in light conditions and the latter by variations in nutrient conditions.

Any useful comparison of observational data from different years depends on temporal/spatial consistency of sampling and as well as suitable frequency (i.e. weekly) of sampling. Thus the chlorophyll data presented in this thesis for NWN in 2002 and 2003, which were based respectively on monthly and weekly sampling, do not provide a valid basis for analyzing annual differences at this station. However, comparison of the weekly observations for Dock in 2002 and 2003 (Chapters 3 and Appendix 2), for NWN in 2003 (Chapter 5) and 2000 (Ali, 2003), and for at Calshot over a longer period (Iriarte and Purdie, 2004) show clearly that the nature of annual

variability in the phytoplankton of Southampton Water is similar to that in other macrotidal temperate estuaries.

It seems probable that climatic factors related to incident irradiance and to fresh water runoff are probably the main cause, directly or indirectly, of annual differences in the succession and abundance of phytoplankton (Heip *et al*., 1995; Iriarte and Purdie, 2004). Light availability is affected by a combination of incident irradiance, water turbidity (also related to runoff) and depth of mixing (see Cole and Cloern, 1987). The factors affecting nutrient availability are more complex as they include biological processes affecting nutrient removal and regeneration as well as physical ones such as runoff that affect initial nutrient levels. Even when initial nutrient levels are the same in late winter/early spring, subsequent differences in rates of nutrient utilization (related to ambient light conditions) and of nutrient input (continuing riverine sources as well as biologically-driven recycling) can lead to very different nutritional conditions for phytoplankton later in the year. Furthermore, the motility of certain species such as dinoflagellates and *Mesodinium* is known to be key behavioural response to bloom development and survival in estuaries (Crawford and Purdie, 1992).

The responses of phytoplankton populations and, more broadly, of the planktonic foodweb to annual differences in their environment are still poorly understood. In general terms it is thought that the abundance and species composition of phytoplankton at any moment in time reflects previous environmental conditions, and that changes in environmental forcing lead to restructuring of populations (see discussions by Smayda, 1998; Underwood and Kromkamp, 1999; Cloern and Difford, 2005). Some evidence from Southampton Water for this process is presented in the following section.

7-2-3 Species succession and occurrence of blooms

The differences in phytoplankton populations between the inner (Dock and NWN) and outer (Calshot and HE) stations of Southampton Water in 2002 are comparable with earlier observations (Iriarte and Purdie 1994; 2004; Ali, 2003) and generally

with other temperate estuaries. In the outer estuary maximum chlorophyll levels in summer are lower as expected from the lower initial nutrient levels and largely attributable to diatom species characteristic of high salinity conditions. In the inner estuary larger flagellates (dinoflagellates, cryptophytes, euglenoids), and the autotrophic ciliate, *Mesodinium rubrum*, are more abundant and, together with diatoms, are important constituents of summer chl maxima. Both parts of the estuary are characterised in winter and summer by nanoflagellates, which are dominant when biomass is low (in summer at times of low abundance of larger autotrophs), and in late winter by populations of large diatoms, such as *Odontella* and *Coscinodiscus* spp, which deplete Si but do not cause significant increases in chl.

The statistical analyses presented in Chapters 3-5 show clearly defined groups of phytoplankton species that are largely related to seasonal changes in PAR. These groups are all characterised by diatoms at the outer stations, Calshot and HE, whereas at NWN the dinoflagellate*, Scrippsiella,* is relatively abundant through much of the summer. At Dock *Eutreptiella* and *Cryptomonas*, as well as dinoflagellates, are prominent throughout much of the year. Thus a gradient from the outer estuary to the inner (mid) estuary and confined environment of the Dock can be seen in terms of the increasing importance of large, motile flagellates from several distinct classes of phytoplankton.

When phytoplankton distributions were considered in terms of the biomass of major taxa, the significance of small (<5μm) and large (>5μm) nanoflagellates became apparent at all stations, with the latter particularly important at Dock. When the other 3 stations were considered on the basis of monthly samples (Chapter 4) again the larger nanoflagellates were a diagnostic feature of the groups but, for the weekly samples from NWN (Chapter 5), it was the smaller nanoflagellates that were picked out. This difference suggests that group definition is sensitive to sampling frequency; when changes in population biomass (chl) structure during the summer are clearly resolved the difference becomes apparent between low biomass samples in which picophytoplankton are relatively abundant and high biomass samples in which larger cells (including diatoms and dinoflagellates) are important. The distributions of

accessory pigments were consistent with those of the major taxa recognized by microsopy but the occurrence of major ones such as fuco and chlb across several taxonomic groups (Jeffery and Vesk, 1997) meant that the pigment data added little extra definitive information about phytoplankton population dynamics.

Analysis of the broad characteristics of phytoplankton populations (total C biomass, taxon-specific biomass, and size biomass defined as </> 5µm) in relation to environmental parameters showed weak but broadly consistent relationships. Thus, as expected, total biomass was best correlated with light (PAR, Kd) and the nutrients, N and P. By contrast, taxon-specific biomass was influenced by Si and tide, representing the direct (essential nutrient) and indirect (cell suspension) effects on diatom growth with diatoms becoming less abundant when Si was depleted. Perhaps the most interesting result was evidence for the influence of P on size biomass, indicating that small cells survived best when P was relatively low in late summer.

One aspect of phytoplankton growth in Southampton Water that could not be examined from the data presented in this thesis is the environmental basis for the occurrence of blooms of organisms other than diatoms, in particular of *Phaeocystis* and the autotrophic ciliate, *Mesodinium rubrum*. The reasons for the apparent absence in 2002 and 2003 of blooms of these species are not known, although low freshwater runoff during winter for 2002 (Figure 5-2) may have led to unfavourable conditions for the growth of *Phaeocystis* (Purdie, unpublished observations). *Phaeocystis* was not observed in 2003 either, although another study did report it for 2003 (Purdie, unpublished data). This is probably because colonies were affected by environmental stress, leaving only small solitary flagellated cells that are unlikely to bloom (see Peperzak, 1993). In the case of *Mesodinium*, small numbers of cells were present in samples from NWN and it is possible that conditions of vertical mixing of the water column were such that motility gave no competitive advantage (see Crawford and Purdie, 1992; Kifle and Purdie, 1993). This could also be the reason for the absence of any high concentrations of dinoflagellates (see Lauria *et al.*, 1999).

7-2-4 Comparison with other estuaries

Southampton Water shows many features expected of phytoplankton distributions in a moderately turbid and eutrophic temperate estuary. A typical range of species is present for the down-estuary gradients in salinity, light and nutrients, and the distributions of biomass (chl) in space and time are consistent with those in other similar estuaries. In particular, chl levels are higher and species of dinoflagellates (and other large motile species) more abundant than in turbid estuaries such as the R. Colne (Kocum *et al.*, 2002a; 2002b) and the Thames estuary (Sanders *et al.*, 2001). On the other hand, estuaries with weak tides and low turbidity, such as found in N America, show much earlier increases in chl, a greater tendency for nutrient depletion, and dominance by picophytoplankton in late summer (e. g. Lewitus *et al.*, 1998; Li and Smayda, 1998). Evidence for P limitation of phytoplankton growth has been found for several estuaries (e. g. Fisher *et al.*, 1992; Westeyn and Kromkamp, 1994) but the causes are likely to be somewhat site-specific depending on the balance between inputs relative to N and on the tendency for abiotic removal through absorption onto particles. The taxonomic composition of the phytoplankton as indicated by HPLC pigments again is typical of temperate estuaries.

7-3 Some Chemical Characteristics of Estuarine Phytoplankton

POC/N Ratio: It is well known that the chemical composition of living marine phytoplankton, in terms of the elements C, N, and P (and Si for diatoms), conforms generally to the Redfield ratios (Tyrrell, 2001), although work with cultures demonstrates considerable variability according to growth conditions (Geider and la Roche, 2002). In most estuarine waters the phytoplankton accounts for a relatively small proportion of the total particulate organic carbon, typically about 30% (Wienke and Cloern, 1987) but less in the winter when much detritus is present. The observations for Southampton Water, presented in Chapters 3-5, show that POC/N decreases from values of ~10 or higher in late winter to somewhat less than the Redfield ratio of 5.7 (by weight) for periods in mid- to late summer, reflecting the trends towards particulate organic material with a higher proportion of living

organisms, and towards an increase in the proportion of heterotrophs (with a relatively high N content) to autotrophs. The seasonal removal of dissolved N and P (and changes in the dissolved N to P ratio) from the water is consistent with the production of organic material according to Redfield ratios. For Si a rather picture emerges as only diatoms have a requirement for Si, with the cells having higher Si/C at low growth rates (Claquin *et al.*, 2002). Thus the dissolved N/Si (or P/Si) is likely to be variable in summer depending on the absolute concentration of Si and on the abundance and growth conditions of diatoms.

