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SCHOOL OF OCEAN AND EARTH SCIENCES
FACULTY OF SCIENCE

SIZE DISTRIBUTION OF CHLOROPHYLL *a* AND COMMUNITY
COMPOSITION OF PHYTOPLANKTON ALONG THE SALINITY GRADIENT
DURING SPRING-SUMMER, 2003 IN SOUTHAMPTON WATER, U.K

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*“A dissertation submitted in partial fulfillment of the requirements for the degree of
M.Sc. (Oceanography) by instructional course”*

September, 2003

As the nominated university supervisors of LAYLA JASSIM HAZEEM M.Sc project, I confirm that I have had the opportunity to comment on earlier draft of the report prior to the submission of the dissertation for consideration of the award of M.Sc (Oceanography)

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ABSTRACT

Variations of total, size- fractioned chlorophyll *a*, species composition and abundance of phytoplankton in Southampton Water were investigated. The study included three segments of Southampton Water representing the different salinity points, Woodmill (freshwater), Northam (brackish water) and NW Netley (saline water).

Factors that are responsible for the seasonal and temporal variations in chlorophyll *a* and species composition in Southampton water are diverse. Light, spring/ neap tidal cycles and nutrients seem to be overriding importance during the present study.

The first part of the research project involved enumeration and identification of the groups present in the phytoplankton samples from the three sites collected at fortnightly intervals during spring and summer, 2003. The phytoplankton community of Southampton water showed differences in species composition and dominance between the two different seasons. The spring phytoplankton communities consisted mainly of diatoms while the summer was dominated by dinoflagellates and ultraphytoplankton.

The biomass indicator, chlorophyll *a* showed three peak times during the sampling period. The maximum value of 32.9mg m⁻³ achieved at NW Netley during the first bloom period.

Variations in the contribution of four size fractions (>20, 5-20, 2-5 and 0.2-2µm) to the total phytoplankton chlorophyll *a* were investigated during spring- summer months. The present observations indicate that the >20µm fraction followed by the 5-20µm fraction were the most important fractions dominating the sampling stations

The distribution of total chlorophyll *a* relative to salinity showed in most cases the existence of considerable horizontal variations. The chlorophyll *a* concentration increases in a seaward direction.

The third part of this research was designed to determine the suitability of the new molecular method (Fluorescent In-Situ Hybridisation) combined with flow cytometry for the enumeration and identification of the small photosynthetic eukaryotes (ultraphytoplankton) that were difficult to be identified in many cases, using the light microscopy because of their small size and simple morphology. The results demonstrate that cells belonging to the division Chlorophytes and the group Haptophyta were dominant in all stations throughout the sampling time.

ACKNOWLEDGEMENT

I would like to express my special thanks to Professor Patrick Holligan and Dr. Mike Zubkov for their supervision and their continuous encouragements throughout the research work period.

I would like to express my special thanks to my husband for his continuous encouragement during this year.

I am very grateful to Mr. Ibrahim Al-Tisan (Ph.D student) for his continuous assistance during the field and Lab. Work.

I am grateful to Dr. Dunkan Purdie, Dr. Cathy Lucas and Mr. Russell D. Davidson for their assistance during the research period.

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INTRODUCTION

1. Phytoplankton ecology in temperate estuaries:

Estuaries are considered to be amongst the most productive ecosystems on earth (Bruno *et al.*, 1980). Estuarine ecosystems are characterised by their high primary productivity and support many organisms that feed and live within those estuaries. The primary productivity might reach up to $190\text{ g C m}^{-2} \text{ y}^{-1}$ in some estuaries (Smith and Hollibaugh, 1993). High rates of primary production in estuaries are sustained by nutrient supply from freshwater input (Ali, 2001).

The main primary producers in aquatic ecosystems are unicellular, non-motile cells of algae and bacteria, motile flagellates, and ciliates. These cells range from less than $1\mu\text{m}$ to greater than 1mm and can attain population abundances exceeding a million cells per litre during bloom periods (Thronsdon, 1997).

Events (e.g. chemical and physical) that occur at freshwater, marine boundaries and on the water surface greatly influence the estuarine ecology. The spatial and temporal variations in species composition, biomass and productivity of phytoplankton are examples of the things that respond to the events that take place in the estuary and its surrounding environment (Hallegraef and Reid, 1986).

It has been found that in some estuaries, more than 80% of the spatial and temporal variability in productivity is correlated with variations in three easily measured parameters: phytoplankton chlorophyll *a*, photic depth, and surface irradiance (Cole and Cloern, 1987). Temporal and spatial dynamics of estuarine phytoplankton exhibit a wide range of seasonal pattern. These changes under natural conditions are controlled by the interplay of environmental, physical, chemical and biological factors (Kifle, 1992; Harris, 1986).

A marked spatial and temporal variability in phytoplankton biomass and / or productivity have been described for a number of temperate estuaries including Chesapeake Bay (Smith and Kemp, 1995), Urdaibai Estuary (Revilla *et al.*, 2000), Mediterranean Estuarine Canal System (Puigserver *et al.*, 2002), San Francisco Bay (Cole and Cloern, 1984) and Southampton Water (Wright *et al.*, 1997). Maximum phytoplankton productivity and/ or biomass is found during winter- spring in San

Francisco Bay (Cole and Cloern, 1984). The development of phytoplankton blooms occurs at different times of the year in different estuarine systems according to the effect of the local environmental conditions (Kifle, 1992). Examples of temperate estuaries and the time of the bloom formation are shown in Table 1.

Table 1: Seasons of phytoplankton bloom in different estuarine environments.

<i>Estuaries</i>	<i>Season of bloom</i>	<i>References</i>
1. Lower St.Lawrence Estuary	Summer	Zakardjian <i>et al.</i> (2000)
2. San Francisco Bay	Summer	Cole and Cloern (1984)
3. Southampton Water	Spring and summer	Holley and Hydes (2002)
4. Chesapeake Bay	Summer	Malone <i>et al.</i> (1988)
5. Mediterranean Estuarine Canal System	Spring and Autumn (innermost part)& summer (the part nearest the sea)	Puigserver <i>et al.</i> (2002)

2. Factors influencing the primary growth and the community structure in temperate estuaries:

Knowledge of the physical, chemical and biological factors that affect and control the phytoplankton productivity is needed to understand how estuaries function as ecological systems.

Primary productivity in economic importance estuaries is influenced by a number of environmental factors (e.g. solar irradiance, temperature and salinity), physical factors (e.g. water column stratification and tidal mixing), chemical factors (e.g. nutrients) and biological factors (e.g. grazing). The following sections are summaries of the most important factors that might affect the growth of phytoplankton and the community structure in estuaries.

2.1 Environmental factors:

2.1.1 Solar irradiance:

Primary production is the rate of carbon fixation by photosynthesis. The primary forcing variable is therefore light. Adaptations of phytoplankton populations to various light regimes also influence primary production. Light acts on the state variable, the biomass of phytoplankton.

In nutrient saturated waters there is a tendency for the phytoplankton to grow until light limitation sets in (Wofsy, 1983; Harris, 1986). The low production of the phytoplankton in the turbidity maximum zones in some estuaries, (Chesapeake Bay for example, is the result of the light limitation (Harding *et al.*, 1986). The turbidity of the water column is also known to affect the phytoplankton biomass. In highly turbid estuaries, the primary production peaks in summer as a result of reduced rainfall and maximum irradiance (Randall and Day, 1987).

In many estuaries, there is a horizontal gradient of increasing productivity in a seaward direction where turbidity decreases and light availability (photoc depth) increases (Cloern *et al.*, 1985), even though nutrient concentrations usually decrease seaward (Nixon, 1981).

In San Francisco Bay, the productivity of the whole phytoplankton population and three size fractions have been found to be highly correlated with biomass and light availability (Cole and Cloern, 1984; Cole *et al.*, 1986).

2.1.2 Temperature:

Temperature is another factor that influences primary production. It is believed that temperature controls the enzyme-mediated dark reaction rates of photosynthesis. Thus, from laboratory experiments, it has been shown that photosynthetic rates in phytoplankton increase with temperature up to an optimal temperature, after which they decrease. In nature, the tendency of primary production rates to increase with temperature is confounded by another effect: upwelling waters with high nutrients tend to have a low temperature, and the increase in primary production in response to the nutrient supply may supersede the counteracting effect of temperature. Boynton *et al.*, (1982) has suggested that estuarine phytoplankton productivity is high during warm periods of the

year, and at times when ratios of nitrogen to phosphorus are low. The Chesapeake Bay is one of the temperate estuaries where the annual pattern of plankton community production follows that of the water temperature (Smith and Kemp, 1995). On the other hand, temperature can have a negative effect on the bloom formation. For example, in the 1998 warm El Nino winter, no winter-spring phytoplankton bloom occurred in Narragansett Bay. The bloom limitation was correlated with warm temperatures, which may have stimulated grazing rates (Oviatt *et al.*, 2002).

2.1.3 Salinity:

It is often assumed that the phytoplankton species composition changes markedly along the salinity gradients (Ali, 2001).

Both field and laboratory data have demonstrated the importance of salinity in controlling phytoplankton distribution (Kifle, 1992).

Freshwater runoff can have a negative effect on the phytoplankton growth, because freshwater creates both horizontal and vertical salinity gradients causing osmotic stress (Kromkamp and Peene, 1995).

Diatoms have been found to adapt a wide range of changes in the salinity of the external medium (Brand, 1984). Also there are many nanoplankton groups that are widely distributed in marine, brackish and freshwater habitats although some are restricted to a definite region of the estuary (Hoek *et al.*, 1995).

In general, the salinity gradient characterising the estuarine environments will lead to spatial changes in the phytoplankton community structure. The turbidity maximum zone is where the freshwater environment changes to marine environment or *vice versa*. In this region, changes in the phytoplankton community structure are observed (KromKamp and Peene, 1995). Phytoplankton production is also known to be minimal in these regions as a result of increasing turbidity.

2.2 Physical factors:

2.2.1 Water column stratification and vertical mixing:

Water column stability is a major regulator of phytoplankton blooms in most temperate estuaries. Vertical mixing in estuaries as a result of tides, wind stress or freshwater runoff

has been shown to have a marked influence on phytoplankton dynamics and productivity (Kifle, 1992).

When the water column is well stratified, the upper illuminated layer is typically low in nutrients, with the deep water acting as a reservoir of nutrients. Mixing processes bring the nutrients to the surface layer, enhancing the primary productivity. In temperate and high latitudes, deep mixing events in winter, and subsequent stratification as the surface warming trend begins, lead to the well known phenomenon of the spring bloom and more generally to a pronounced seasonal cycle in primary production. Water column stratification leads to the initiation of the plankton blooms in temperate areas (Ryther, 1963). In general, the flagellates species found to be predominant in stratified waters and diatoms are dominant in the more turbulent water (Smayda, 1980).

Monbet (1992) has noticed that the tidal mixing of the water column is one of the factors responsible for the differing responses of phytoplankton population to nutrient input observed in estuaries. The increased tidal mixing, during spring tides leads to a reduction in the water column stratification and hence to a decrease in the estuarine phytoplankton biomasses (Monbet, 1992). Wind is also an important mixing force in many estuarine environments (Kifle, 1992). Southampton Water, UK, is one of the estuaries that show the development of bloom in different areas of the estuary, through the spring and summer, in relation to tidal and weather conditions (Wright *et al.*, 1997; Holley and Hydes, 2002).

2.3 Chemical factors:

2.3.1 Nutrients:

Seasonal variations in freshwater runoff are known to drive the annual cycles of phytoplankton through effects on the concentration of nutrients, and on the stability and turbidity of the water column in estuaries (Kifle, 1992). It is well known that both nitrate and phosphate control the phytoplankton growth in the sea. But usually the estuaries are not nutrient limited because of the fertilising effects of river inputs (Howarth, 1988).

The innermost part of the Mediterranean Estuarine Canal System has been found to show two bloom periods throughout the annual cycle, one in the spring and the other in autumn, both associated with water input from the Albufera River (Puigserver *et al.*, 2002). Riverine nutrient input and phytoplankton biomass have been found to peak during spring, but phytoplankton productivity peaks during summer in Chesapeake Bay, a partially stratified estuary. The seasonal variations in biomasses in the Bay are correlated with the riverine nitrate input while seasonal variations in productivity are correlated with light and temperature (Malone *et al.*, 1988).

It has been found that phosphorus is limiting in freshwater environments, whereas marine and estuarine phytoplankton are limited by nitrogen (Heckey and Kilham, 1988). The highest phytoplankton growth rates have been found to occur under high nitrate concentration (Pinckney *et al.*, 1999). In some estuaries where the nutrient concentrations are low, phosphorus (Jaworski, 1981), and nitrogen (Boynton *et al.*, 1982) have been found to be major factors controlling the phytoplankton production.

The freshwater inputs of nutrients could result in both a spatial and temporal succession of phytoplankton due to seasonal fluxes of nutrients, as the different phytoplankton groups would utilise these inorganic nutrients.

2.4 Biological factors:

2.4.1 Grazing:

One of the factors responsible for the mortality of phytoplankton is grazing by zooplankton (Kifle, 1992), and it is believed that grazing affect both primary productivity and community structure in estuaries. The degree to which phytoplankton are affected by zooplankton grazing is dependent on the growth conditions of the phytoplankton and the life history of the major grazers (Heinrich, 1962).