Chla/Tpig ratio: Analysis of diverse data sets for the pigment content of marine phytoplankton measured by HPLC has shown that chla makes a relatively constant proportion of the total pigment (Tpig) (Trees *et al.*, 2000). A closer examination of this relationship has demonstrated that microalgae synthesise chla preferentially to other pigments when growing actively, leading to a corresponding increase in the chla to Tpig ratio (Aiken *et al.*, 2004). The chla/Tpig ratios for Southampton Water (Figures 3-16 and 4-11) were similar to the mean annual value observed by Aiken *et al.* (2004) for the western English Channel but there was no evidence for systematic variations in relation to PAR, K_d or depth of mixing. It is possible that persistent thermal stratification in the western English Channel in summer creates a sufficiently different surface light environment to affect the pigment composition. This process appears not to happen in the estuary where tides permit only relatively short periods of stratification and, in summer, the mean light level in the surface mixed layer is lower than in the surface layer of offshore waters.

C/chl Ratio: The C/chl ratio is an important cellular property in relation to the energetic capacity of autotrophic cells to fix C and to the determination of their growth rate (Geider *et al.*, 1997). Knowledge of variability in the C/chl ratio is essential for interpreting the distributions of biomass (chl) and carbon fixation rates (e.g. P/E curves) in terms of potential population growth rates. Thus, for a given chl-specific rate of carbon fixation, the growth rate will vary inversely to the C/chl ratio.

Estimates of the C/chl ratio for natural populations of estuarine phytoplankton include a mean of 51 (Wienke and Cloern, 1987), and ranges of 17-48 (Westeyn and Kromkamp, 1994) and 20-30 for small diatoms and 40-70 for large diatoms (Ali, 2003). In a review of information from cultures (mainly diatoms) Cloern *et al*. (1995) showed a range of greater than 100 to less than 25. Although there is much uncertainty in the values for natural populations due to the difficulties in estimating phytoplankton C, it is clear that the C/chl is variable according to the species, size and physiological state of the cells present. Also estimates for mixed populations may have only limited value as different components are likely to have quite different C/chl ratios so that their growth responses to any perturbation of environmental conditions will not be the same.

In this work the correlation of chl against estimates of phytoplankton C from microscope counts for high chl samples showed poor regression coefficients but gave values towards the lower end of those quoted above, suggesting that they were representative of the diatoms present. However, analysis of the flow cytometer data for small flagellates (Chapter 6) indicated much larger C/chl ratios (>100) especially at the beginning and end of the sampling period when light was low. The P/E data (in Chapter 5) indicated that the total phytoplankton population had higher production than the size-fractionated one. The result suggests that any mean value incorporates relatively low numbers for diatoms and relatively high numbers for flagellates, and that flagellates may show photoadaptive responses in their chl content between winter and summer.

7-4 Characterisation of Small Nanophytoplankton

In temperate estuaries, where small nano- and pico-phytoplankton have been investigated, they have been found as important constituents of populations in winter when light is low and in late summer when nutrients are low especially in the outer regions (e. g. Cole *et al.*, 1986; Madariaga and Orive, 1989; Iriarte and Purdie, 1994; Tamigneaux *et al.*, 1999). Up to 50% of biomass can be attributed to cells <5µm in diameter at these times. Also they are typically associated with relatively low

concentrations of chlorophyll. In reviews of the structure of marine phytoplankton communities, Kiorboe (1993) and Riegman *et al*. (1993) concluded that small autotrophic cells are better competitors for light and nutrients, due in part to their high surface area to volume ratio, but their abundance is tightly controlled by microzooplankton throughout the year. Therefore in spring, when light increases, larger cells such as diatoms increase faster than small cells as they can escape grazing and so tend to dominate phytoplankton populations when chl is high. This general picture is fully consistent with observations reported here for Southampton Water, and suggests that the small autotrophs play an important role throughout the year in the microbial component of the planktonic food chain and the cycling of key elements such as N and P.

Remarkably little is known about the taxonomic affinity of this component of estuarine phytoplankton populations except in cases where cyanobacteria have been investigated (Iriarte and Purdie, 1994; Lewitus *et al.*, 1998). HPLC pigment data indicate that Chlorophytes, Prymnesiophytes and Cryptophytes are probably the most important groups but their diversity at the species level and the source of some pigments such as 19'but (Ansotegui *et al.*, 2001) remain unknown. Also significant differences exist between estuaries in their pigment signatures (Lemaire *et al.*, 2002). Some of the major accessory pigments, such as fuco, are found in several groups of algae so that HPLC data alone is unlikely to provide unambiguous data about the taxonomy of nanoflagellates. For this reason the development and application of new molecular taxonomic methods will become very important for gaining a better understanding of the roles of this group of organisms in the food chains and biogeochemistry of estuaries.

7-5 Conclusions and Recommendations for Future Work

7-5-1 Conclusions

1. It has been observed that phytoplankton biomass (chl or carbon) cycle is similar to that in other temperate estuaries, following seasonal patterns as well as irradiance and nutrients. The year cycle was characterized by four phases (I-IV), largely in response to light level and nutrient levels. Statistical analyses showed the key role that both light and nutrients played in the structuring of communities. Moreover, it also revealed that turbidity and tidal cycle were significant environmental factors. The chl fluctuation during phase III for 2002 and 2003 was related to many factors, such as the level of PAR, water column transparency and tidal range.

2. Due to climatic factors (PAR and fresh water input) and nutrient levels, chl value and phytoplankton community composition varied significantly from year to year.

3. Diatoms were present year around and bloomed during spring and summer. However when Si was depleted, the composition of the phytoplankton community shifted to flagellate species becoming dominant, and they do not require Si for growth. The nanophytoplankton were present year round and their percentages increased when the environmental parameters (light and nutrient) were not favourable for large cells.

4. Nanophytoplankton <5μm size contributed 35 to 40% of the total chlorophyll. However, the nanophytoplankton <5μm contribution to total Chl was inversely proportional to that of the larger size fraction. The small nanoplankton (2-5μm) and occasionally larger nanoplankton (>5μm) dominated the biomass when chlorophyll was relatively high. The picoplankton included cyanobacteria (probably *Synechococcus*), which were most numerous in the summer. Cryptophytes were found in most of the samples and contributed highly to nanophytoplankton biomass in late summer.

5. A numerical model was constructed by Cloern (1995) to study the interaction between light and nutrients in relation to phytoplankton biomass. A similar model may be required in the future for Southampton Water, to explain the complex interaction between them.

6. Ratios of chla to Tpig were found to be relatively constant, a result that is in agreement with other research, the effect of light at the surface being reduced

by the effect of the tides. C/chl ratios give an indication about community production and vary according to community size structure and species composition

7-5-2 Recommendations

- Other studies reported the bloom of *Phaeocystis* and *Mesodinium rubrum* which were required more evaluation in relation to various environmental parameters. However, in the present study the bloom was not observed.

- Nanophytoplankton is part of the community structure requiring more investigation in terms of their identity and survival, using new techniques such as the flow cytometer and FISH combined with HPLC analysis.

- Models to investigate the relationships between phytoplankton structure and the interaction of light, nutrients and grazing are required.

- Increasing sampling frequency (once a week) provided reliable data for monitoring chl fluctuation (see Figure 5-9). For comparison, sampling frequency and time should be similar.

REFERENCES

Aiken, J., Fishwick, J., Moore, G., and Pemberton, K. 2004. The annual cycle of phytoplankton photosynthetic quantum efficiency, pigment composition and optical properties in the western English Channel. *Journal of Marine Biology Association of United Kingdom* **84**:1-13.

Ali, E. M. 2003. Processes and conditions influencing phytoplankton growth and bloom initiation in a macrotidal estuary, Southampton Water. PhD thesis, Southampton Oceanography Centre. University of Southampton, Southampton. 221

Amann, R. I. 1995. Fluorescently Labeled, Ribosomal-Rna-Targeted Oligonucleotide Probes in the Study of Microbial Ecology. *Molecular Ecology* **4**:543-553.

Amann, R. I., Ludwig, W., and Schleifer, K. H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *FEMS Microbial Review* **59**:143-169.

Ansotegui, A., Sarobe, A., Trigueros, J. M., Urrutxurtu, I., and Orive, E. 2003. Size distribution of algal pigments and phytoplankton assemblages in a coastal-estuarine environment: contribution of small eukaryotic algae. *Journal of Plankton Research* **25**:341-355.

Ansotegui, A., Trigueros, J. M., and Orive, E. 2001. The use of pigment signatures to assess phytoplankton assemblage structure in estuarine waters. *Estuarine, Coastal and Shelf Science* **52**:689-703.

Azam, F., Fenchel, T., J.G.Field , J.S.Gray, Meyer-Reil, L. A., and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology - Progress Series* **10**:257-270.

Barlow, R. G., Mantoura, R. F. C., Cummings, D. G., and Fileman, T. W. 1997. Pigment Chemotaxonomic distributions of phytoplankton during summer in the Westem Mediterranean. *Deep Sea Research Part II* **44**:833-850.

Barlow, R. G., Mantoura, R. F. C., Gough, M. A., and Fileman, T. W. 1993. Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. *Deep-Sea Research II* **1/2**:459-477.

Beman, J. M., Arrigo, K. R., and Matson, P. A. 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *NATURE* **434**:211-214.

Boenigk, J., and Arndt, H. 2000. Comparative studies on the feeding behaviour of two heterotrophic nanoflagellates: the filter feeding choanflagellate *Monosiga ovata* and the raptorial-feeding kinetoplastid *Rhynchomonas nasuta*. *Microbial Ecology* **22**:243-249.

Booth, B. C. 1993. Estimating cell concentration and biomass of autotrophic plankton using microscopy. *in,* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, editors. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, New York. Pages 199-205

Boynton, W. R., Kemp, W. M., and Keefe, C. W. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. *in,* V. S. Kennedy, editor. Estuarine Comparisons. Academic Press, San Diego CA. Pages 69-90

Brandt, S. M., and Sleigh, M. A. 2000. The quantitative occurrence of different taxa of heterotrophic flagellates in Southampton Water, U.K. *Estuaries, Coastal and Shelf Science* **51**:91-102.

Breton, E., Brunet, C., Sautour, B., and Brylinski, J.-M. 2000. Annual variations of phytoplankton biomass in the Eastern English Channel: comparison by pigment signatures and microscopic counts. *Journal of Plankton Research* **22**:1423-1440.

Brussaard, C. P. D. 2004. Viral control of phytoplankton populations - a review. *Journal of Eukaryotic Microbiology* **51**:125-138.

Calliari, D., Gomez, M., and Gomez, N. 2005. Biomass and composition of the phytoplankton in the Rio de la Plata: large-scale distribution and relationship with environmental variables during a spring cruise. *Continental Shelf Research* **25**:197-210.

Cameron, W. M., and Pritchard, D. W. 1963. Estuaries. *in,* M. N. Hill, editor. The Sea. Wiley, New York. Pages 306-324

Carr, J. F., Turville, C. M., Jarman, R. T., and Spencer, J. F. 1980. Water temperatures in the Solent estuarine system. *in,* The Solent Estuarine System: An Assessment of Present Knowledge, NERC Publications Series C No. 22. Pages 36-43

Chan, T. U., and Hamilton, P. 2001. Effect of freshwater flow on the succession and biomass of phytoplankton in a seasonal estuary. *Marine and Freshwater Research* **52**:869-884.

Claquin, P., Martin-Jezequel, V., Kromkamp, J. C., Veldhuis, M. J. W., and Kraay, G. W. 2002. Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophceae) under light, nitrogen, and phosphorus control. *Journal of Phycology* **38**:922-930.

Clarke, K. R., and Warwick, R. M. 1994. Change in marine communities: An approach to statistical analysis and interpretation (PRIMER). Plymouth Marine Laboratory, Plymouth, 144pp

Cloern, J. E. 1982. Does the benthos control phytoplankton biomass in south San Francisco Bay. *Marine Ecology - Progress Series* **9**:191-202.

Cloern, J. E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Continental Shelf Research* **7**:1367-1381.

Cloern, J. E. 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology* **33**:3-15.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology - Progress Series* **210**:223-253.

Cloern, J. E., and Difford, R. 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. *Marine Ecology-Progress Series* **285**:11-28.

Cloern, J. E., Grenz, C., and VidergarLucas, L. 1995. An empirical model of the phytoplankton chlorophyll:carbon ratio - The conversion factor between productivity and growth rate. *Limnology and Oceanography* **40**:1313-1321.

Cole, B. E. 1989. Temporal and spatial patterns of phytoplankton production in Tomales Bay, California, U.S.A. *Estuarine, Coastal and Shelf Science* **28**:103-115.

Cole, B. E., and Cloern, J. E. 1987. An Empirical-Model for Estimating Phytoplankton Productivity in Estuaries. *Marine Ecology-Progress Series* **36**:299-305.

Cole, B. E., Cloern, J. E., and Alpine, A. E. 1986. Biomass and Productivity of 3 Phytoplankton Size Classes in San-Francisco Bay. *Estuaries* **9**:117-126.

Crawford, D. W., and Purdie, D. A. 1992. Evidence for avidence of flushing from an estuary by planktonic phototrophic ciliate. *Marine Ecology - Progrgress Series* **79**:259-265.

Crawford, D. W., Purdie, D. A., Lockwood, A. P. M., and Weissman, P. 1997. Recurrent red-tides in the Southampton Water Estuary caused by the phototrophic ciliate *Mesodinium rubrum*. *Estuarine, Coastal and Shelf Science* **45**:799-812.

Ducklow, H. W. 2001. Bacterioplankton. *in,* J. H. Steele, S. A. Thorpe, and K. K. Turekian, editors. Encyclopaedia of Ocean Sciences. Academic Press, San Diego. Pages 217-224

Dyer, K. R. 1973. *Estuaries: A Physical Introduction*. John Wiley and Sons, London. 183

Dyer, K. R. 1982. Localized mixing of low salinity patches in a partially mixed estuary (Southampton Water, England). *in,* V. S. Kennedy, editor. 6th International Estuarine Research Conference. Academic Press New York, Gleneden Beach, Oregon. Pages 21-36

Dyer, K. R. 1997. *Estuaries: A Physical Introduction*. John Wiley and Sons, London. 179

Edgar, G. J., Barrett, N. S., and Last, P. R. 1999. The Distribution of Macroinvertebrates and Fishes in Tasmanian Estuaries. *Journal of Biogeography* **26**:1169-1189.

Ficheza, R., Jickells, T. D., and Edmunds., H. M. 1992. Algal blooms in high turbidity, a result of the conflicting consequences of turbulence on nutrient

cycling in a shallow water estuary. *Estuarine, Coastal and Shelf Science* **35**: 577-592.

Fisher, T. R., Jr., L. W. H., Stanley, D. W., and Warda, L. G. 1988. Phytoplankton, nutrients, and turbidity in Chesapeake, Delaware and Hudson estuaries. *Estuarine, Coastal and Shelf Science* **27**:61-93.

Fisher, T. R., Peele, E. R., Ammerman, J. W., and Jr., L. W. H. 1992. Nutrient limitation of phytoplankton in Chesapeake Bay. *Marine Ecology - Progress Series* **82**:51-63.

Froneman, P. W. 2001. Seasonal changes in zooplankton biomass and grazing in a temperate estuary, South Africa. *Estuaries, Coastal and Shelf Science* **52**:543-553.

Froneman, P. W. 2004. Food web dynamics in a temperate temporarily open/closed estuary (South Africa). *Estuarine Coastal and Shelf Science* **59**:87-95.

Gaarder, J., and Gran, H. H. 1927. Investigations of the production of plankton in the Oslo Fjord. *Rapports et Proces-Verbaux Des Reunios* **42-46**:1-48.

Gallegos, C. L., Jordan, T. E., Hines, A. H., and Weller, D. E. 2005. Temporal variability of optical properties in a shallow, eutrophic estuary: Seasonal and inter annual variability. *Estuaries, Coastal and Shelf Science* **64**:156-170.

Garibotti, A., Vernet, M., Kozlowski, W. A., and Ferrario, M. E. 2003. Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses. *Marine Ecology - Progress Series* **247**:27-42.

Garnier, J., Billen, G., and Coste, M. 1995. Seasonal Succession of Diatoms and Chlorophyceae in the Drainage Network of the Seine River: Observations and Modeling. *Limnology and Oceanography* **40**:750-765.

Gattuso, J.-P., Frankignoulle, M., and Wollast, R. 1998. Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics* **29**:405-434.

Gazeau, F., Smith, S. V., Gentili, B., Frankignoulle, M., and Gattuso, J.-P. 2004. The European coastal zone: characterization and first assessment of ecosystem metabolism. *Estuarine, Coastal and Shelf Science* **60**:673-694.

Geider, R. J., MacIntyre, H. L., and Kana, T. M. 1997. Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll *a*:carbon ratio to light, nutrient-limitation and temperature. *Marine Ecology - Progrgress Series* **148**:148-187.

Geider, R. J., and La, Roche, J. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal Phycology* **37**:1-17.

Gillanders, B. M., and Kingsford, M. J. 2002. Impact of changes in flow of freshwater on estuarine and open coastal habitats and the associated organisms. *in,* Oceanography and Marine Biology, Vol 40. Taylor & Francis LTD, London. Pages 233-309

Goosen, N. K., Kromkamp, J., Peene, J., Rijswik, P. v., and Breugel, P. v. 1999. Bacterial and phytoplankton production in the maximum turbidity zone of three European estuaries: the Elbe, Westerschelde and Gironde. *Journal of Marine Systems* **22**:151-171.

Grenz, C., Cloern, J. E., Hager, S. W., and Cole, B. E. 2000. Dynamics of nutrient cycling and related benthic nutrient and oxygen fluxes during a spring phytoplankton bloom in South San Francisco Bay (USA). *Marine Ecology - Progress Series* **197**:67-80.

Heip, C. H. C., Goosen, N. K., and Herman, P. M. J. 1995. Production and consumption of biological particles in temperate tidal estuaries. *Oceanography and marine Biology: An annual review* **30**:1-149.

Hendey, N. I. 1964. *An Introductory Account of the Smaller Algae of British Coastal Waters, Part V : Bacillariophyceae (Diatoms)*. Her Majesty's Stationery Office, London. 319pp

Hessen, D. O. 1999. Catchment properties and the transport of major elements of estuaries. *Advances in Ecological Research Estuaries* **29**:1-41.