Zooplankton grazing has been found to be one of the factors that limit the bloom initiation in the Narragansett Bay (Oviatt *et al.*, 2002).

The differences that occur in the phytoplankton species composition are assumed to represent a considerable difference in the feeding conditions of the zooplankton (M'harzi

et al., 1998). Sautour *et al.*, (2000) has estimated that, on average, 26% of the total primary production was grazed daily by mesozooplankton in the Gironde Estuary.

3. Methods for enumerating and identifying phytoplankton in different size classes:

It is increasingly recognised that the physicochemical characteristics prevailing in the water column play an important role in selecting the size structure of the phytoplankton community (Legendre and Le Fevre, 1989). The spatio-temporal gradients in environmental parameters within estuaries make these ecosystems suitable for examining the relationship between physicochemical parameters and phytoplankton structure (Iriarte, 1993). In any seawater sample, the silica-walled diatoms, the armoured dinoflagellates and the calcareous coccolithophorids are the most obviously robust and abundant forms. They are always identifiable microscopically in samples preserved by formalin or Lugol's fixatives. However, the fragile phytoflagellates, which are often equally abundant, are only recognised when gentle fixatives (e.g. buffered glutaraldehyde) are used for their preservation (Jeffrey and Vesk, 1997).

Phytoplankton can be differentiated into three main size classes: the microplankton (20-200 μm), nanoplankton (2-20 μm) and picoplankton (0.2-2.0 μm) (Table 2). The nanoplankton at times account for up to 90% of the total phytoplankton chlorophyll *a* in coastal and open ocean waters (Jeffery and Hallegraeff, 1987).

Recognition of the importance of the delicate flagellates (5-20 μm) and minute coccoid forms (0.2-2.0 μm) has come only in the past two decades when more appropriate techniques for studying the phytoplankton were developed. These included examination of species in the living state after concentrating them in a plankton centrifuge, gentle methods of fixation, examination by electron microscopy, size fractionation of phytoplankton, use of enrichment cultures, onboard flow cytometry and chromatographic pigment analysis (TLC, HPLC) for assessment of algal pigment signatures (Jeffrey and Vesk, 1997)

New approaches, such as immunolabelling (Campbell *et al.* 1994) or the design of taxon- specific rRNA probes (Amann *et al.*, 1990a) are thus needed to help identify small phytoplanktonic cells and further to estimate the contributions of the different taxonomic groups to a given community (Simon *et al.* 1995). The use of rRNA probes for the detection and identification of bacterial and archeal species by epifluorescence microscopy is now common (Amann *et al.*, 1990a). Most recently, this technique was combined with flow cytometry for the detection of bacteria (Amann *et al.*, 1990b) and nanoheterotrophs (Lim *et al.*, 1993).

In the present study, a combination of different methods will be used for both enumerating and identifying the different size classes of the phytoplankton.

Table 2 : Algal types in phytoplankton size classes (from Jeffrey and Hallegraeff, 1990, cited in Jeffrey and Vesik , 1997)

<i>Algal division/class</i>	<i>Microplankton</i>	<i>Nanoplankton</i>	<i>Picoplankton</i>
	<i>20-200+ μm</i>	<i>2-20 μm</i>	<i>0.2-2 μm</i>
Bacillariophyta	+	+	+
Chlorophyta	+	+	+
Chrysophyceae	+	+	+
Cryptophyta	+	+	-
Cyanophyta	+	+	+
Dinophyta	+	+	-
Euglenophyta	+	-	-
Prymnesiophyceae	-	+	-
Pelagophyceae	?	?	+
Bolidophyceae	?	?	+

4. Description of the research area:

The sampling sites are shown in Figure 1. Southampton Water is located on the south coast of the United Kingdom. It is fed by two main rivers, Test and Itchen, with an average annual discharge equivalent to $1.54 \times 10^6 \text{ m}^3 \text{ day}^{-1}$. It is a partially mixed, macrotidal estuary with a maximum tidal range of about 4.5m, and a tidal excursion of 2.5km. The estuary is between 6 and 8m deep. The salinity structure depends on the tidal state and to a lesser degree, the seasonal freshwater flows from the rivers Test and Itchen. Salinities in the main body of the estuary vary between 30 and 33. In addition to river flows, Southampton water receives a consented sewage discharge of about $0.1 \times 10^6 \text{ m}^3 \text{ day}^{-1}$ (Wright *et al.*, 1997).

Southampton Water is characterised by the twice-daily ebb and flood of tidal currents. Tidal currents can be very strong, with near- surface current reaching over 1 m s^{-1} during spring tides (Sharples, 2000). In a partially-mixed estuary, the rapid tidal current is sufficient to allow the stratification and vertical mixing to happen in few hours. Southampton Water is characterised by the double high water, and a semi- diurnal, asymmetric cycle of ebb currents that leads to a doubling of the velocity of the flood (Webber, 1980).

One of the sampling sites intensively studied NW Netley. This Buoy is in a sheltered high salinity estuarine environment, receiving a large rainfall-dependent river input. This results in stratification, with the less-saline surface water containing elevated levels of nutrients. The sheltered nature of this site leads to high biological activity during summer months and the development of a large bloom of phytoplankton (Howard *et al.*, 1995).

Southampton Water is the most intensively studied part of the Solent estuarine system. The physical, chemical and biological aspects of this estuary have been studied in varying detail. All studies indicate a seaward decrease of the temperature of the water. In respect to salinity, Dyer (1973) has seen that the estuary is vertically homogenous at high water in the lower part with stratification developing further up the estuary. Both the tidal cycle and the seasonal cycle in freshwater inflows affect the salinity structure in Southampton Water.

Previous studies of nutrients in Southampton Water show highest concentration at the top of the estuary and during winter and lower concentration at the mouth and during summer (Antaie, 1989).

It has been found that photosynthetic processes in Southampton Water are dominated by planktonic rather than benthic algae (Williams, 1980). The area has been documented as experiencing yearly algal blooms in both spring and summer. Chlorophyll *a* levels are generally highest on spring tides and lowest on neap tides (Wright *et al.*, 1997). The annual spring diatom bloom occurs over the period of a spring tide. The timing of the bloom coincides with sufficient light, water stratification and good weather conditions.

Tidal variations and weather conditions within the estuary play a dominant role in governing the growth of phytoplankton. The development of a bloom depends on a balance between irradiance driving plankton growth and tidal energy tending to disperse the bloom (Holley and Hydes, 2002).

Studies of seasonal and spatial variations of phytoplankton species composition in Southampton Water have shown differences in species composition and dominance between periods of cold turbulent water and warm, relatively calm water column. The temporal variations in species composition occurring in Southampton Water are shown in Table 3.

Table 3: Dominant phytoplankton groups occurring in Southampton water.

(From Kifle(1992)

<i>Months</i>	<i>Dominant groups</i>
April-June	Microflagellates
	Diatom (<i>Skletonema costatum</i>)
May-June	Diatom (<i>Rhizosolenia</i> (<i>Guinardia</i>) <i>delicatula</i>)
May	Euglenoid (<i>Eutreptiella marina</i>)
June-August	Autotrophic ciliate (<i>Mesodinium</i> <i>rubrum</i>)

5. Research objectives:

Recent studies of the phytoplankton of Southampton Water and their seasonal and spatial variation have concentrated on the large phytoplankton groups (e.g. diatoms and ciliates), only few dealing with the small organisms (both the nano- and picoplanktonic phytoplankton, collectively known as ultraphytoplankton). Light microscopy and the epifluorescence microscope have been used for the identification of the different groups of phytoplankton. In the present study combinations of different methods including light microscopy, high performance liquid chromatography (HPLC) and the new molecular method (Fluorescent In Situ-Hybridisation) were applied for the identification of the different size classes of phytoplankton.

In order to investigate the relationship between the environmental parameters and phytoplankton size distribution, which is a vital step towards a comprehensive analysis of spatial and temporal variability in the community structure of chlorophyll was size-fractionated and the different environmental, physical, chemical and biological parameters were analysed.

The relative abundance of chlorophyll *a* and the main accessory pigments have been compared with the microscopic observations, as the photosynthetic pigments have been widely used recently as taxonomic markers (Ansotegui *et al.*, 2001).

Light microscopy was used for both the identification and enumeration of the different size classes of phytoplankton. The Fluorescent In-Situ Hybridisation (FISH) method and the detection of the phytoplankton by flow cytometry were used mainly for enumerating and identifying nano and picophytoplankton.

So, the objectives of the present study were as follows:

1. To understand the effect of the environmental, physical and chemical parameters in the estuary on the temporal and spatial distribution of the total phytoplankton abundance and size-fractionated chlorophyll *a*.

2. To examine the seasonally important phytoplankton species in relation to the total phytoplankton abundance along the salinity gradient of Southampton water.
3. To evaluate the importance of the new molecular technique (Fluorescent In- Situ Hybridisation) in the identification and enumeration of some phytoplankton groups belonging to the nano and picophytoplankton.

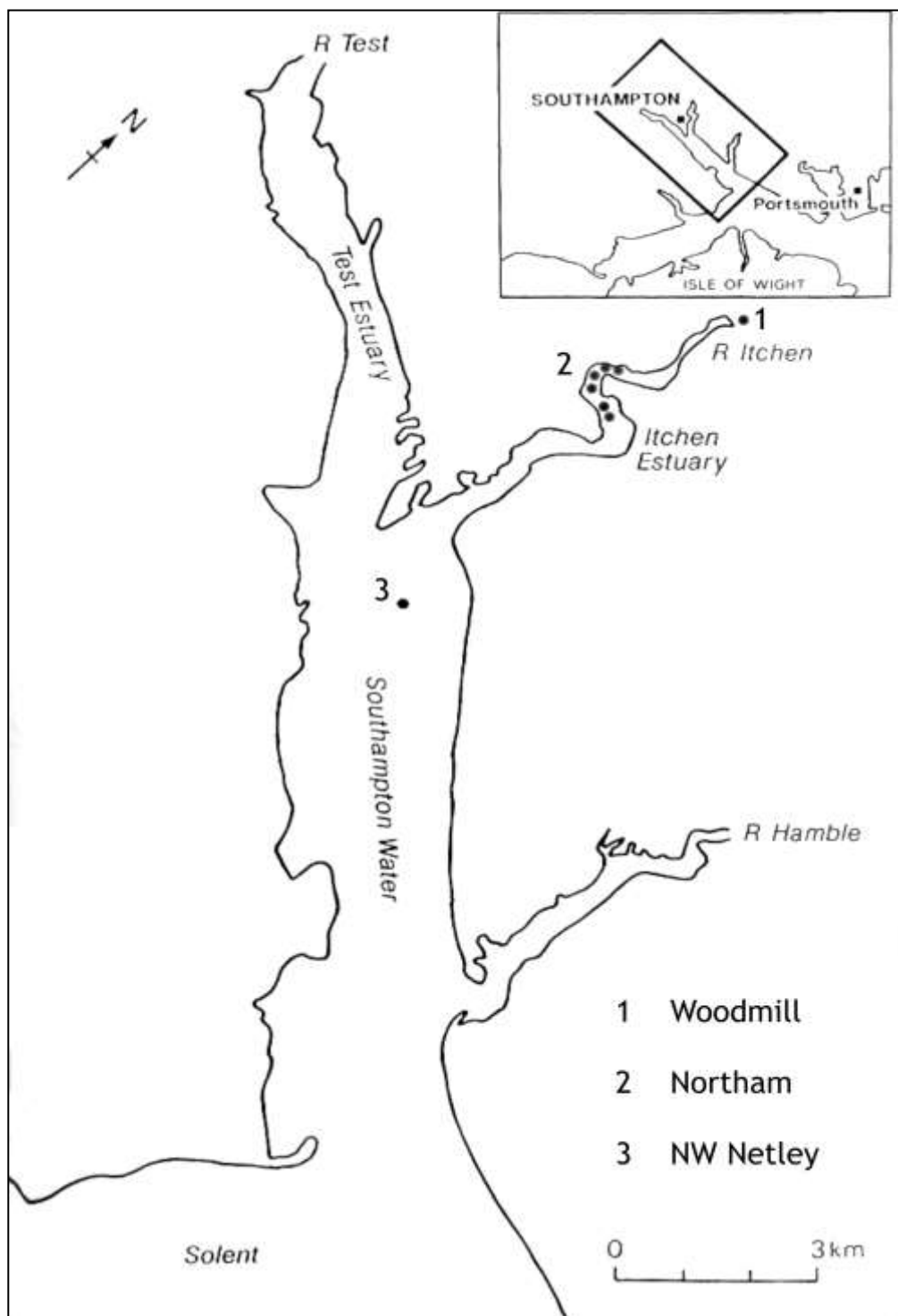


Figure 1: Map of the research area showing the three sampling stations (River Itchen (Woodmill, freshwater), Northam (brackish water) and NW Netley (saline water)).