Holley, S. E., and Hydes, D. J. 2002. Ferry-Boxes and data stations for improved monitoring and resolution of eutrophication-related processes: application in Southampton Water UK, a temperate latitude hypernutrified estuary. *Hydrobiologia* **475/476**:99-110.

Hopkinson, C. S., and Hoffman, R. 1984. The estuary extended - A recipient - system study of estuarine outwelling Georgia. *in,* V. S. Kennedy, editor. The Estuary As A Filter. Academic Press, New York. Pages 313-330

Horner, R. A. 2002. *A Taxonomic Guide to Some Common Marine Phytoplankton*. Biopress Limited, Bristol-England. 195pp

Hughes, J. E., Deegan, L. A., Peterson, B. J., Holmes, R. M., and Fry, B. 2000. Nitrogen Flow through the Food Web in the Oligohaline Zone of a New England Estuary. *Ecology* **81**:433-452.

Hydes, D. 2000. Nutrients in the Solent. *in,* M. Collins and K. Ansell, editors. Solent Science - A Review. Elsevier Science. Pages 135-148

Hydes, D. J., and Wright, P. N. 1999. SONUS: The Southern Nutrients Study 1995 -1997. Southampton Oceanography Centre, Southampton, Report, No.7. 130

Iriarte, A. 1991. Picophytoplankton ecology and physiology studies in culture and natural coastal and estuarine waters. PhD thesis, Oceanography centre. University of Southampton, Southampton, U. K. 279

Iriarte, A. 1993. Size fractionated chlorophyll a biomass and picophytoplankton cell density along a longitudinal axis of a temperate estuary (Southampton Water). *Journal of Plankton Research* **15**:485-500.

Iriarte, A., and Purdie, D. 1994. Size distribution of chlorophyll a biomass and primary production in a temperate estuary (Southampton Water): The contribution of photosynthetic picoplankton. *Marine Ecology - Progress Series* **115**:283-297.

Iriarte, A., and Purdie, D. A. 2004. Factors controlling the timing of major spring bloom events in an UK south coast estuary. *Estuarine, Coastal and Shelf Science* **61**:679-690.

Jeffery, S. W. 1997. Application of pigment methods to oceanography. *in,* S. W. Jeffery, R. F. C. Mantoura, and S. W. Wright, editors. Phytoplankton Pigments in Oceanography: A Guide to Advanced Methods. SCOR-UNESCO, Paris. Pages 360

Jeffery, S. W., and Humphrey, G. F. 1975. New spectrophotometer equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**:191-194.

Jeffery, S. W., and Vesk, M. 1997. Introduction to marine phytoplankton and their pigment signatures. *in,* S. W. Jeffery, R. F. C. Mantoura, and S. W. Wright, editors. Phytoplankton Pigments in Oceanography: A Guide to Advanced Methods. SCOR-UNESCO, Paris. Pages 127-166

Joint, I. R., Owens, N. J. P., and Pomroy, A. J. 1986. Seasonal production of photosynthetic picoplankton and nanoplankton in the Celtic sea. *Marine Ecology - Progress Series* **28**:251-258.

Jorgensen, E. G. 1968. The adaptation of algae. IV. light adaptation in different algal species. *Physiology and plantology* **22**:1307-1315.

Justic, D., Rabalais, N. N., and Turner, R. E. 1995a. Stoichiometric Nutrient Balance and Origin of Coastal Eutrophication. *Marine Pollution Bulletin* **30**:41-46.

Justic, D., Rabalais, N. N., Turner, R. E., and Dortch, Q. 1995b. Changes in Nutrient Structure of River-Dominated Coastal Waters - Stoichiometric Nutrient Balance and Its Consequences. *Estuarine Coastal and Shelf Science* **40**:339-356.

Karl, D. M., and Michaels, A. F. 2001. Nitrogen cycle. *in,* J. H. Steele, S. A. Thorpe, and K. K. Turekian, editors. Encyclopedia of ocean sciences. Academic Press, San Diego. Pages 1876-1883

Karlson, B. 1995. *On the role of pico- and nanoplankton in the Skagerrak*. Goeteborg University (Sweden). 126

Kennish, M. J. 1986. *Ecology of Estuaries Volume II Biological Aspect*. CRC Press, Boca Raton. 395

Kennish, M. J. 1997. *Estuarine and Marine Pollution*. CRC Press, New York. 509pp

Kennish, M. J. 2000. *Estuary Restoration and Maintenance : The National Estuary Program*. CRS press, USA.

Ketchum, B. H. 1983. Estuarine characteristics. *in,* B. H. Ketchum, editor. Ecosystems of the World 26 Estuaries and Enclosed Seas. Elsevier Science Publishers B. V., Amsterdam. Pages 1-13

Kifle, D. 1992. Seasonal and spatial variation species composition, abundance, biomass and primary production of phytoplankton in Southampton Water,

U.K. PhD thesis, Oceanography. University of Southampton, Southampton. 306

Kifle, D., and Purdie, D. A. 1993. The seasonal abundance of the phototrophic ciliate *Mesodinium rubrum* in Southampton Water, England. *Journal of Plankton Research* **15**:823-833.

Kiorboe, T. 1993. Turbulence, Phytoplankton Cell size and the Structure of Pelagic Food Webs. *in,* J. H. S. Blaxter and A. J. Southward., editors. Advances in Marine Biology. Academic Press, London NW1 7DX.

Kocum, E., Nedwell, D. B., and Underwood, G. J. C. 2002a. Regulation of phytoplankton primary production along a hypernutrified estuary. *Marine Ecology Progress Series* **231**:13-22.

Kocum, E., Underwood, G. J. C., and Nedwell, D. B. 2002b. Simultaneous measurement of phytoplanktonic primary production, nutrient and light availability along a turbid, eutrophic UK east coast estuary (the Colne Estuary). *Marine Ecology Progress Series* **231**:1-12.

Kolber, Z. S., Prasil, O., and Falkowski, P. G. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1367**:88-106.

Lagus, A., Suomela, J., Weithoff, G., Heikkila, K., Helminen, H., and Sipura, J. 2004. Species-specific difference in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago sea, northern Baltic sea. *Journal of Plankton Research* **26**:779-798.

Lalli, C. M., and Timothy, R. P. 1997. *Biological Oceanography an Introduction*, second edition. Butterworth Heinemann, Oxford. 341

Landry, M. 2001. Microbial loops. *in,* J. H. Steele, S. A. Thorpe, and K. K. Turekian, editors. Encyclopaedia of Ocean Sciences. Academic Press, San Diego. Pages 1763-1770

Lauria, M. L. 1998. Physical constraints on phytoplankton in estuaries and shallow coastal waters. PhD thesis, Southampton Oceanography Centre. University of Southampton, Southampton. 180

Lauria, M. L., Purdie, D. A., and Sharples, J. 1999. Contrasting phytoplankton distributions controlled by tidal turbulence in an estuary. *Journal of Marine Systems* **21**:189-197.

Legendre, L. 1990. The significance of microalgal bloom for fisheries and for export of particulate organic carbon in oceans. *Journal of Plankton Research* **12**:681-699.

Lehman, P. W. 2000. The Influence of Climate on Phytoplankton Community Biomass in San Francisco Bay Estuary. *Limnology and Oceanography* **45**:580-590.

Lemaire, E., Abril, G., De Wit, R., and Etcheber, H. 2002. Distribution of phytoplankton pigments in nine European estuaries and implications for an estuarine typology. *Biogeochemistry* **59**:5-23.

Lewitus, A. J., Koepfler, E. T., and Morris, J. T. 1998. Seasonal Variation in the Regulation of Phytoplankton by Nitrogen and Grazing in a Salt-Marsh Estuary. *Limnology and Oceanography* **43**:636-646.

Li, Y., and Smayda, T. J. 1998. Temporal variability of chlorophyll in Narragansett Bay, 1973-1990. *ICES Journal of Marine Science* **55**:661-667.

Little, C. 2000. *The Biology of Soft Shores and Estuaries*, First edition. Oxford University Press, Oxford. 252

Llewellyn, C. A., Fishwick, J. R., and Blackford, J. C. 2005. Phytoplankton community assemblage in the English Channel: a comparison using chlorophyll a derived from HPLC-CHEMTAX and carbon derived from microscopy cell counts. *J. Plankton Res.* **27**:103-119.

Lucas, C. H., and Holligan, P. M. 1999 a. Nature and ecological implications of algal pigment diversity on the Molenplaat tidal flat (Westerschelde estuary, SW Netherlands). *Marine Ecology Progress Series* **180**:51-64.

Lucas, L. V., Koseff, J. R., Cloern, J. E., Monismith, S. G., and Thompson, J. K. 1999a. Processes governing phytoplankton blooms in estuaries. I: The local production-loss balance. *Marine Ecology-Progress Series* **187**:1-15.

Lucas, L. V., Koseff, J. R., Monismith, S. G., Cloern, J. E., and Thompson, J. K. 1999b. Processes governing phytoplankton blooms in estuaries. II: The role of horizontal transport. *Marine Ecology-Progress Series* **187**:17-30.

Maclsaac, E. A., and Stockner, J. G. 1993. Enumeration of phototrophic picoplankton by autofluorescece microscopy. *in,* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, editors. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, New York. Pages 187-197

Madariaga, I. d., and Orive, E. 1989. Spatio-temporal variations of size-fractionated primary production in the Gernika estuary. *Journal of Experimental Marien Biology and Ecology* **127**:273-288.

Mann, K. H. 2000. *Ecology of Coastal Waters: with Implications for Management*, 2nd edition. Blackwell Science, USA. 390

Martin-Jezequel, V., Hildebrand, M., and Brzezinski, M. A. 2000. Silico metabolism in diatoms: implication for growth. *Journal of Phycology* **36**:821-840.

May, C. L., Koseff, J. R., Lucas, L. V., Cloern, J. E., and Schoellhamer, D. H. 2003. Effects of spatial and temporal variability of turbidity on phytoplankton blooms. *Marine Ecology-Progress Series* **254**:111-128.

Menden-Deuer, S., and Lessard, E. J. 2000. Carbon to volume relationship for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography* **45**:569-579.

Menden-Deuer, S., Lessard, E. J., and Satterberg, J. 2001. Effect of preservation on dinoflagellate and diatom cell volume and consequences for carbon biomass predictions. *Marine Ecology Progress Series* **222**:41-50.

Middelburg, J. J., Klaver, G., Nieuwenhuize, J., and Vlug, T. 1995. Carbon and nitrogen cycling in intertidal sediments near Doel, Scheld Estuary. *Hydrobiologia* **311**:57-69.

Monbet, Y. 1992. Control of phytoplankton biomass in estuaries: A comparative analysis of microtidal and macrotidal estuaries. *Estuaries* **15**:563-571.

Montagnes, D. J. S., Berges, J. A., Harrison, P., Harrison, P. J., and Taylor, F. J. R. 1994. Estimating carbon, nitrogen, protein, and chlorophyll-a from volume in marine phytoplankton. *Limnology and Oceanography* **39**:1044-1060.

Muxagata, E. 2005. Seasonal and spatial distribution of the meszooplankton of Southampton Water with particular reference to the contribution of copepods and barnacle larvae to pelagic carbon flux. PhD thesis, Southampton Oceanography Centre. University of Southampton, Southampton. 237

Muylaert, K., and Sabbe, K. 1999. Spring phytoplankton assemblages in and around the maximum turbidity zone of the estuaries of the Elbe (Germany), the Schelde (Belgium/The Netherlands) and the Gironde (France). *Journal of Marine Systems* **22**:133-149.

Muylaert, K., Sabbe, K., and Vyverman, W. 2000. Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). *Estuarine, Coastal and Shelf Science* **50**:673-687.

Nedwell, D. B., Dong, L. F., Sage, A., and Underwood, G. J. C. 2002. Variations of the Nutrients Loads to the Mainland U.K. Estuaries: Correlation with Catchment Areas, Urbanization and Coastal Eutrophication. *Estuarine, Coastal and Shelf Science* **54**:951-970.

Nedwell, D. B., Jickells, T. D., Trimmer, M., and Sanders, R. 1999. Nutrients in Estuaries. *Advances in Ecological Research Estuaries* **29**:43-92.

Nixon, S. W. 1981. Fresh water input and estuarine productivity. *in,* R. a. D. W. Cross, editor. Proceeding of the national symposium on fresh water inflow to estuaries. Service and Biological Services, U.S. Fish and Wildlife.

Not, F., Simon, N., Biegala, F., and Vault, D. 2002. Application of fluorescent *in situ* hybridisation coupled with tyramide signal amplification (FISH-TSA) to assess eukaryotic picoplankton composition. *Aquatic Microbial Ecology* **28**:157-166.

Officer, C. B. 1992. Physics of estuarine circulation. *in,* B. H. Ketchum, editor. Ecosystems of the worlds 26 estuaries and enclosed seas. Elsevier Science Publishers B. V., Amsterdam. Pages 15-39

O'Mahony, J., and Weeka, A. 2000. Phytoplankton - annual sequences in the Hamble estuary. *in,* M. Collins and K. Ansell, editors. Solent Science - A review. Elsevier, Amsterdam. Pages 287-294

Ornolfsdottir, E. B., Lumsden, S. E., and Pinckney, J. L. 2004a. Nutrient pulsing as a regulator of phytoplankton abundance and community composition in Galveston Bay, Texas. *Journal of Experimental Marine Biology and Ecology* **303**:197-220.

Ornolfsdottir, E. B., Lumsden, S. E., and Pinckney, J. L. 2004b. Phytoplankton community growth-rate response to nutrient pulses in a shallow turbid estuary, Galveston Bay, Texas. *J. Plankton Res.* **26**:325-339.

Parsons, T. R., Maita, Y., and Lalli, C. M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford. 173pp

Parsons, T. R., and Takahashi, M. 1973. Environmental control of phytoplankton cell size. *Limnology and Oceanography* **18**:511-515.

Pennock, J. R., and Sharp, J. H. 1986. Phytoplankton production in the Delaware Estuary: temporal and spatial variability. *Marine Ecology Progress Series* **34**:143-155.

Peperzak, L. 1993. Daily irradiance governs growth rate and colony formation of *Phaeocystis* (Prymnesiophyceae). *Journal of Plankton Research* **15**:809-521.

Peperzak, L., Colijn, F., Gieskes, W. W. C., and Peeters, J. C. H. 1998. Development of the diatom-*Phaeocystis* spring bloom in the Dutch costal zone of the north Sea: the silicon depletion versus the daily irradiance threshold hypothesis. *Journal of Plankton Research* **20**:517-537.

Pinckney, J. L., Paerl, H. W., and Harrington, M. B. 1999. Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. *Journal of Phycology* **35**:1455-1463.

Pinckney, J. L., Paerl, H. W., Tester, P., and Richardson, T. L. 2001. The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives* **109**:699-706.

Pitkanen, H., and Tamminen, T. 1995. Nitrogen and phosphorus as production limiting factors in the estuarine waters of the eastern Gulf of Finland. *Marine Ecology Progress Series* **129**:283-294.

Platt, T., Gallegos, C. L., and Harrison, W. G. 1980. Photoinhibition of photosynthesis assemblages of marine phytoplankton. *Journal of Marine Research* **38**:687-701.

Pritchard, D. W. 1967. Observations of circulation in coastal plain estuaries. *in,* G. H. Lauff, editor. Estuaries. American Association for the Advancement of Science, Washington DC. Pages 37-44

Ragueneau, O., C. Lancelot, V. Egorov, J. Vervlimmeren, A. Cociasu, G. Déliat, A. Krastev, N. Daoud, V. Rousseau, and al., V. P. e. 2002. Biogeochemical transformations of Inorganic nutrients in the mixing zone between the Danube River and the North-western Black Sea. *Estuaries, Coastal and Shelf Science* **54**:321-336.

Raymont, J. E. G. 1980. *Plankton and Productivity in the Oceans*, 2 edition. Pergamon Press, Oxford. 660

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *American Scientist* **Autumn 1958**:205-221.

Reid, P. C., Planque, B., and Edwards, M. 1998. Is observed variability in the long term results of the continuous plankton recorder a response to climate change? *Fisheries Oceanography* **7**:282-288.

Riegman, R., Bouwe, E., Kuipers, Noordeloos, A. M. A., and Harry, J. 1993. Size-differential control of phytoplankton and the structure of plankton communities. *Netherlands Journal of Sea Research* **31**:255-265.

Roberts, E. C., Davidson, K., and Gilpin, L. C. 2003. Response of temperate microplankton communities to N : Si ratio perturbation. *Journal of Plankton Research* **25**:1485-1495.

Rodriguez, F., Varela, M., and Zapata, M. 2002. Phytoplankton assemblages in the Gerlache and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data. *Deep Sea Research Part II: Topical Studies in Oceanography* **49**:723-747.

Samuelsson, K. 2003. Mechanism structuring the pelagic microbial food web - Importance of resources and predation, Ecology and Environmental Science. Umea University, Sweden. 129

Sanders, R., Jickells, T., and Mills, D. 2001. Nutrients and chlorophyll at two sites in the Thames plume and southern North Sea. *Journal of Sea Research* **46**:13-28.

Shi, L. 2000. Development and application of a three-dimensional water quality model in a partially-mixed estuary, Southampton Water, UK. PhD thesis, Southampton Oceanography Centre. University of Southampton, Southampton. 213

Simon, N., Barlow, R. G., Marie, D., Partensky, F., and Vaulot, D. 1994. Characterization of Oceanic Photosynthetic Picoeukaryotes by Flow-Cytometry. *Journal of Phycology* **30**:922-935.

Simon, N., Lebot, N., Marie, D., Partensky, F., and Vaulot, D. 1995. Fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes to identify small phytoplankton by flow cytometry. *Applied and Environmental Microbiology* **61**:2506-2513.

Sin, Y., Wetzel, R. L., and Anderson, I. C. 2000. Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). *Journal of Plankton Research* **22**:1945-1960.