Materials and methods

1. Sampling programme:

Surface water samples were collected from three sites in Southampton water, UK (around 51°N 01°W) along the salinity gradient. The location of the sampling sites is shown in Figure 1. The sampling programme was carried out approximately at fortnightly intervals between April and August 2003 (Julian days 91-223). Surface water samples were usually checked at high tide to minimise tidal influences and transported back to the laboratory in Thermos flasks and plastic containers. Both salinity and temperature were measured *in situ*, using a high precision salinometer. The surface irradiance data were obtained from the Meteorological Office through the British Atmosphere Data Centre web site.

2. Laboratory analyses:

2.1 Nutrient analyses:

Surface water sub-samples were taken (25ml in duplicate) from the plastic bottles and filtered through glass fibre filters (GF/F) one to two hours after the time of collection. Filtered samples were then stored in plastic bottles, one in the freezer (for phosphate and nitrate) and the other in a dark place at room temperature, (for silica which breaks down during freezing). The nutrients were then analysed using BURKARD auto-analyser (model 80 Plus), following (Hydes and Wright, 1999).

The nutrient concentrations were measured in duplicate with standard deviation not exceeding $\pm 24.6\%$ (see Appendix I).

2.2 Pigment analyses:

2.2.a Fluorometric measurements:

Total chlorophyll *a* concentration was measured in triplicate by fluorometry (Parsons *et al.*, 1984). 25 ml water samples were filtered on 25 mm glass fibre filter (GF/F). The filters were folded in half, with algal material inside, placed in a sealable plastic bag, labelled and stored at –20 °C until later analysis.

On the day of analysis, the filters were sonicated in 5ml of 90% acetone for 30 seconds and centrifuged at 3000 rpm for about ten minutes. Fluorescence was then measured using AMINCO fluorometer. The fluorometer was calibrated using standard chlorophyll *a* solution (Sigma Chemicals Co.) The concentration of the standard was determined by spectrophotometry according to the equation given by Parsons *et al.* (1984):

$$\text{Standard chlorophyll } a = 11.85E_{664} - 1.54E_{647} - 0.08E_{630}$$

Where **E** is the absorbance reading of the spectrophotometer at 664, 647 and 630nm.

Chlorophyll *a* concentration were then determined from the following equation:

$$\text{Chl.}a \text{ (mg/m}^3\text{)} = R * v/V$$

Where **R** is the fluorometer reading, **v** is the volume of acetone extract (in ml), **V** is the volume of the seawater filtered (in ml).

Chlorophyll *a* concentrations for all the collected water samples were expressed as the averaged mean for triplicates with a standard deviation not exceeding ($\pm 6.5\%$).

Chlorophyll *a* was size fractionated by passing a 25 ml water samples through 25mm polycarbonate filters with pore sizes 20, 5, 2 and 0.2 μm . A hand vacuum pump was used for the 2 and 0.2 μm filters. The filters were wrapped in plastic bags, labelled and stored at –20°C for later analysis. The fluorescence technique for measuring chlorophyll *a* is followed as mentioned in the above section.

Total chlorophyll *a* concentration was compared to the sum of the four size fractions. The linear regression between the two parameters was ($r^2 = 0.6536$ at station 1, $r^2 = 0.5634$ at station 2 and $r^2 = 0.8321$ at station 3). In some cases, the sum of the four size fractions was more than the total chlorophyll *a* concentration.

2.2.b HPLC (High Performance Liquid Chromatography) pigment analysis:

The High Performance Liquid Chromatography (HPLC) was used to analyse the pigment content of the samples collected. 250 ml water samples were filtered through 25mm glass fibre filters (GF/F) and immediately frozen until analysis. The procedure was followed as described by Barlow *et al.*, (1993).

On the day of analysis, the frozen filters were extracted in 5ml of 90% acetone using sonication (30 seconds) and centrifuged to remove cellular debris. 100µl of the extract was injected into a ThermoFinnigan HPLC system (P2000 gradient pump, vacuum degasser, AS 3000 autosampler, UV6000 diode- array detector, FL3000 fluorescence detector, SN4000 system controller) incorporating a 3µm Adsorbosphere column (3X 0.45cm, Alltech). Chlorophylls and carotenoids were detected by absorbance at 440 nm. Data collection and integration utilised the Chromquest 3.0 software on a Dell computer.

Pigment identity was secured through co-elution with authentic pigments: chlorophyll *a* and *b* and β- carotene were obtained from Sigma Chemical Co., chlorophyll *c*2, chlorophyll *c*3, peridinin, 19' butanoyloxyfucoxanthin, fucoxanthin, 19' hexanoyloxyfucoxanthin, diadinoxanthin, violaxanthin, prasinoxanthin, alloxanthin and zeaxanthin from DHI, Denmark.

The concentration of the signature pigments was calculated according to the following equation:

$$\text{Concentration (Ng/L)} = \frac{[\text{Peak area} \times \text{Volume extracted (ml)} \times 1000]}{[\text{Pigment response factor} \times \text{Volume injected (}\mu\text{l)} \times \text{Volume filtered (l)} \times 0.5]}$$

(Barlow *et al.*, 1993)

2.3. Particulate organic Carbon/Nitrogen analyses:

Triplicate 25 ml of water samples were filtered through 13mm glass fibre filters (GF/F) pre-combusted at 460 °C for four hours for POC/PON analysis. The filters were then dried in a 60 °C oven for two hours and stored in a dessicator for further analysis.

The analysis of POC/PON was done in a Carlo ERBA EA element analyser (model 1108). A standard compound with a known C-H-N-S composition (sulphanilamide) was

analysed after every tenth unknown samples in order to constrain machine drift and allow estimation of relative and absolute errors. The method used according to Wilkinson (1991).

2.4.Light- Microscopic analysis of phytoplankton:

100ml of the water samples taken from each sampling station were fixed with 1ml of Lugol-acetic acid solution (1% final concentration, (Parsons *et al.*, 1984)), and then stored in dark bottles for later identification of the phytoplankton and determination of cell counts.

The preserved water samples were mixed well to loosen any organisms attached to the wall of the glass bottle and to distribute the phytoplankton throughout the whole sample. 10 ml were then poured into a Sedgewick Rafter counting chamber and left for about 24 hours, to allow the cells to settle. The phytoplankton cells identified and counted using a FLUOVERT inverted microscope. The large phytoplankton cells including diatoms and ciliates were identified and counted at magnification of 200x (20x objective lens and 10x ocular lens) in the whole counting chamber. The small flagellate cells were identified and counted in vertical and horizontal transects at magnification of 400x (40x objective lens and 10x ocular lens). To convert the counted cells in transects into number of cells per ml, the following calculations were used:

1. The area of the counted transects = 20.83mm^2 (Calculated by measuring the diameter of the whole chamber and of one field of view).

2. Area of the whole chamber = πr^2 , where r^2 is the radius of the chamber
 $= 3.14 \times (11.97)^2$
 $= 450.13 \text{ mm}^2$

3. Number of cells per ml = $(n \times 100) / (P \times V)$

Where: **n** = number of cells in both the horizontal and vertical transects, **P** = the % of the counted area in relation to the area of the whole chamber, which is about 4.64%, **V** = Volume of the chamber (10ml).

To assess counting errors, two replicates from the first and second chlorophyll *a* maximum periods at each of the three stations were counted. The replicate counts gave maximum standard deviation of ($\pm 20\%$).

2.5. Cell fixation and Fluorescent In-Situ Hybridisation:

100 ml of the water samples were pre- filtered through 60 μm Millipore filters and fixed with 5 ml of 20% paraformaldehyde (PFA) to a final concentration of 1% and stored at room temperature for one hour. The water was then filtered through a 1.0 μm polycarbonate 47mm filter (Whatman), and the filter rinsed with Milli.Q.water (Millipore) and finally dehydrated with 5ml of 96% ethanol. The filters then left to dry, labelled, divided into small segments and stored at $-20\text{ }^{\circ}\text{C}$ until further analysis. The procedure followed as described by Not *et al.*, (2002) with some modification.

Oligonucleotide probes: The probes used were chosen to encompass a range of nanoplanktonic and picoplanktonic divisions and classes (collectively known as ultraphytoplankton) , EUB338 probe was used as a negative control (Table 4).

Table 4: Characteristics of oligonucleotide probes used in this study.

<i>Probe</i>	<i>Sequence(5' $\rightarrow$ 3')</i>	<i>Target group</i>	<i>Source</i>
EUK516	ACCAGACTTGCCCTCC	Eukarya	Amann <i>et al</i> (1990a)
EUB338	GCTGCCTCCCGTAGGAGT	Prokaryota (MOST BACTERIA)	Amann <i>et al</i> (1990a)
CHLO01	GCTCCACGCCTGGTGGTC	Chlorophyta	Simon <i>et al.</i> (1995)
PRYM02	GGAATACGAGTGCCCCTGAC	Haptophyta	Simon <i>et al.</i> (2000)
PELA01	ACGTCCTTGTTGACGCT	Pelagophyceae	Simon <i>et al.</i> (2000)
BOLI02	TACCTAGGTACGCAAACC	Bolidophyceae	Guillou <i>et al.</i> (1999)

Fluorescent In- Situ Hybridisation (FISH):

The protocol used for in- situ hybridisation with FITC (fluorescein isothiocyanate) mono labelled probes was adapted from Amann *et al.*, (1995).

On the day of analysis, the filters were thawed and cut into segments. The filter face that supports the cells was marked with a black pencil.

In brief, for hybridisation with FITC- labelled probes, filters were covered by 20 µl of formamide hybridisation buffer contained 2 µl of oligonucleotide probes (stock at 50 ng µl⁻¹) and incubated at 46°C for 2 hours. To enhance the probe specificity of CHLO01, 2.5ng of competitor were used (Simon *et al.*, 1995). 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. The hybridisation buffer and the washing buffer used for the different oligonucleotide probes are shown in Table 5.

The filters were dried before mounted in anti-fading reagent (Citifluor-DAPI). The hybridised and DAPI-stained filters were observed with a Zeiss epifluorescence microscope equipped with a mercury light source and a 40*UV 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 5: A list of the chemicals used to make up the hybridisation buffer and the washing buffer for the different oligonucleotide probes (in μ l); SDS: sodium dodecyl sulfate; EDTA: Ethylenediaminetetraacetic acid; M.Q water: Milli-Q(Millipore) water.

	<i>Oligonucleotide probes</i>		
	<i>Chemicals</i>	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.water	1200	900
	10% SDS	2	2
2.Washing buffer	5M NaCl	2150	700
	1M Tris HCl	1000	1000
	EDTA	500	500
	M.Q.water	add M.Q.water up to 50 ml	
	10% SDS	50	50

2.6. Flow cytometry:

Cell concentrations were estimated using flow cytometry following Marie *et al.*, (1999). For flow cytometry analysis, 1.8 ml of 60 μ m filtered water and 1.8 ml of unfiltered water samples were fixed with 90 μ l of 20% PFA and stored at -20°C until further analysis. On the day of analysis, 100 μ l of beads (0.95 μ m- diameter, Polyscience, Inc.) were added to 1ml of water sample and analysed with a FACScan flow cytometer (Becton Dickinson). Eukaryotic phytoplankton was discriminated from bacteria and enumerated using CELLQUEST software.

The flow cytometry data were analysed to determine the number of the four categories of ultraphytoplankton (Chlorophyta, Haptophyta, Pelagophyceae and Bolidophyceae)

identified by using the Fluorescent In-situ Hybridisation (FISH). The number of total eukaryotic cells calculated in filtered water samples was compared with the number of cells calculated in unfiltered water samples (linear regression $r^2 = 0.979$, 0.6121 and 0.4863 for station 1, 2 and 3 respectively). The relatively high number of cells that is calculated in filtered samples compared to unfiltered samples could be due to breaking down large colonies during filtration.

RESULTS

1. Physico- chemical parameters, chlorophyll *a* and phytoplankton cell abundance:

Changes in water temperatures at the three sampling sites are shown in Figure 2. The lowest water temperature (9.7°C) was measured during April. Then the water temperature started to increase during summer.

The salinity showed little variation at station 1 (0 to 2.4) and 3 (30 to 33.2). At station 2 the salinity ranged between 13.5 and 26.

The four days averaged surface irradiation for the research area ranged between ($608 \text{ KJ m}^{-2} \approx 169 \text{ W m}^{-2}$) and ($1202 \text{ KJ m}^{-2} \approx 334 \text{ W m}^{-2}$) during the sampling time (Figure 2). These findings indicate that the irradiance levels were sufficient to initiate a spring bloom during the first week of June. A critical mean light level of about 174.6 W m^{-2} is needed for a pronounced increase in phytoplankton growth rate (Riley, 1967).

During the sampling period, three maximum chlorophyll *a* events (blooms) were recorded. The first and second blooms occurred at a spring tide, whereas the third bloom occurred at a neap tide (Figure 3).

The seasonal variations in phosphate, nitrate and silicate concentrations of surface water collected from the three sampling sites are shown in Figure 4. The concentrations of nutrients were generally high at the start of the sampling period, and gradually declined to the lowest values at or just after the major chlorophyll *a* bloom events. Furthermore, there was a spatial variation in the nutrient concentrations. The first sampling site (River Itchen) showed always the highest nutrient concentrations; the river input of nutrients is sufficient to maintain the nutrients at relatively high levels even when the chlorophyll *a* concentration increases.