Smayda, T. J. 1983. The phytoplankton of estuaries. *in,* B. H. Ketchum, editor. Ecosystems of the world 26 estuaries and enclosed seas. Elsevier science publishers B. V., Amsterdam. Pages 1-13

Smayda, T. J. 1989. Novel and nusisan phytoplankton bloom in the sea: evidence for a global epidemic. *in,* E. Graneli, B. Sunstrom, L. Edler, and D. M. Anderson, editors. Toxic Marine Phytoplankton. Elsevier, Lun, Sweden. Pages 29-39

Smayda, T. J. 1998. Patterns of variability characterizing marine phytoplankton, with examples from Narragansett Bay. *Ices Journal of Marine Science* **55**:562-573.

Smith, S. V., and Hollibaugh, J. T. 1993. Coastal metabolism and the oceanic carbon balance. *Review of Geophysics* **31**:75-89.

Smith, V. H. 2003. Eutrophication of freshwater and costal marine ecosystems: a global problem. *Environment Science Pollution Research International* **10**:126-139.

Soetaert, K., and Herman, P. M. J. 1995a. Carbon flows in the Westerschelde estuary (The Netherlands) evaluated by means of an ecosystem model (MOSES). *Hydrobiologia* **311**:247-266.

Soetaert, K., and Herman, P. M. J. 1995b. Nitrogen dynamics in the Westerschelde estuary (SW Netherlands) estimated by mean of the ecosystem model MOSES. *Hydrobiologia* **311**:225-246.

Suggett, D., Kraay, G., Holligan, P., Davey, M., Aiken, J., and Geider, R. 2001. Assessment of photosynthesis in a spring cyanobacterial bloom by use of a fast repetition rate fluorometer. *Limnology and Oceanography* **46**:802-810.

Sylaios, G., and Boxall, S. R. 1998. Residual currents and flux estimates in a partially-mixed estuary. *Estuaries,Coastal and Shelf Science* **42**:371-416.

Tamigneaux, E., Legendre, L., Klein, B., and Mingelbier, M. 1999. Seasonal Dynamics and Potential Fate of Size-fractionated Phytoplankton in a Temperate Near shore Environment (Western Gulf of St Lawrence, Canada). *Estuarine, Coastal and Shelf Science* **48**:253-269.

Tarran, G. A., Zubkov, M. V., Sleigh, M. A., Burkill, P. H., and Yallop, M. 2001. Microbial Community structure and standing stocks in the NE Atlantic in June and July of 1996. *Deep-Sea Research II* **48**:963-985.

Tett, P. B. 1987. Plankton. *in,* J. M. Baker and W. J. Wolf, editors. Biological Surveys of Estuaries and Coasts. Cambridge University Press, Cambridge. Pages p

Tomas, C. R. 1997. *Identifying Marine Phytoplankton.* Academic Press, San Diego, California. 858pp

Trees, C. C., Clark, D. K., Bidigare, R. R., Ondrusk, M. E., and Mueller, J. L. 2000. Accessory pigment versus chlorophyll *a* concentration within euphotic zone: A ubiquitous relationship. *Limnology and Oceanography* **45**:1130-1143.

Trees, C. C., Kennincutt, M. C., and Brooks, J. M. 1985. Errors associated with standard fluorometric determination of chlorophyll and phaeopigment. *Marine Chemistry* **17**:1-12.

Tyrrell, T. 2001. Redfield ratio. *in,* J. H. Steele, S. A. Thorpe, and K. K. Turekian, editors. Encyclopaedia of Ocean Sciences. Academic Press, San Diego. Pages 2377-2386

Underwood, G. J. C., and Kromkamp, J. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research Estuaries* **29**:93-139.

Vernberg, W. B. 1992. Responses to estuarine stress. *in,* B. H. Ketchum, editor. Ecosystems of the world 26 estuaries and enclosed seas. Elsevier science publishers B. V., Amsterdam. Pages 34-60

Webb, W. M., Newton, M., and Star, D. 1974. Carbon dioxide exchange of *Alnus rubra*. A mathematical model. *Oecologica* **17**:281-291.

Westeyn, L. P. M. J., and Kromkamp, J. C. 1994. Turbidity, nutrients and phytoplankton primary production in the Oosterschele (The Netherlands) before, during and after a large- scale coastal engineering project (1980-1990). *Hydrobiologia* **282/283**:61-78.

Wienke, S. M., and Cloern, J. E. 1987. The phytoplankton component of seston in San Francisco Bay. *Netherlands Journal of Sea Research* **21**:25-33.

Wilkinson, J. J. 1991. Volatile production during contact metamorphism: the role of organic matter in pelites. *Journal of the Geological Society* **148**:731-736.

Williams, P. J. L. 1980. Phytoplankton in Southampton Water. *in,* The Solent Estuarine System: An Assessment of Present Knowledge. NERC Publications Series C No. 22. Pages 73-75

Wyman, M., Davies, J. T., Weston, K., Crawford, D. W., and Purdie, D. A. 1998. Ribulose-1,5-biosphosphate carboxylase/oxygenase (RubisCO) gene expression and photosynthetic activity in nutrient-enriched mesocosm experiments. *Estuaries, Coastal and Shelf Science* **46**:23-33.

Zehr, J. P., and Ward, B. B. 2002. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. *Applied Environmental Microbiology* **68**:1015-1024.

APPENDIX 1- PHYTOPLANKTON SPECIES

1-1 Dock station 2002

Samples Day		**carbon**	**6/2**	**13/2**	**20/2**	**27/2**	**6/3**	**13/3**	**20/3**
Days	**cell**		**30**	**37**	**44**	**51**	**58**	**65**	**72**
Chl	**volume**	**content**	**0.67**	**1.42**	**1.5**	**0.51**	**0.6**	**1.42**	**1.51**
Diatoms	**µm-^{3}**	**pg/cell**							
Asterionella glacialis	1470	107							
Asterionella kariana	2550	167							
Bacillaria paxillifera	4400	260							0.2
Bellerochea malleus	4400	260	0		1.5		1.8		2.5
Biddulphia pulchella	650	55		0.2		0.1			
Cerataulina pelagica	28274	1173							
Chaetoceros curvisetus	1767	124							
Chaetoceros debilis	1045	81							
Chaetoceros eibenii	21206	929							
Chaetoceros vistulae	65	9			0.2				
Chaetoceros wighamii	696	58							
Coscinodiscus	79851	2724							
Cylindrotheca closterium	22	4	0.4	0.1	0.3	0.2			
Ditylum brightwelli	5805	325			0.1		0.1		0.1
Eucampia spp	4665	272		0.2			1.3		2
Grammatophora marina	7725	410							
Guinardia delicatula	53011	1954							
Guinardia flaccid	29372	1210							
Guinardia striata	47517	1788							
Helicotheca tamesis	6000	334							
Lauderia	9425	481					0.3		
Lithodesmium undulatum	4330	256							
Melosira sp		0							
Navicula sp	300	29			2	1			
Nitzschia longissima	158	17		0.1			0.4		0.3
Odontella aurita	650	55							
Odontella mobiliensis	280686	7549					0.1		
Odontella sinensis	892699	19294							
Pennate	35	5	1	2	0.9		0.8	0.4	
Pleurosigma	13433	642			0.4		0.2		1.2
Pseudo –nitzschia sp	34	5			0.2		0.4		
Rhizosolenia imbricate	94248	3115							
Rhizosolenia setigera L	94248	3115				0.1			
Rhizosolenia setigera S	19635	873							
Skeletonema costatum	79	10	1.6		1.1	0.7			
Thalassionema frauenfeldii	1250	94							
Thalassionema Nitzschioides	1250	94						0.1	
Thalassiosira hylina	5890	329							
Thalassiosira rotula	3421	212					0.3		
Dinoflagellates + Ciliate									
Gymnodinium	3644	477						0.1	0.9
Gymnodinium sp2	188	30		3.5	30	4		20	11
Prorocentrum micans	11780	1436							
Scrippsiella trochoidea	2927	389	0.2	0.6		5.5			
Mesodinium rubrum	1767	242	0.2	3	1	1.4	2		5
Flagellates									
Cryptomonas spp	67	11	1.4	15	14	4	6	39	11
Eutreptiella sp	1374	191	0	0	4.2	0	0.2	109	4
Flagellates<5	14	3	2771	1935	1688	1975	308	9958	9905
Flagellates>5	113	18	3714	2941	3412	4647	480	3667	1810

Samples Day	**27/3**	**4/4**	**10/4**	**17/4**	**24/4**	**2/5**	**9/5**	**14/5**	**22/5**
Days	**79**	**86**	**94**	**100**	**107**	**114**	**122**	**129**	**134**
Chl	**0.56**	**0.49**	**2.16**	**2.48**	**1.85**	**0.68**	**1.12**	**1.68**	**2.92**
Diatoms									
Asterionella glacialis									
Asterionella kariana			2.5		2				
Bacillaria paxillifera			1.4	1.1					
Bellerochea malleus					1.5				
Biddulphia pulchella									
Cerataulina pelagica									
Chaetoceros curvisetus									
Chaetoceros debilis									
Chaetoceros eibenii									
Chaetoceros vistulae									
Chaetoceros wighamii									
Coscinodiscus		1	0.2	2					
Cylindrotheca closterium		0.5	2	5.1	1	2.4	0.3	0.6	0.2
Ditylum brightwelli		0.2	0.2		0.2				
Eucampia spp									
Grammatophora marina					0.7	0.4			0.1
Guinardia delicatula								0.2	
Guinardia flaccid									
Guinardia striata									
Helicotheca tamesis									
Lauderia									
Lithodesmium undulatum									0.2
Melosira sp		1							
Navicula sp	0.1		0.5	1	0.2	0.2	0.1	0.6	0.3
Nitzschia longissima	0.2								
Odontella aurita									
Odontella mobiliensis									
Odontella sinensis									
Pennate		3.8	1.7	3.2		1.1	0.6	2.5	1.8
Pleurosigma	0.3								
Pseudo –nitzschia sp			0.2						0.1
Rhizosolenia imbricate									
Rhizosolenia setigera L	0.1		0.1				0.3	0.1	
Rhizosolenia setigera S									
Skeletonema costatum		5	21.5	21.5	29.5	15	4	10.2	14.5
Thalassionema frauenfeldii					1	0.3			
Thalassionema Nitzschioides						0.2			0.2
Thalassiosira hylina		1	0.5	5.8	2			1.5	
Thalassiosira rotula			0.4	0.7		0.4		0.8	0.3
Dinoflagellates + Ciliate									
Gymnodinium								2.6	
Gymnodinium sp2	35		22		6		6		
Prorocentrum micans									0.1
Scrippsiella trochoidea	5.5		2	0.2		2	0.5	0.4	2.7
Mesodinium rubrum	2		2	1.2	2	0.3	3	2	6.5
Flagellates									
Cryptomonas spp	25	12	32	27	43	2.1	71	40	29
Eutreptiella sp	19	0	25	3	325	1	72	27	4.5
Flagellates<5	1335	1276	1694	1200	1523	1185	1529	1429	1217
Flagellates>5	3412	2941	3059	2823	3882	1762	2529	4706	2647

Samples Day	**30/5**	**5/6**	**12/6**	**20/6**	**27/6**	**2/7**	**10/7**	**17/7**	**23/7**
Days	**142**	**150**	**156**	**163**	**171**	**178**	**184**	**191**	**198**
Chl	**1.55**	**2.57**	**8.69**	**1.10**	**10.5**	**9.63**	**1.02**	**6.98**	**5.93**
Diatoms									
Asterionella glacialis			0.4					0.9	7.5
Asterionella kariana	3								0.5
Bacillaria paxillifera									
Bellerochea malleus				0.2					
Biddulphia pulchella								2	0.4
Cerataulina pelagica				5.4	45.8				
Chaetoceros curvisetus									
Chaetoceros debilis				0.6	4.2			5.5	17.2
Chaetoceros eibenii								2.2	1.3
Chaetoceros vistulae								0.4	0.7
Chaetoceros wighamii								4.8	1.6
Coscinodiscus		0.2	0.2	0.3	4	8.1			1.4
Cylindrotheca closterium	0.7		0.2					0.7	1.1
Ditylum brightwelli					0.1			0.6	0.1
Eucampia spp									
Grammatophora marina	1.2								
Guinardia delicatula					4				7.2
Guinardia flaccid									
Guinardia striata									
Helicotheca tamesis									
Lauderia									
Lithodesmium undulatum		2	0.8	0.4				2	7.9
Melosira sp									4
Navicula sp	0.6			1.2		1.2	0.3		4.5
Nitzschia longissima									
Odontella aurita									0.2
Odontella mobiliensis									0.1
Odontella sinensis									
Pennate	2.5			0.6			1.6	0.8	0.3
Pleurosigma		0.1	0.7	1	5.3		0.1	1	0.4
Pseudo –nitzschia sp								3	2.3
Rhizosolenia imbricate									
Rhizosolenia setigera L	0.9	2.3			0.1			1	2
Rhizosolenia setigera S								6	
Skeletonema costatum	8.7							47.3	601.
Thalassionema frauenfeldii							0.3		
Thalassionema Nitzschioides	1.3					7.4		1.2	
Thalassiosira hylina	0.4				6.4				
Thalassiosira rotula		2.6		1.3	0.9			0.7	
Dinoflagellates + Ciliate									
Gymnodinium	0.1			0.3	0.7		0.1	0.4	8
Gymnodinium sp2		28	2189			63		360	96
Prorocentrum micans						0.3			10
Scrippsiella trochoidea	3.5	2.3	90	1.8	7.5	69	10	25	8
Mesodinium rubrum	3.2	2	5	0.3	20	15	3.5	16	8
Flagellates									
Cryptomonas spp	18	13	20	12	150	100	60	535	210
Eutreptiella sp	3.6	11	39	6.5	16.9	2	5	6	3.1
Flagellates<5	1550	1128	1066	1329	6095	6481	1238	1929	5882
Flagellates>5	1714	1857	1857	2417	1143	1889	1000	4529	1500

Appendix

Samples Day	**31/7**	**8/8**	**14/8**	**20/8**	**28/8**	**4/9**	**11/9**	**19/9**
Days	**204**	**212**	**220**	**226**	**232**	**240**	**247**	**254**
Chl	**6.53**	**7.11**	**2.91**	**19.13**	**2.99**	**3.35**	**1.43**	**2.93**
Diatoms								
Asterionella glacialis	13.8	4.3		41				
Asterionella kariana								
Bacillaria paxillifera								
Bellerochea malleus								
Biddulphia pulchella								
Cerataulina pelagica								
Chaetoceros curvisetus								
Chaetoceros debilis				90	3	3		2
Chaetoceros eibenii	1							
Chaetoceros vistulae		2.1		4				
Chaetoceros wighamii		19.1						85
Coscinodiscus	4			3	1	1	1	
Cylindrotheca closterium	3.2	10.6	1	125	4	31	9	
Ditylum brightwelli	1							
Eucampia spp								
Grammatophora marina					1			
Guinardia delicatula			4	7	4			5
Guinardia flaccid								
Guinardia striata								
Helicotheca tamesis								
Lauderia								
Lithodesmium undulatum	3		1				0.2	
Melosira sp								
Navicula sp								
Nitzschia longissima								
Odontella aurita								
Odontella mobiliensis							0.1	
Odontella sinensis								
Pennate	19	4	15.5	7	5	14	36	18
Pleurosigma	13.8							1
Pseudo –nitzschia sp				2	1		0.1	
Rhizosolenia imbricate								
Rhizosolenia setigera L	1			1		1		
Rhizosolenia setigera S	17							
Skeletonema costatum	388	1669	101	16189	76	248	67	240
Thalassionema frauenfeldii			3.2					0.5
Thalassionema Nitzschioides								
Thalassiosira hylina			15	10		5	2	31
Thalassiosira rotula		16.2					2	3
Dinoflagellates + Ciliate								
Gymnodinium	10					8		
Gymnodinium sp2	869		581	88	100	20	10	38
Prorocentrum micans	6.4	9	2	2				
Scrippsiella trochoidea	8	1	1	8				0.5
Mesodinium rubrum	11			14	0			
Flagellates								
Cryptomonas spp	275	6	44	323	158	101	42	23
Eutreptiella sp	0		4	25		4	11	
Flagellates<5	7852	2283	12000	13705	9952	14000	19706	7452
Flagellates>5	2667	1787	1952	8235	1333	2000	2058	2387

Samples Day	**25/9**	**2/10**	**9/10**	**17/10**	**23/10**	**29/10**
Days	**262**	**268**	**275**	**282**	**296**	**302**
Chl	**2.5**	**2.47**	**1.94**	**0.98**	**1.32**	**1.98**
Diatoms						
Asterionella glacialis						
Asterionella kariana						
Bacillaria paxillifera			8			
Bellerochea malleus						
Biddulphia pulchella	4			1.4	2	
Cerataulina pelagica						
Chaetoceros curvisetus						
Chaetoceros debilis	3	2				
Chaetoceros eibenii						
Chaetoceros vistulae				1.2		
Chaetoceros wighamii						
Coscinodiscus						
Cylindrotheca closterium	27	4		4	0.3	1
Ditylum brightwelli						
Eucampia spp						
Grammatophora marina	1					
Guinardia delicatula	2					
Guinardia flaccid						
Guinardia striata						
Helicotheca tamesis						
Lauderia						
Lithodesmium undulatum						
Melosira sp						
Navicula sp						
Nitzschia longissima						
Odontella aurita						
Odontella mobiliensis				0.1		0.1
Odontella sinensis						
Pennate	12		14	12	45	23
Pleurosigma			2			
Pseudo –nitzschia sp				0.4		
Rhizosolenia imbricate						
Rhizosolenia setigera L				1	0.1	2
Rhizosolenia setigera S						
Skeletonema costatum	47	18	19	2		
Thalassionema frauenfeldii						
Thalassionema Nitzschioides						
Thalassiosira hylina						
Thalassiosira rotula						
Dinoflagellates + Ciliate						
Gymnodinium	2					
Gymnodinium sp2	31		21		4	6
Prorocentrum micans					1	
Scrippsiella trochoidea	6	10	36	13	16	4
Mesodinium rubrum	2	0	4	2	0.2	3
Flagellates						
Cryptomonas spp	163	233	94	109	119	108
Eutreptiella sp		11	3	11		4
Flagellates<5	11571	22058	8875	4570	7889	10714
Flagellates>5	2476	2529	2375	1275	1432	1762