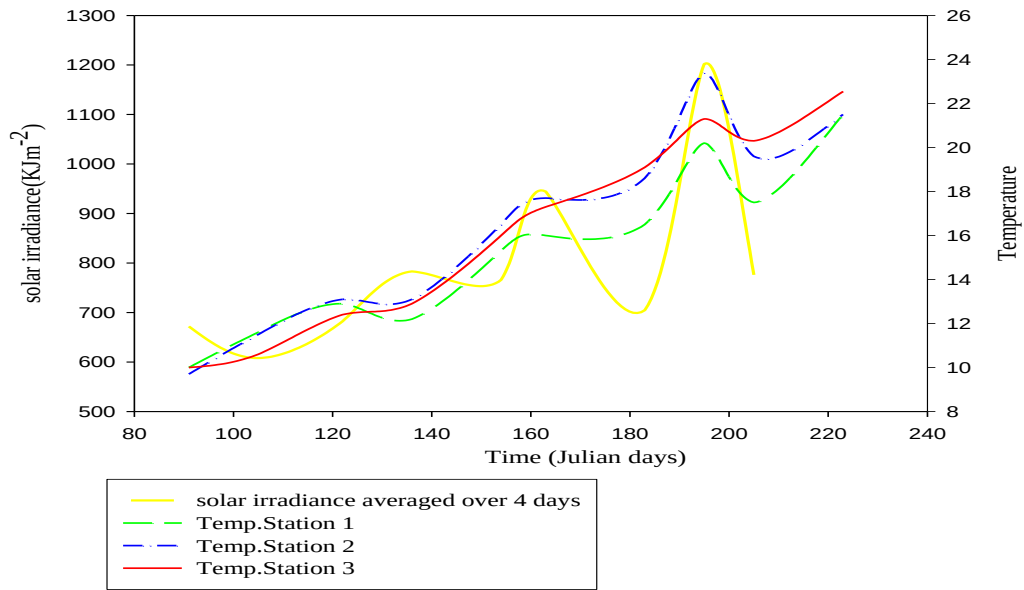


Figure 2: Variations in solar irradiance and surface water temperature in the three sampling sites through the spring- summer periods

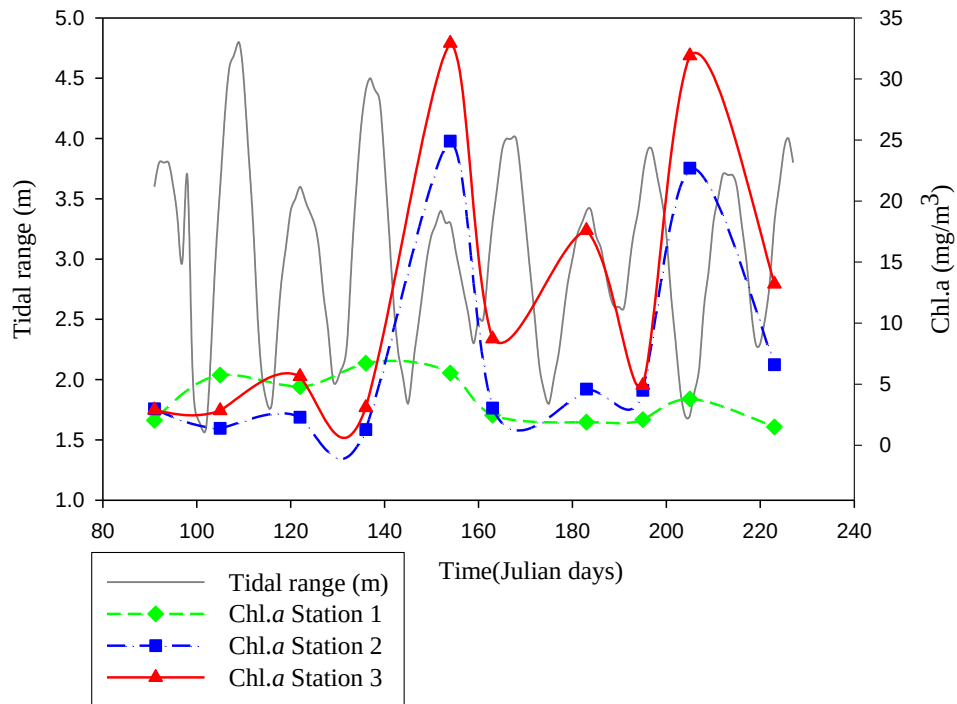


Fig.3: Temporal and spatial variations in chlorophyll *a* concentration showing the spring and summer blooms in relation to spring and neap tidal cycles.

The nutrient concentrations were found to decrease in a seaward direction (the lowest nutrient concentrations were measured at NW Netley during summer time (8.3 μ M silicate and 20 μ M nitrate). Phosphate concentration reached a minimal value of about 0.1 μ M after the third bloom at station 3. The temporal changes in the nutrient concentrations are shown in Figure 4.

Relatively low concentrations of both silicate and nitrate were measured during the first week of June during a spring bloom. The seasonal depletion of silicate at the bloom time is associated with the diatom bloom (*Navicula spp* at station 1, *Thalassiosira spp.* at both station 2 and 3). Although the diatom, *Thalassiosira spp.* at station 3 showed high peaks in cell number (39 cells ml⁻¹), its effect on silicate concentration was not as dramatic as that of the relatively large-celled *Guinardia delicatula*. Figure 5 shows the concentrations of nutrients at the chlorophyll *a* peak times in relation to salinity, and indicates that there is a good trend between the nutrient concentration and salinity (as an indicator of the freshwater dilution). This agreement reflects the effect of freshwater dilution on the temporal and spatial variations in nutrient concentrations in Southampton Water (Kifle, 1992). The removal of nutrients, especially phosphate, at salinities >15 is likely to be due to biological uptake (i.e. chlorophyll *a* concentration is higher at salinities >15).

Total plankton community chlorophyll *a* biomass ranged between 1.5 and 6.7 at station 1, between 1.3 and 24.9 at station 2 and between 2.8 and 32.9 mgm⁻³ at station 3. The lowest values were recorded at low salinities (station1), the maximum concentration at station 3 (saline water). The low chlorophyll *a* concentration measured at station 1 is due to the high turbidity, which decreases the light intensity in the water column.

Along the salinity gradient, the seasonal distribution of chlorophyll *a* showed low values in April and May followed by a maximum peak during the first week of June, and two successive peaks during summer of a relatively lower magnitude.

Light microscope observations showed that the total phytoplankton cell abundance was high during the entire period of study, ranging between 9.7 cells ml⁻¹ at station 1 during the first week of April and 45688 cells ml⁻¹ at station 2 during August. Although the maximum chlorophyll *a* concentration occurred during the first bloom, the maximum

concentration of cells occurred during summer (August). Total chlorophyll *a* concentration showed considerable spatial and temporal variations (see Figure 3).

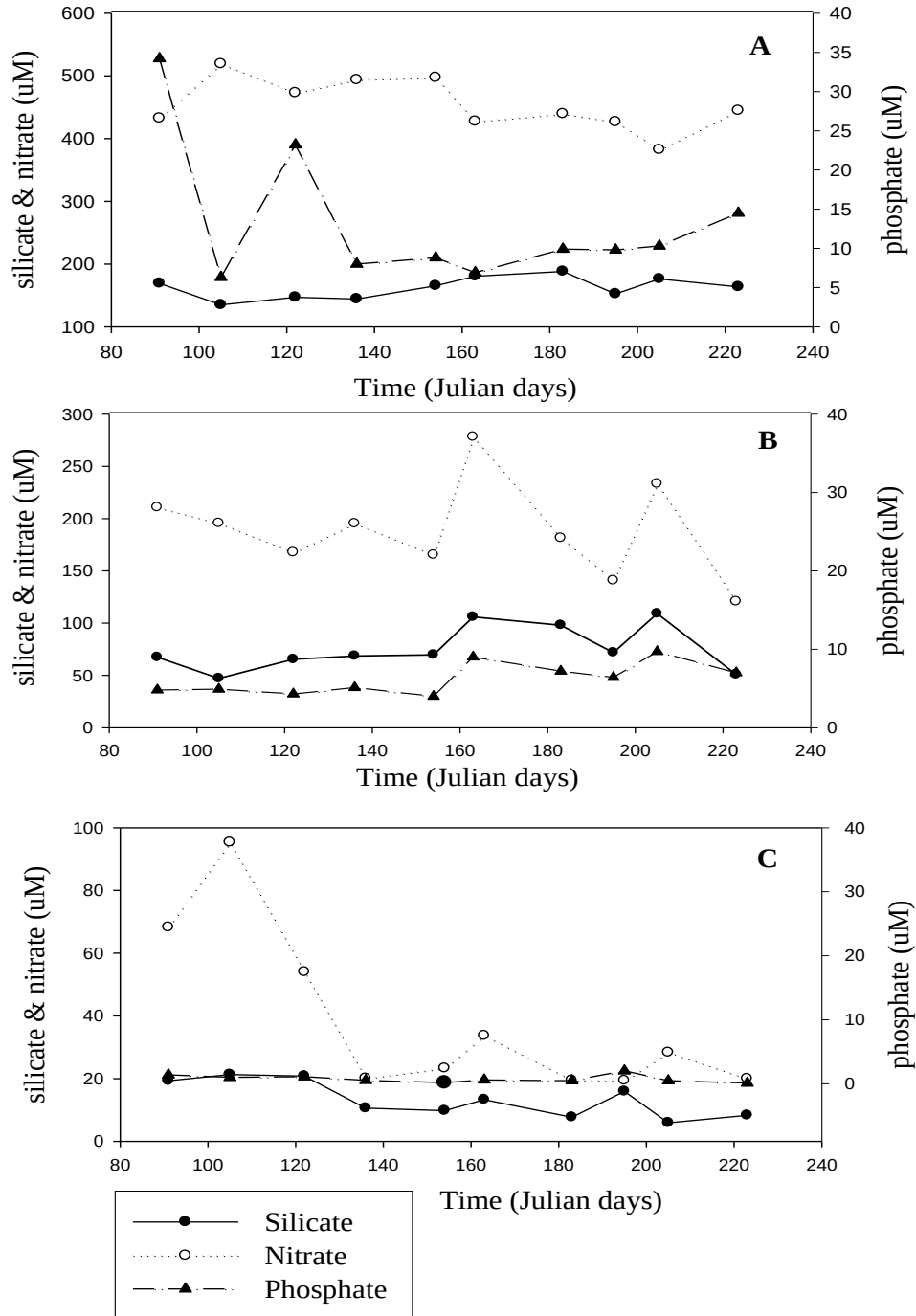
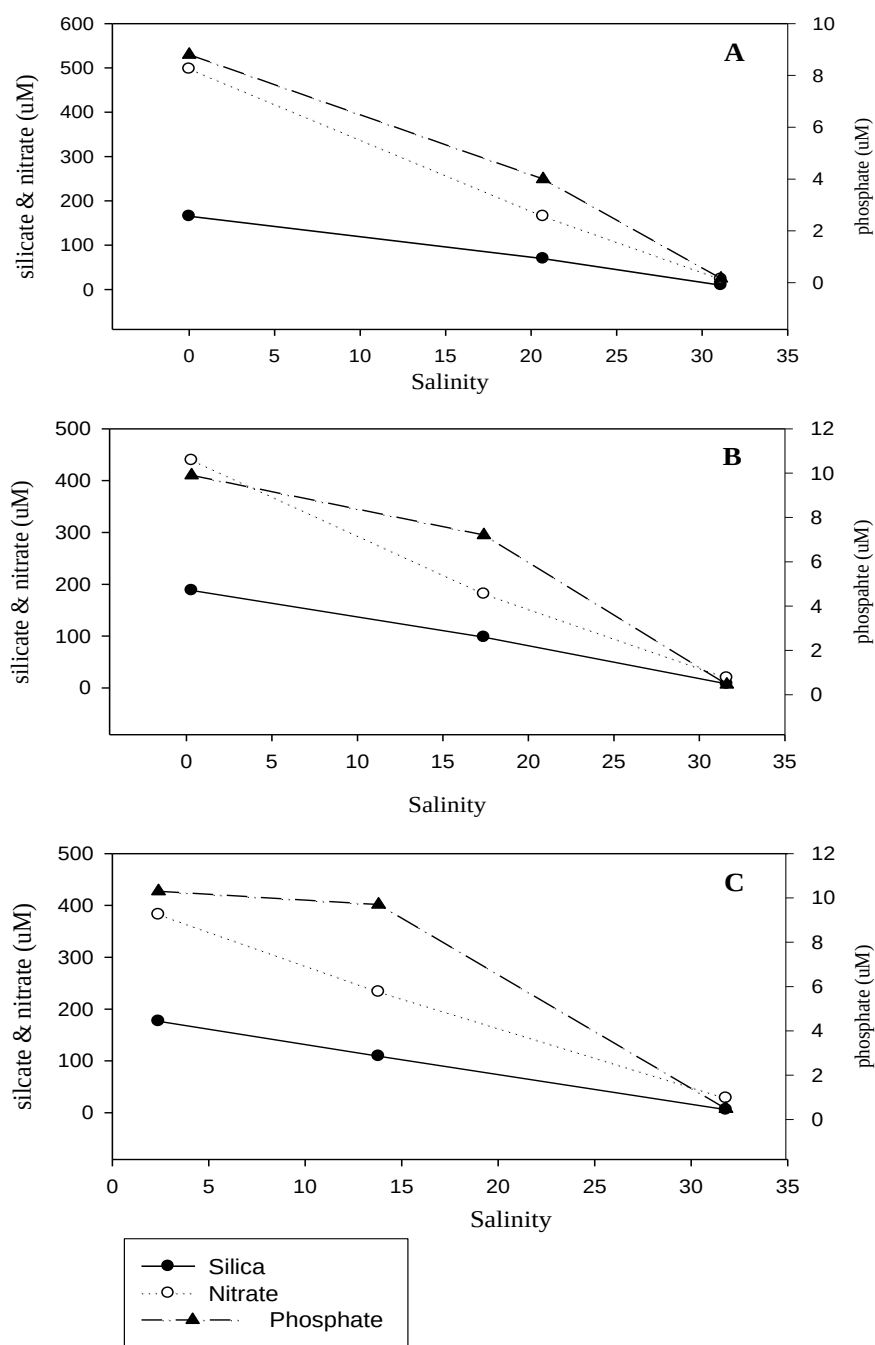


Figure 4: Temporal and spatial variations in silicate, nitrate and phosphate concentrations during the sampling period (A:station 1; B:station 2; C:station 3) (notice the different scales on the left Y axis)



Figures 5: Changes in nutrient concentrations in relation to salinity during the bloom periods (**A**: julian day= 154; **B**: julian day= 183; **C**: julian day= 205). Salinity values < 5 corresponds to station 1, **10 to 25** to station 2 and > **30** to station 3.

The temporal changes in the carbon to nitrogen ratios of particulate organic material measurements of POC (particulate organic carbon) and PON (particulate organic nitrogen) are summarised in Figure 6. Carbon to nitrogen ratios ranged between 3.5 and 9.3 at station 1, between 5.0 and 11.4 at station 2 and between 5.8 and 9.6 at station 3. The relatively high carbon to nitrogen ratio for the particulate material at station 2 indicates some preferential removal processes of nitrate by bacterial population within that station.

In general, there was a decrease in the carbon to nitrogen ratio over time due to the increase in the productivity and the accumulation of nitrogen by phytoplankton at station 2 and 3, whereas the decrease at station 1 was mainly due to the relative decrease of the detritus material. Examining the correlation between POC and PON yielded the following ($r^2 = 0.9022$ for station 1, $r^2 = 0.6355$ for station 2 and $r^2 = 0.6705$ for station 3).

Data on the temporal changes in the standing stock of chlorophyll *a* and particulate organic carbon (POC) for the three stations are shown in Figure 7. The correlation between POC and chlorophyll *a* was relatively low in the three stations. ($r^2 = 0.1564$ at station 1, $r^2 = 0.692$ at station 2 and $r^2 = 0.3635$ at station 3), indicating that there is no consistent relationship between the two parameters. The highest carbon to chlorophyll *a* ratio calculated was (POC: chl.*a* = 1063) at station 1 in August (see Appendix II).

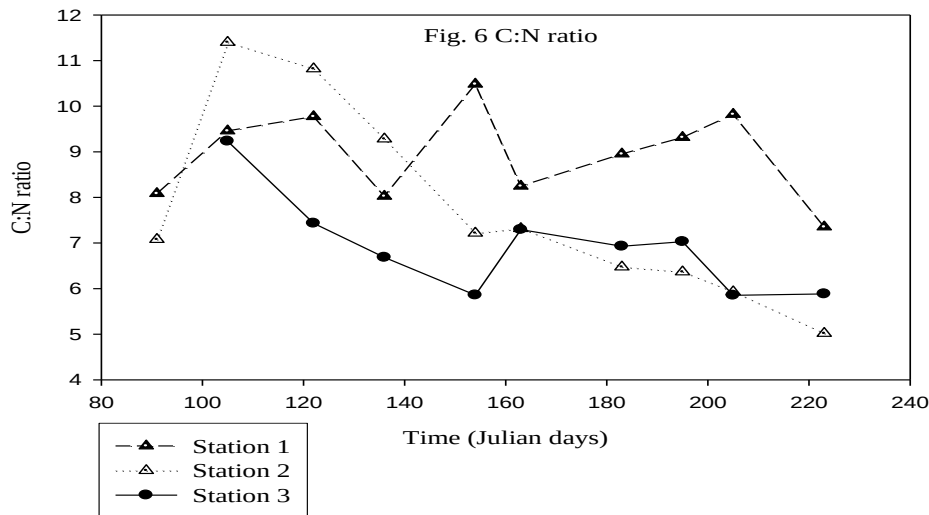
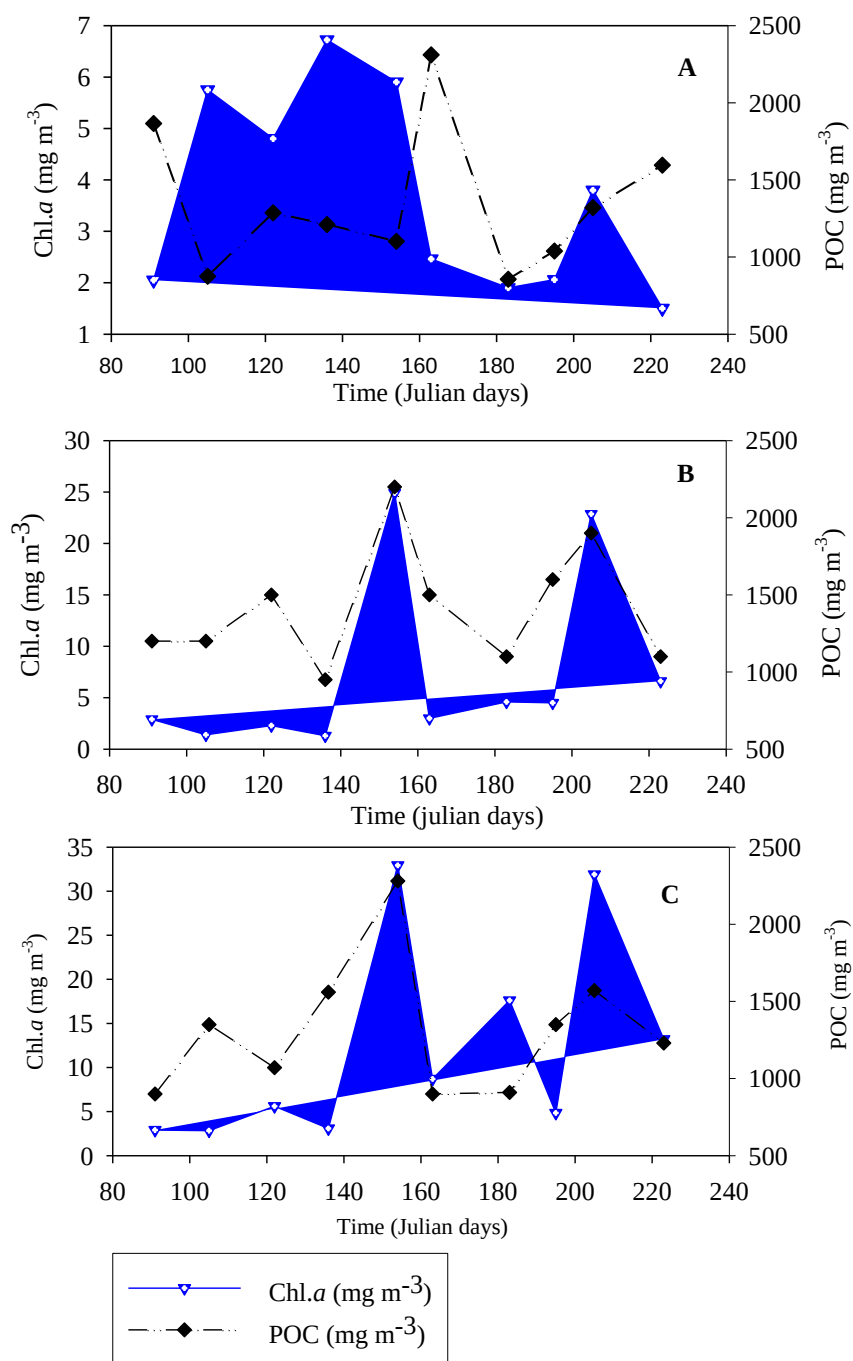


Figure 6: Temporal changes in POC: PON ratio in the three stations.



Figures 7: Temporal changes in particulate organic carbon and chlorophyll *a* at the sampling stations. **A**: station 1; **B**: station 2; **C**: station 3. (notice the different scales on the left Y axis in the different stations)

2. Temporal and spatial distribution of size-fractionated chlorophyll *a*:

a. Size fractionated chlorophyll a:

An analysis of the distribution of chlorophyll *a* biomass by size fractions (>20 , 5-20, 2-5 and $0.2-2\mu\text{m}$) showed both spatial and temporal variations during the sampling period. Total chlorophyll *a* and the four size fractions concentrations are shown in Table 6. The contribution of the different size fractions to total chlorophyll *a* during the bloom periods is shown in Figure 8.

During the first bloom, the $>20\mu\text{m}$ fraction was maximal at station 1 (5mg m^{-3}) and at station 3 (14.8mg m^{-3}). In contrast, the 5-20 μm fraction was dominating station 2 (5mg m^{-3}).

During the second bloom, the $>20\mu\text{m}$ fraction was maximal at station 2 (2.5mg m^{-3}) and at station 3 (10.2mg m^{-3}). The 5-20 μm fraction was the dominant fraction (1.2mg m^{-3}) at station 1 during this bloom time.

Finally, during the last bloom, the $>20\mu\text{m}$ fraction was maximal at station 1 (1.9mg m^{-3}) and at station 2 (15.2mg m^{-3}). The 5-20 μm fraction (18.8mg m^{-3}) was the dominant fraction at station 3.

The 2-5 μm fraction showed a maximum concentration (3.1 mg m^{-3}) at station 2 on the 2nd of May (Julian day = 122) (Table 6). The more abundant phytoplankton species on that day were *Eutreptiella* sp. (67 cells ml^{-1}) and *Cryptomonans* sp. (24 cells ml^{-1}).

When expressed as a percentage of total chlorophyll *a*, the $>20\mu\text{m}$ fraction was the dominant along the salinity gradient, the proportion of which reached a maximum of 88% of the total chlorophyll *a* at station 1 during the first bloom. During the second bloom, the proportion of the $>20\mu\text{m}$ fraction was maximal at station 3 (58%) of total chlorophyll *a*. Finally, during the last bloom period, the $>20\mu\text{m}$ fraction was maximal at station 2 (66%). The 5-20 μm fraction achieved maximum contribution to total chlorophyll *a* at station 1 during the second bloom (63%) and at station 3 during the last bloom (59%). The seasonal variations of the percentage contribution to the whole sample chlorophyll *a* content showed that the $0.2-2\mu\text{m}$ fraction to be the least significant during the bloom

periods (Figure 8) at all stations (ranges of 7% to 13% at station1, 6 to 7% at station 2 and 4% to 9% at station 3) with a tendency to become more prominent during periods of lowest overall chlorophyll *a* biomass in summer (e.g. during August, 17% at station1, 21% at station 2) and reached up to 33% at station 3 on the 14th of July (Julian day= 195).

Table 6: Temporal variations of total (average values) and size-fraction of chlorophyll *a* biomass (mg m⁻³) at station 1, 2 and 3, (N/A: not done).