1-2 NWN station 2003

Sampling Day			**1/4**	**14/4**	**28/4**	**6/5**	**12/5/**	**19/5**
Days	**cell**	**carbon**	**91**	**104**	**118**	**126**	**132**	**139**
Chl	**volume**	**content**	**3.3**	**2.8**	**3.8**	**5.1**	**15.5**	**1.0**
Diatoms	**µm-^{3}**	**pg/cell**						
Asterionella glacialis	2550	173	42.5	12.8				
Asterionella kariana	2550	173	5.3	0.8				
Bacillaria paxillifera	4400	261						
Bellerochea malleus	4400	261						
Biddulphia pulchella	650	61						
Cerataulina pelagica	28274	1074						
Chaetoceros curvisetus	1767	64		1.2				
Chaetoceros debilis	1045	88	0.2					
Chaetoceros eibenii	21206	863						
Chaetoceros vistulae	65	11	0.1					
Chaetoceros wighamii	696	64		0.6	21.3			
Coscinodiscus	267865	7323						
Cylindrotheca closterium	22	5			0.2			
Ditylum brightwelli	27245	1044		0.2				
Eucampia spp	4665	273						
Grammatophora marina	7725	401						
Guinardia delicatula	6185	338				307.6	561.6	20.2
Guinardia flaccid	294524	6376				0.4	6.3	2.1
Guinardia striata	47517	1594				0.4	1	3.2
Helicotheca tamesis	6000	331						
Lauderia	9425	466						
Lithodesmium undulatum	4330	258						
Melosira sp								
Navicula sp	300	34						
Nitzschia longissima	210	26	3.1	21.3	8.5	0.5		
Odontella aurita	650	61	2.2					
Odontella mobiliensis	280686	6147						
Odontella sinensis	892699	14809						
Pennate	35	7	10.4	1.2	54.2	1.3	3.2	8.5
Pleurosigma	18600	781						1.1
Pseudo –nitzschia sp	34	6					2.1	3.2
Rhizosolenia imbricate	94248	2682					0.3	
Rhizosolenia setigera L	94248	2682					0.4	
Rhizosolenia setigera S	19635	814			2.1			
Skeletonema costatum	188	24	1162	693	10.6			
Thalassionema frauenfeldii	1250	100						
Thalassionema	1250	100						
Thalassiosira hylina	3421	216	39.7	5.6				
Thalassiosira rotula	15080	666	19	4.8				
Dinoflagellates + Ciliate								
Gymnodinium	3644	173	5.3		25.5	0.4	5.2	
Gymnodinium sp2	188	35	108	25	143.6	29.5		6.4
Prorocentrum micans	11780	1686					0.1	
Scrippsiella trochoidea	2927	456	4.2		10.6			
Mesodinium rubrum	1767	283	25.5	44.6	42.5	3.5	2.1	7.4
Flagellates								
Cryptomonas spp	67	13	120		345.7	43.9		44.7
Eutreptiella sp	26180	3572	28.7		80.9	14.9		7.4
Flagellates<5	34	7	1925	1542	4191	6476	5095	5625
Flagellates>5	524	90	379	63	524	1142	476	333

Sampling Day	**27/5**	**3/6**	**9/6**	**16/6**	**23/6**	**30/6**	**7/7**	**14/7**
Days	**147**	**153**	**160**	**167**	**174**	**181**	**188**	**195**
Chl	**3.4**	**37.1**	**8.9**	**8.1**	**6.0**	**12.3**	**2.1**	**2.0**
Diatoms								
Asterionella glacialis								
Asterionella kariana								
Bacillaria paxillifera								
Bellerochea malleus					0.8			
Biddulphia pulchella								
Cerataulina pelagica								
Chaetoceros curvisetus	1.8	38.2			263.8			
Chaetoceros debilis			26.6	417	463.8	319.2		
Chaetoceros eibenii								
Chaetoceros vistulae				3.2				
Chaetoceros wighamii		106.4	128.7	143.6	797.9	1220.2		
Coscinodiscus				4.2	0.1			
Cylindrotheca closterium	1.1					1.1		2.2
Ditylum brightwelli				5.3	9.5	10.6	2.1	
Eucampia spp		60	3.8		11.9			24.5
Grammatophora marina								
Guinardia delicatula	3.2	10.6		3.2	3.1			36.2
Guinardia flaccid	2.1	2.1						
Guinardia striata	3.1	2.7	0.8	3.2				
Helicotheca tamesis								
Lauderia	64.9	334						
Lithodesmium undulatum					0.6	8.5	51.1	
Melosira sp								
Navicula sp								
Nitzschia longissima	2.1	3.2			2.1	1.1		1.1
Odontella aurita								
Odontella mobiliensis								
Odontella sinensis								
Pennate		3.3			4.2		3.2	
Pleurosigma								1.1
Pseudo –nitzschia sp	2.2	35.1	103.2		308.5		8.5	
Rhizosolenia imbricate	0.1	3.2		0.1				
Rhizosolenia setigera L	0.1		0.1		17			
Rhizosolenia setigera S		0.1	8.5	16	9.6	1.1		
Skeletonema costatum		103		22.3	39.4	25.5		17
Thalassionema frauenfeldii	0.8	12.8			17		4.2	5.3
Thalassionema Nitzschioides						1.2		
Thalassiosira hylina		127	309.6	448	0.3	4.2		
Thalassiosira rotula	2	50	678	194.7	33		0.6	13.8
Dinoflagellates + Ciliate								
Gymnodinium					1.2			
Gymnodinium sp2	52		21.3	122.3		69.1	225.8	142.6
Prorocentrum micans	0.1	0.1		0.1		0.1	2.1	2.2
Scrippsiella trochoidea	68	43.6	10.6	41.5	8.5	26.6	5.3	34
Mesodinium rubrum	5.1	29.6		29.8	17	3.7	11.7	25.4
Flagellates								
Cryptomonas spp	140.4	70	137.2	202.1	25.9	77.7	354.8	230.9
Eutreptiella sp	220.2	0.4	0.4	53.2		10.6	5.3	7.4
Flagellates<5	3857	9458	4583	12809	6208	7958	5292	4647
Flagellates>5	1286	1166.7	1208.5	2238	777.8	1000	580	676.5

Sampling Day	**21/7**	**28/7**	**4/8**	**11/8**	**18/8**	**26/8**	**8/9**	**22/9**
Days	**202**	**209**	**216**	**223**	**230**	**238**	**251**	**265**
Chl	**21.1**	**3.6**	**3.1**	**10.9**	**2.8**	**10.1**	**3.7**	**2.3**
Diatoms								
Asterionella glacialis								
Asterionella kariana								
Bacillaria paxillifera								
Bellerochea malleus								
Biddulphia pulchella						8.5		
Cerataulina pelagica								
Chaetoceros curvisetus								
Chaetoceros debilis				604.3				
Chaetoceros eibenii					26.6	30.9	34	
Chaetoceros vistulae								
Chaetoceros wighamii	20208							
Coscinodiscus					1	0.6		
Cylindrotheca closterium	1.1							
Ditylum brightwelli	1.1							
Eucampia spp								
Grammatophora marina								
Guinardia delicatula			20.2	8.5				
Guinardia flaccid								
Guinardia striata								
Helicotheca tamesis								
Lauderia		9.6						
Lithodesmium undulatum					4.2			
Melosira sp								
Navicula sp								
Nitzschia longissima			5.3	0.3	180.	5.2		0.3
Odontella aurita								
Odontella mobiliensis			2.2	4.3				
Odontella sinensis	0.1							
Pennate			5.2		4	3.2	4.3	
Pleurosigma	1.1		2.2		2.2	0.3		0.2
Pseudo –nitzschia sp	1.1							
Rhizosolenia imbricate								
Rhizosolenia setigera L						1.1		
Rhizosolenia setigera S			3.2			2.1		
Skeletonema costatum			129.8	1257.	17			
Thalassionema frauenfeldii	0.8			8.5				
Thalassionema Nitzschioides								
Thalassiosira hylina	23.4							
Thalassiosira rotula	23.4	8.5	2.1	13.5				
Dinoflagellates + Ciliate								
Gymnodinium	0.1	3.2	7.4	8.2		5.2		2.5
Gymnodinium sp2	74.5	391.5	1148?	30.9		74.5	7.5	
Prorocentrum micans	4.3	7.4	89	52.1	1.1			0.4
Scrippsiella trochoidea	36.1	43.6	71.3	33	5.3	4.3	3.2	2.5
Mesodinium rubrum	12.8	9.4	47.9		7.2		3.2	
Flagellates								
Cryptomonas spp	155.3	107.5	518.5	31.9	375	244.7	31.9	666.6
Eutreptiella sp	4.3	12.8	42.6	5.3				
Flagellates<5	2791.7	1100	2593	5416.	5333	8450	1014	20765
Flagellates>5	458.3	400	963	2416.	1458	2416.7	1476	1647

APPENDIX 2- CHLOROPHYLL

2-1 Chlorophyll a distribution at Dock 2002 and 2003

Not there is massing data on Day 210