Julian days	Average total chl. <i>a</i>	Station 1					sum of size fractions
		st.dev	size fraction				
			>20um	5-20um	2-5um	0.2-2um	
91	2.1	±0.1	1.1	0.3	0.3	N/A	1.8
105	5.8	±1.5	3.8	0.8	0.6	0.5	5.7
122	4.8	±0.8	1.9	1.3	0.7	0.7	4.6
136	6.7	±0.9	1.6	1.5	0.2	1	4.2
154	5.9	±0.5	5.2	2.3	0.9	0.8	9.1
163	2.5	±0.5	1.1	0.7	0.3	0.3	2.3
183	1.9	±0.5	0.9	1.2	0.2	0.1	2.5
195	2.1	0	0.5	0.5	0.2	0.3	1.5
205	3.8	±0.8	1.9	0.4	0.3	0.4	2.7
223	1.5	±0.2	0.6	0.3	0.2	0.3	1.3
Julian days	Average total chl. <i>a</i>	Station 2					sum of size fractions
		st.dev	size fraction				
			>20um	5-20um	2-5um	0.2-2um	
91	3	±0.1	0.6	0.8	0.6	N/A	1.9
105	1.4	±0.1	0.8	0.5	0.3	0.4	1.6
122	2.3	±0.2	1.3	0.5	3.1	0.6	5.7
136	1.3	±0.2	0.4	0.3	0	0.3	1
154	24.9	±7.1	2.6	5	2.1	1.7	11.5
163	3.1	±0.6	0.6	1.3	0.7	0.4	2.9
183	4.6	±0.2	2.5	0.9	0.4	0.3	4.1
195	4.5	±0.8	1.3	0.6	0.5	1.4	3.8
205	22.9	±1.1	15.2	3	1.5	1.3	19.6
223	6.6	±1.1	1.2	1.1	0.7	1.4	4.4
Julian days	Average total chl. <i>a</i>	Station 3					sum of size fractions
		st.dev	size fraction				
			>20um	5-20um	2-5um	0.2-2um	
91	2.9	±0.7	1.3	0.4	0.6	N/A	2.4
105	2.8	±1.2	1.2	0.6	0.3	0.3	2.5
122	5.6	±1.1	3.8	1.1	0.4	0.6	7
136	3.1	±1.1	4.3	0.3	0.2	0.3	5.1
154	32.9	±6.5	14.8	0.8	0.9	2.4	19
163	8.7	±0.1	3.5	2	0.6	0.2	6
183	17.6	±2.6	10.2	2.2	1.7	1.5	15.6
195	4.9	±1.1	2.2	0.1	0.7	1.6	4.6
205	31.9	±1.9	13.3	18.8	2.7	1.3	34.8
223	13.2	±2.4	3.3	3.9	0.8	1.1	9.1

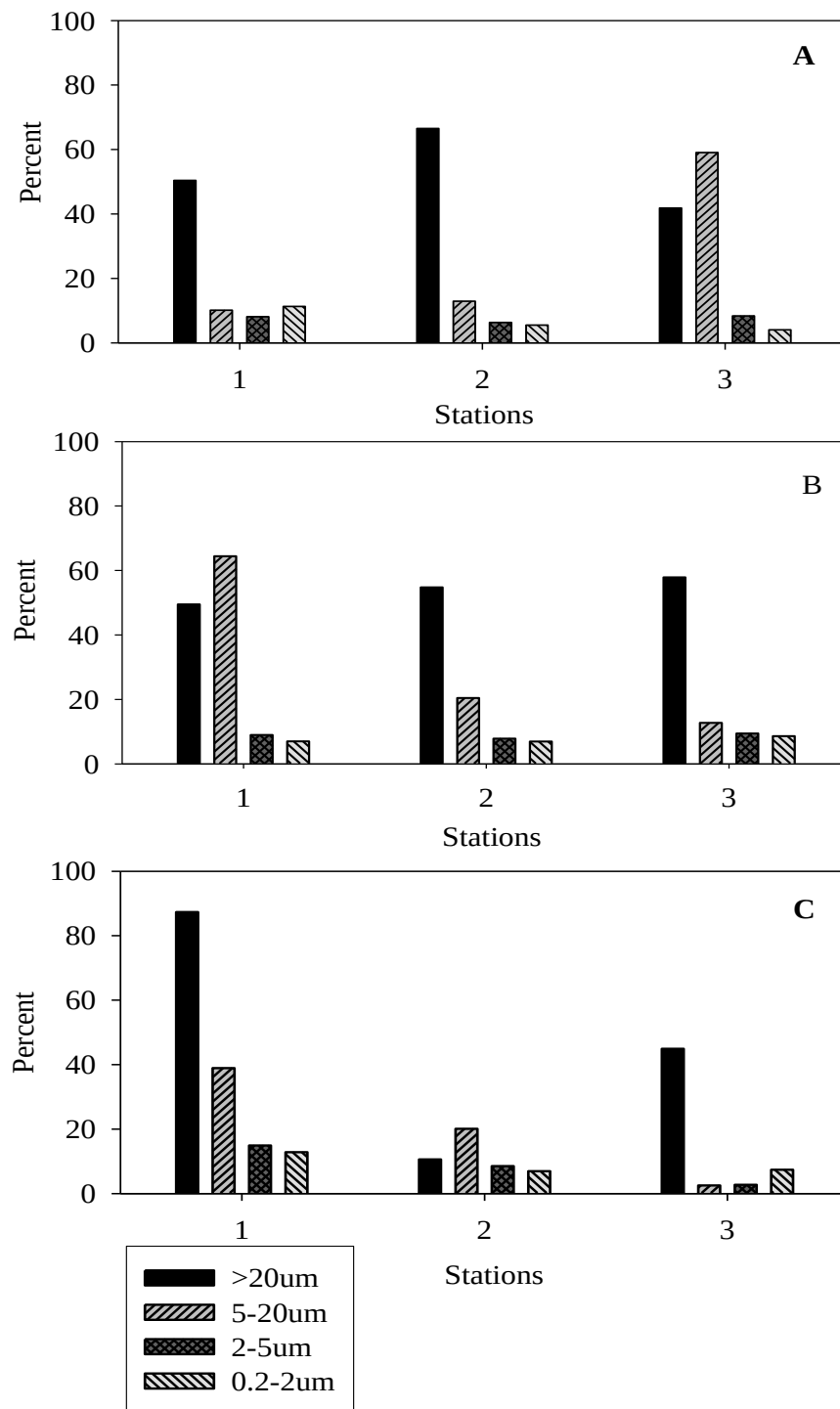


Figure 8: The contributions of the different size fractions to total chlorophyll *a* in the three stations during the bloom periods (**A**: Julian day= 154; **B**: Julian day= 183; **C**: Julian day= 205)

b. Size- fractionated chlorophyll *a* and flow cytometry:

The standing stocks of the small phytoplankton cells in the 0.2-5 μ m diameter (nano and picophytoplankton) were quantified using flow cytometry. The flow cytometer is not used to detect the larger cells (>5 μ m)

In samples collected during the spring-summer periods, total eukaryotic cell concentrations determined by flow cytometry ranged between 539 and 86637 cells ml⁻¹ at station 1, between 1054 and 75486 cells ml⁻¹ at station 2 and between 1220 and 33275 cells ml⁻¹ at station 3 (Table 8). Cell concentrations showed a general increase with time, the highest concentrations of cells being found during July and August. This indicates that there is a significant contribution of the small eukaryotic cells to the total phytoplankton community in the research area during the summer period. The number of eukaryotic cells measured by flow cytometry showed a good trend with the total chlorophyll *a* concentration, measured by fluorometry (Figure 9).

The flow cytometry data was compared also to the size-fractionated chlorophyll *a* in the 0.2 to 5 μ m size range during the bloom periods (Figure 10). There is a good trend between the two parameters in most cases. The low chlorophyll *a* concentration compared to the total cell concentration detected at station 2 on day 205 could be due to the presence of a high concentration of dead cells that are detected by flow cytometry.

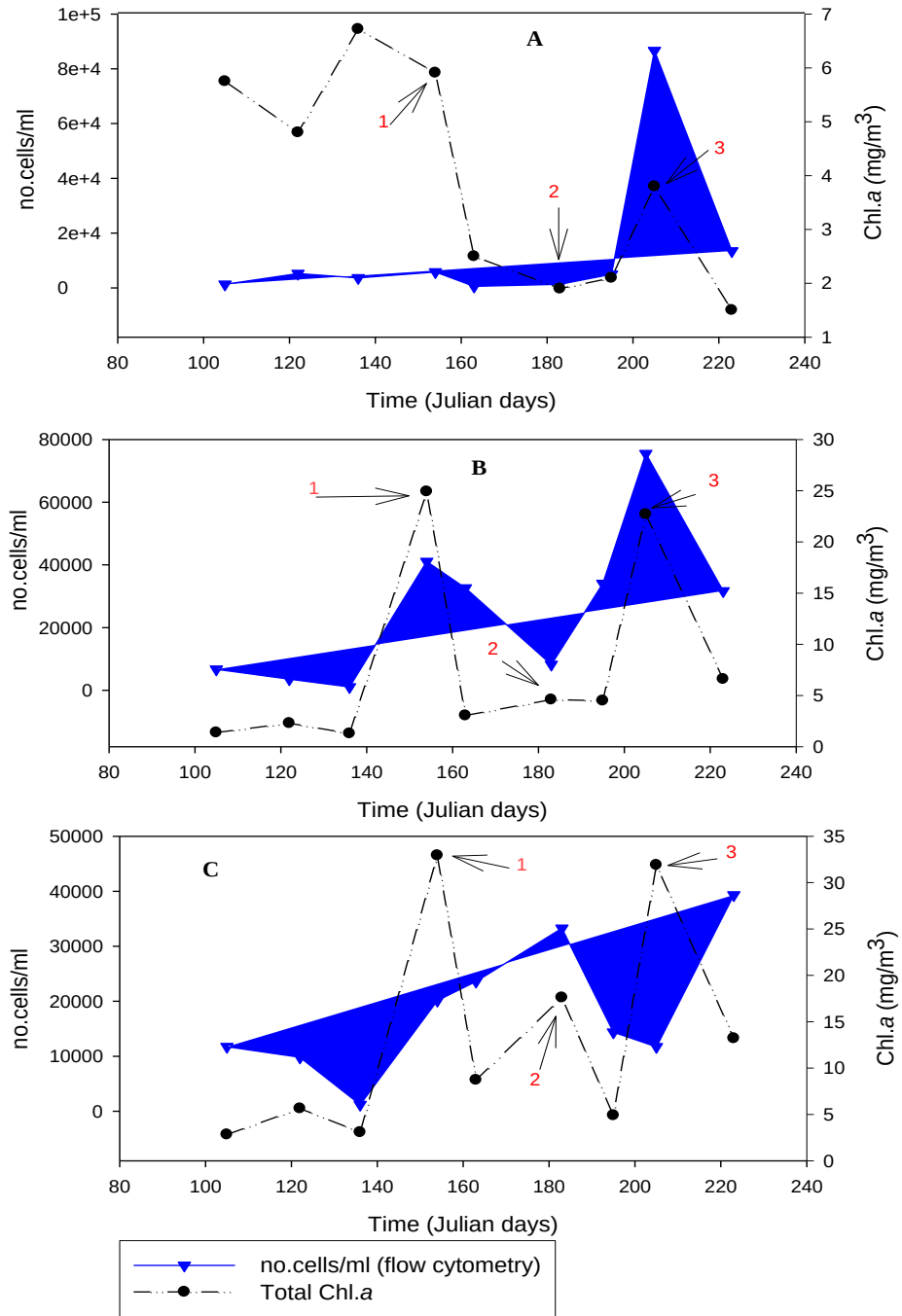


Figure 9: The distribution of the nano- and picophytoplankton detected by flow cytometry in relation to total chlorophyll *a* measured by fluorometry. (Arrows indicate bloom conditions at each station as defined by chlorophyll concentrations, see Figure 2)(A: station 1; B: station 2; C: station 3). (notice the different scales on the left and right Y axis in the different stations)

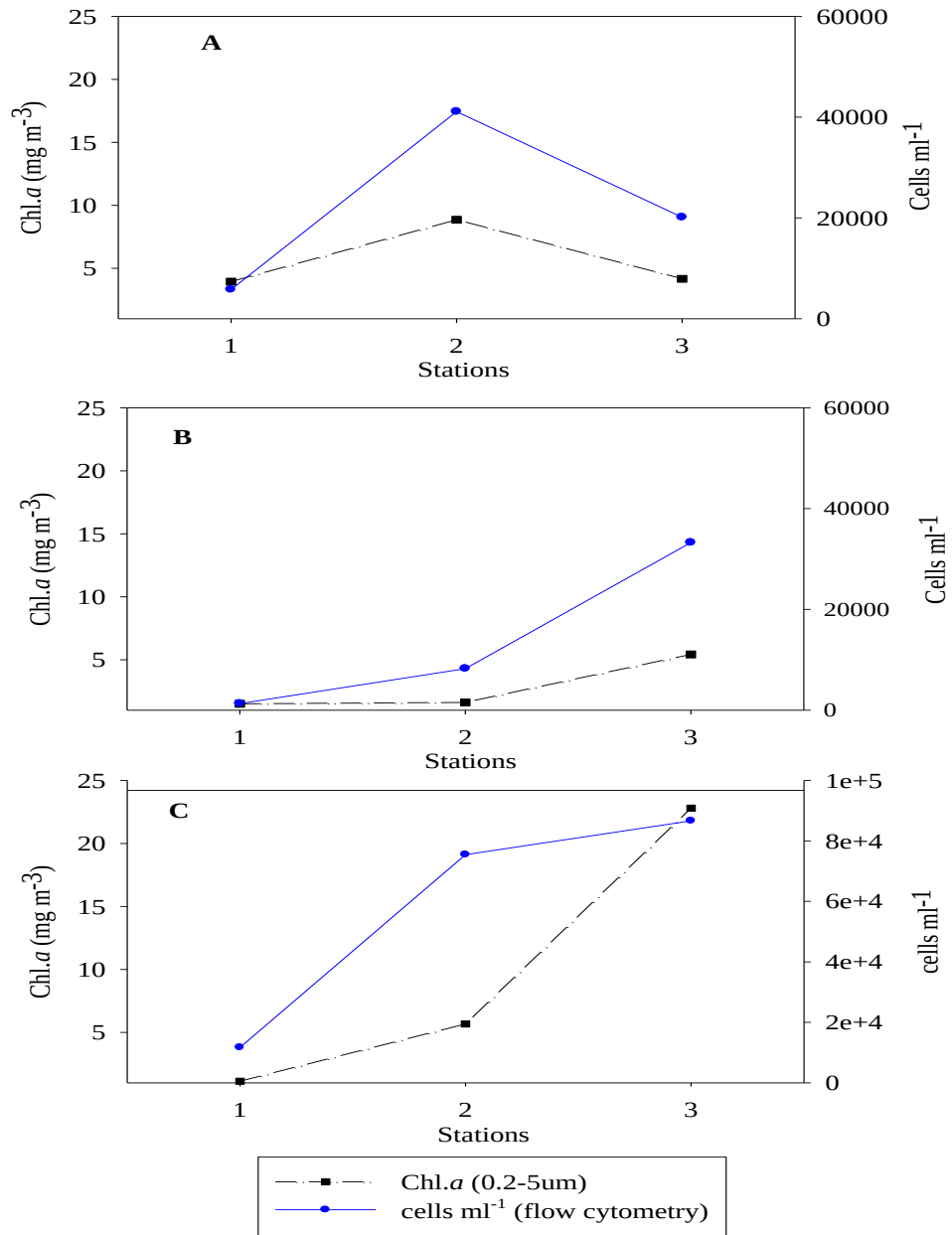


Figure 10: The relationship between the abundance of eukaryotic cells detected by flow cytometry (FC) and the concentration of chlorophyll *a* in the 0.2-5 μ m size fraction (measured by fluorometry), (**A**: julian day =154, **B**: julian day =183), **C**: julian day = 205), (notice the different scales on the left and right Y axis at julian day =205).

3. Signature pigments and phytoplankton taxa:

a. Pigment distribution and abundance:

Variations in HPLC pigment concentrations revealed the dynamic nature of the phytoplankton in terms of quantity, community structure and physiological status and were closely related to the salinity gradients in the estuary.

Fucoxanthin (FUC) was the most prominent carotenoid in fresh, brackish and saline waters. Other important pigment biomarkers were 19'hexanoyloxyfucoxanthin (HEX), 19'butanoyloxyfucoxanthin (BUT) and chlorophyll *b* (CHL*b*), alloxanthin and peridinin.

Concentrations of key chemotaxonomic marker pigments are summed to facilitate a broad assessment of the relative importance of the two functional classes of phytoplankton along the salinity gradient in Southampton Water.

[FUC + PER] - abundance of large eukaryotes i.e. diatoms and dinoflagellates.

[BUT + HEX + CHL *b*] – abundance of eukaryotic nanoflagellates i.e. chrysophytes, prymnesiophytes and chlorophytes (Gibb *et al.*, 2000).

The distribution of these two classes of organisms during the bloom periods is shown in Figure 11. The concentration of chlorophylls and carotenoid pigments during the bloom periods are summarised in Table 7. (see Appendix IIIA for the complete data set, Appendix IIIB for a list of the signature pigments that are useful as markers of algal groups, and Appendix IIIC for correlation between chlorophyll *a* measured by HPLC method and by fluorometry).

The abundance of (FUC+PER) closely follows that of chlorophyll *a* at the three stations, indicating the predominance of diatoms and dinoflagellates throughout the entire estuary. The highest concentration of (FUC +PER) (almost 6.4µg l⁻¹) was recorded at station 3 during the first bloom, and coincided with a high number of diatoms cells. High concentration of (FUC+PER) at station 3 during the third bloom was coincided with the high abundance of both diatoms and dinoflagellates.

The distribution of (BUT+HEX+ CHL.*b*) exhibits a different pattern between the different stations during the bloom periods. The concentration of (BUT+HEX +CHL.*b*) was greatest at station 1 during the last bloom ($1.0\mu\text{g l}^{-1}$) compared to $0.9\mu\text{g l}^{-1}$ for (FUC+PER) at the same station. This indicates that eukaryotic nanoflagellates dominate the phytoplankton community in the summer time at station 1. The concentration of (BUT+HEX+CHL.*b*) along the salinity gradient were markedly lower than those observed for (FUC+PER).

Table 7: The concentration of chlorophylls and carotenoid pigments during the bloom periods ($\mu\text{g m}^{-3}$).

Pigment	Julian day								
	154			183			205		
	station 1	Station 2	station 3	station 1	Station 2	station 3	station 1	Station 2	station 3
Peridinin	9.2	6.8	260	1.5	111	6	0	0	11.5
19'but	0	0	9.5	0	0	4.2	5	0	35.6
Fucoxanthin	2214	5641	6179	436	1315	2244	926	4269	4128
19Hex	55.5	36	42	19.2	11.1	80.3	466	38.6	2.4
Alloxanthin	73.9	225	256	4.6	12.6	55.9	11.7	227	61.5
Zeaxanthin	281	4.9	18.2	138	225	4.9	2338	28.9	2.8
Chl <i>b</i>	370	288	395	55.4	18.1	82.2	547	442	33.8

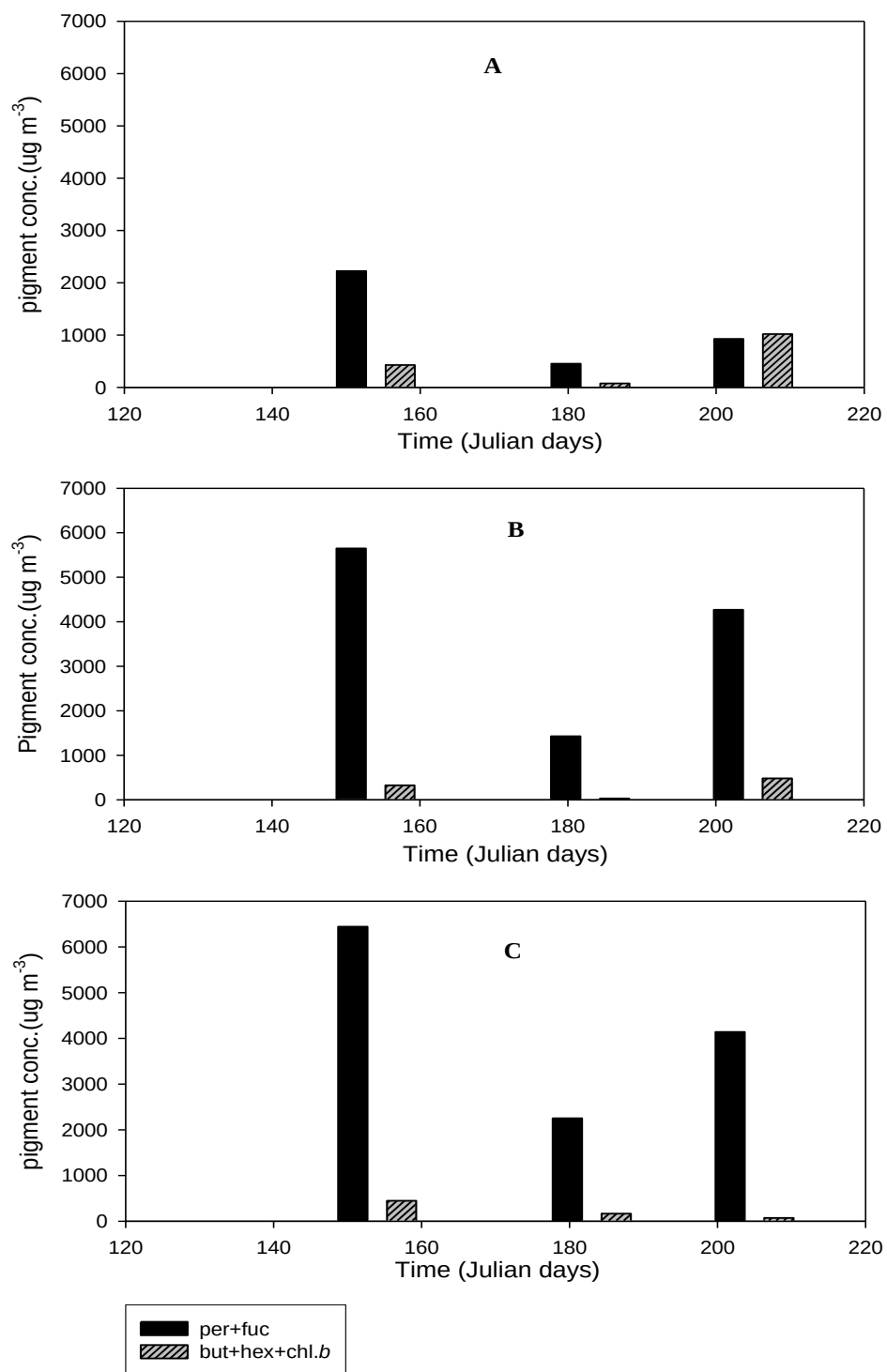


Figure 11: The distribution of (FUC+ PER) and (BUT+ HEX+ CHL b) during the bloom periods. (A: station 1; B: station 2; C: station 3).

b. Relationship between signature pigments and phytoplankton taxa:

Due to the limitations of time, only selected water samples from April to August, 2003 have been analysed by light microscopy for cell counts and identification. Phytoplankton can be classified into two major groups of forms, i.e. larger than 5µm (e.g. diatoms, dinoflagellates and ciliates), smaller than 5 µm, the ultraphytoplankton (e.g. small flagellates).

In the upper estuary (station 1), microscope observations revealed that the peaks of chlorophyll *a* and those of fucoxanthin were mainly associated with the diatoms *Navicula* spp. (2nd of May, up to 17 cells ml⁻¹), *Skeletonema costatum* and *Thalassiosira* spp. (August samples, up to 8 and 10 cells ml⁻¹, respectively).

In the middle estuary (station 2), peaks in chlorophyll *a* and fucoxanthin corresponded with maximum concentration of diatoms of the genera *Guinardia delicatula* (9 cells ml⁻¹) on the 2nd of May, *Thalassiosira* spp. (6 cells ml⁻¹) at the first bloom period and *Skeletonema costatum* (5 cells ml⁻¹) during August.

In the lower estuary (station 3), the most prominent peaks of fucoxanthin concentration corresponded to mixed assemblages of diatoms and to a lesser extent prymnsiophytes (*Phaeocystis*). Diatoms of the genera *Thalassiosira* spp. reached up to 39 cells ml⁻¹ during the first bloom, while the second bloom was dominated by *Chaetoceros* spp. (25 cells ml⁻¹).

Algae associated with 19'hex- and 19'butanolyloxyfucoxanthin could generally not be distinguished by light microscopy. Among the chlorophyll *b* containing groups observed along the estuary, the most prominent were euglenophytes of the genus *Eutreptiella* (up to 4555 cells ml⁻¹ at station 2 in August). Among peridinin containing dinoflagellates, the most important was the genus *Prorocentrum* (up to 8 cells ml⁻¹ at station 1 in August). Among the cryptophytes, the most conspicuous were *Cryptomonas*- like cells in station 1 and 2. A maximum number of *Cryptomonas* cells were found at station 2 in August (1400 cells ml⁻¹). Low numbers of the alloxanthin- containing ciliates (*Mesodinium rubrum*, (Ansotegui *et al.*, 2001) were observed at all stations during the sampling period with a

maximum concentration found in station 3 during the first bloom (29 cells ml⁻¹). HPLC analysis showed that the corresponding alloxanthin concentration was 256 µg m⁻³.

The phytoplankton species diversity was found to be higher in station 3. During most of the sampling period, a mixed assemblage of diatoms, dinoflagellates with peridinin, chlorophyll *b* containing euglenophytes and cryptophytes was present. Unidentified small flagellates (0.2-2 µm in diameter) increased during the sampling period and were most abundant during the summer time.

The identified phytoplankton groups and their relative abundance are listed in Appendix IV.

4. Molecular taxonomy (Fluorescent in situ- hybridisation):

a. FISH results:

The photosynthetic nano and picophytoplankton (0.2-20 µm) contributed significantly to the total phytoplankton community. Their small size and simple morphology make their identification by the traditional methods (e.g. light microscopy) quite difficult.

The results of the FISH method combined with flow cytometry showed that the ultraphytoplankton (nano and picoplankton cells) contributed significantly to the total community structure in Southampton Water throughout the sampling period, especially during summer months and during low total chlorophyll *a* values.

Target species displayed a very bright fluorescence signal, while non-target species showed only a faint fluorescence. The hybridisation signal was better with samples collected at station 3 (the turbidity in that station is lower than station 1 and 2). Sometimes the signal intensity was too low to distinguish labelled cells from the auto-fluorescence of non-target cells and from background fluorescence especially at more turbid water samples from station 1 and 2. For this reason, the standard deviation for microscopic counts was up to 31% at station 2 (maximum turbidity zone).

The contribution of the group specific probes to the total eukaryotic community at the three stations is shown in Figure 12.

The CHLO01 probe has been found to target about 37%, 64% and 41% of the eukaryotes at station 1, 2 and 3 respectively on day 223. Probe (PRYM02) targeted about 65%, 62.8% of the eukaryotes at station 1 and 2 respectively during the first bloom period. The highest contribution of PRYM02 has been detected at station 3 during the first bloom (about 81%, STD $\pm 13.3\%$). This result is supported by light microscope analysis, where *Phaeocystis sp.* (belongs to Haptophyta) was detected in large numbers ($1012 \text{ cells ml}^{-1}$) at station 3, but was difficult to be seen, under the light microscope in water samples collected from station 1 and 2 due to the presence of detrital materials.

Compared to the probes (CHLO01 and PRYM02), only a small fraction of eukaryotic cells were labelled by the class specific probes (PELA01 and BOLI02) with one exception (BOLI02 targeted about 39.6% of eukaryotes at station 3 on the last sampling day, STD $\pm 24.7\%$). (see Appendix V for the contribution of the group specific probes in relation to total eukaryotic cells).

b. Ultraphytoplankton abundance detected by FISH and flow cytometry:

Data obtained from flow cytometry were analysed to determine the number of the four categories of the nano-picophytoplankton (Chlorophyta, Haptophyta, Pelagophyceae and Bolidophyceae). Table 8 shows the abundance of these groups in the water samples determined by FISH and flow cytometry results. Both Chlorophyta and Haptophyta were found to be the most numerous phototrophic organisms in the water samples throughout the sampling period.

At station 1, CHLO01 concentration ranged between 89 cells ml^{-1} during the second bloom to about $8671 \text{ cells ml}^{-1}$ in August. At station2, CHLO01 concentration reached a maximum concentration ($20381 \text{ cells ml}^{-1}$) and ($16124 \text{ cells ml}^{-1}$) at station 3 in August. PRYM02 was found to have a maximum concentration during the first bloom in all stations ($3772 \text{ cells ml}^{-1}$) at station 1 and ($14663 \text{ cells ml}^{-1}$) at station 2 and ($5361 \text{ cells ml}^{-1}$) at station3.

Table 8: The relative abundance of the nano and picophytoplankton in natural samples determined by FISH and flow cytometry.

		122		154		183		223	
Julian day		cell/ml	% of probe	cell/ml	% of probe	cell/ml	% of probe	cell/ml	% of probe
Station1	Flow cytometry	5227		5790		1299		13478	
	CHLO01	1536	29.38	1400	24.18	89	6.85	8671	37.06
	PRYM02	487	9.32	3772	65.15	14	1.09	429	4.48
	PELA01	0	0	1377	23.79	34	2.65	697	38.08
	BOLI02	0	0	0	0	6	0.43	178	0.81
	Flow cytometry	3571		41085		8236		31682	
Station2	CHLO01	154	15.85	6512	4.3	379	4.6	20381	64.33
	PRYM02	2244	35.69	14663	62.83	285	12.36	1007	3.18
	PELA01	208	5.07	2083	5.83	86	1.05	1638	5.17
	BOLI02	0	0	456	1.11	1018	12.36	418	1.32
	Flow cytometry	9805		20132		33275		39317	
Station3	CHLO01	1833	9.26	1864	18.69	3151	9.47	16124	41.01
	PRYM02	7967	26.63	5361	81.25	779	39.6	830	2.11
	PELA01	654	3.74	753	6.67	2589	7.78	370	0.94
	BOLI02	0	0	211	1.05	13177	39.6	259	0.66

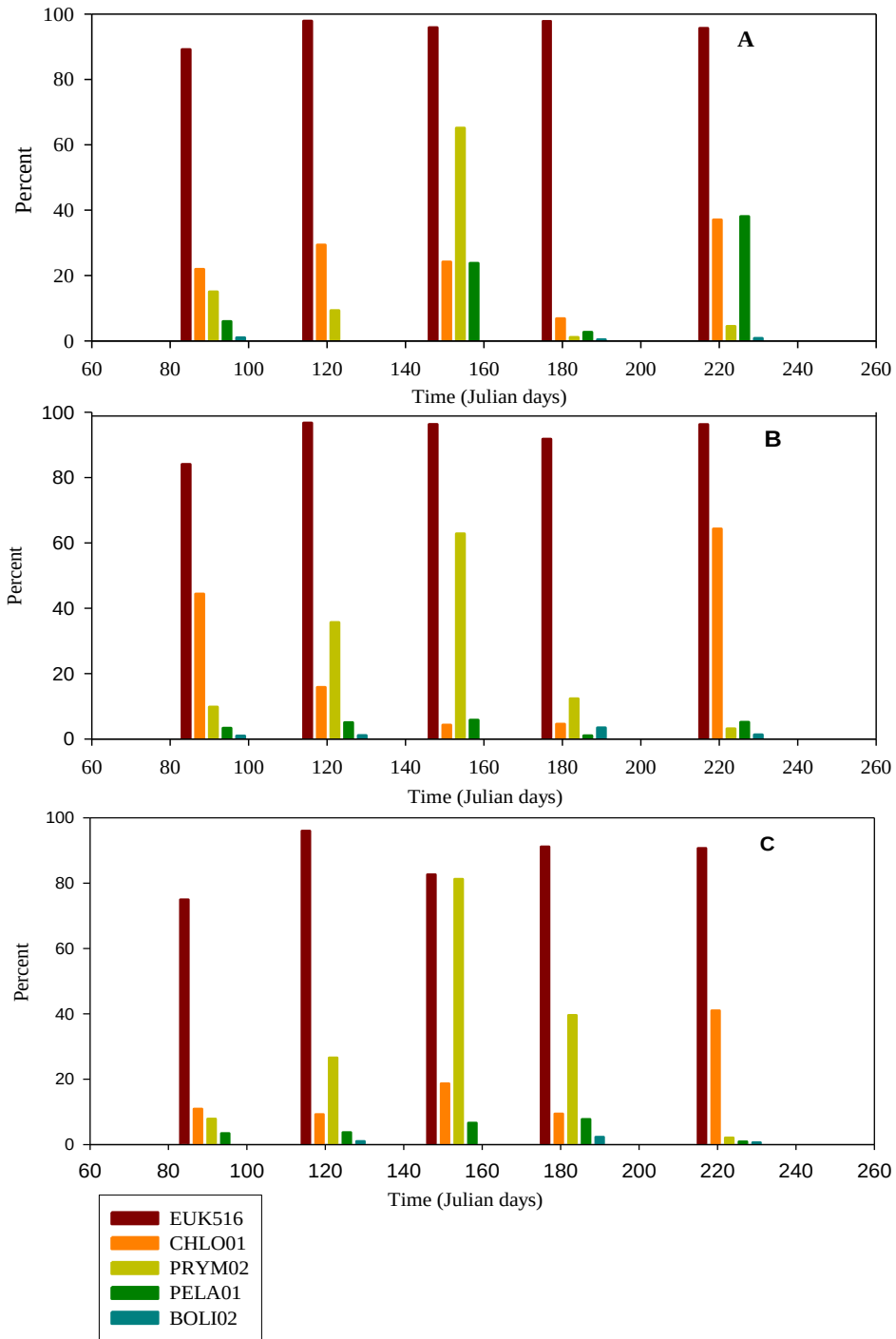


Figure 12: The spatial and temporal variations in the contribution of the different nano and picophytoplankton groups in relation to the total eukaryotic cells (**A**: station 1, **B**: station 2, **C**: station 3)

DISCUSSION

1. Factors controlling the seasonal abundance of total and size- fractionated chlorophyll *a* distribution:

a. Total chlorophyll a:

The annual cycle of phytoplankton production in Southampton Water is characterised by a spring-summer maximum when solar irradiance is high, water temperature is increasing and nutrient concentrations remain above the limiting levels. A pattern is typical of a broad spectrum of estuarine environments reviewed by Boynton *et al.*, (1982).

In the present study, which has been conducted between April and August, the chlorophyll *a* peaked in the first week of June and in the first and last weeks of July. The first and second bloom coincided with a spring tide, whereas the third bloom was during a neap tide. Over the sampling period, the solar irradiance ranged between ($169\text{W m}^{-2}\text{ day}^{-1}$ and $334\text{W m}^{-2}\text{ day}^{-1}$).

Earlier data have shown that this pattern of events is quite typical (Kifle, 1992). Holley and Hydes (2002) also reported that the development of blooms in different areas of Southampton Water do occur through the spring and summer, in relation to tidal and weather conditions. In 1999, conditions were such that the spring bloom increased in intensity through a spring tide (maximum chlorophyll *a* 55mgm^{-3}), which coincided with calm weather conditions and high light levels (averaged irradiance had been above $193\text{W m}^{-2}\text{ day}^{-1}$). It was followed by a sequence of seven blooms, the development of which can be related to changes in the tidal energy, irradiance and nutrient supply (Holley and Hydes, 2002).

During the present study, nutrient data indicated that phytoplankton in Southampton Water estuary are not nitrogen or silicon limiting during the sampling period (spring-summer), while phosphate was depleted during the last bloom especially in the saline water (NW Netley).

The SONUS data (Hydes and Wright, 1999) showed that nutrient concentrations in Southampton Water are generally high enough to support phytoplankton growth

throughout the whole year. The same trend in seasonal variations in nutrient concentrations was previously observed by Antai (1990), and Kifle and Purdie (1993).

During periods of depleted nutrients in estuaries due to either reduced river input during summer (Holmes *et al.*, 2000) or increased biological activity (Kifle, 1992), additional nitrogen can be derived from benthic regeneration (Holmes *et al.*, 2000). In Urdaibai Estuary, Spain inorganic nutrient availability as well as tidal flushing are considered to be the controlling factors of phytoplankton biomass (Revilla *et al.*, 2000).

The spatial distribution of total chlorophyll *a* concentration was in general in good agreement with observations of the regional variation of primary production rate reported by Bryan (1979) for Southampton Water.

The spring-neap tidal cycle has been identified as one major factor influencing phytoplankton growth in estuaries and shallow coastal seas (Haas, 1981). Kifle and Purdie (1993) conducted intensive studies on the spatial and seasonal distribution of phytoplankton in Southampton Water, UK. They observed that phytoplankton populations established themselves on the low neap tides and were then apparently flushed from the system during the spring tides. Species tended to establish themselves on the neap tides when water stability is enhanced, with a succession of blooms generally occurring between May and August (Lauria *et al.*, 1999). Cloern (1991) also showed that blooms occur in South San Francisco Bay during periods of low mixing during the neap tides.

It has been found during the present study that there was a decrease in total chlorophyll *a* concentration toward the upper limits of the estuary (River Itchen, station 1). The decrease in the total chlorophyll *a* concentration in the upper estuaries, despite enhanced nutrient levels (river input of nutrients), could be explained in terms of increased turbidity and thus a reduction in light availability. The same pattern is found in San Francisco Bay (Cole and Cloern, 1984) and Chesapeake Bay (Harding *et al.*, 1986).

A further factor contributing to the low chlorophyll *a* concentration in the upper estuary can be osmotic stress. Pennock and Sharp (1986) observed a decline in chlorophyll *a* concentration, in freshwater phytoplankton cell numbers, and in chlorophyll specific primary production rate, from the riverine region down to the beginning of the mesohaline region in the Delaware estuary. In the present study, it has been found that

overall chlorophyll *a* levels were lower in the freshwater region, the pattern of variation in the number of freshwater algae were also lower than the other two regions (brackish and saline waters).

Some freshwater algae showed a degree of tolerance to salinity (some diatoms found in all stations, e.g. *Nitzschia* spp. and *Skeletonema costatum*). In general, freshwater phytoplankton does not seem to be capable of growing well in the estuary (Rice and Ferguson, 1975; Brand, 1984).

It is thus likely to be a combination of light limitation by turbidity and osmotic stress of phytoplankton can explain the low chlorophyll *a* levels in the low salinity region of the upper estuary.

At intermediate salinities, as light availability increases and nutrients are still in excess of phytoplankton demands, total chlorophyll *a* concentration and phytoplankton abundance increase. However, the high turbidity caused by the accumulation of suspended particles in the intermediate salinity zone would limit the increase in the total chlorophyll *a* concentration.

In the high salinity region (NW Netley), the light availability is maximal but nutrient concentrations are lowest compared to the freshwater and intermediate salinity waters, especially during summer time.

In Chesapeake Bay and Delaware Bay, chlorophyll *a* is maximal in clearer waters, seaward of the turbidity maximum, and a nutrient-depleted zone at the highest salinities. Phytoplankton accumulation was associated with inorganic nutrient removal, suggesting that the high salinity zone near and in the shelf plume, is an index of nutrient limitation and widespread limitation in the Chesapeake Bay especially for phosphate (Fisher, 1988).

b. Size- fractionated chlorophyll a:

On analysing the spatial distribution of chlorophyll *a* biomass by size fractions, the most important feature was the distribution of the >20µm fraction along the salinity gradient. The >20µm fraction was the dominant fraction along the salinity gradient at chlorophyll *a* maximum events in most cases. The 5-20µm fraction became dominant in

three occasions, in the brackish water during the first bloom, in the freshwater during the second bloom and in the saline water during the third bloom.

In general, the relative significance of the 0.2-2 and 2-5 μm fractions were not correlated with total chlorophyll *a* biomass.

The results of the present study indicate the effect of the turbulence, light intensity and nutrient availability on the distribution of the different size fractions of phytoplankton along the salinity gradient during the spring- summer periods. Generally, diatoms (>20 μm) are sinking organisms, which rely on physical mixing to ensure sufficient irradiance levels to sustain production. As the thickness of the mixed layer increases during the summer months beyond a critical depth, net production ceases due to irradiance limitation. Dinoflagellates and small flagellates found to inhabit water columns where stable stratification, high irradiance levels and reduced nutrients are characteristic, typically during summer months in Southampton Water. These findings are consistent with other studies conducted in Southampton Water (e.g. Kifle, 1992 and Ali, 2001).

The nano and picophytoplankton were the least significant in relation to the larger phytoplankton during the bloom periods. The impact of the small phytoplankton has been found to decline from oligotrophic open ocean water (>50% of the total chlorophyll *a*) (Odate and Maita, 1988), to more eutrophic coastal (20-30%) (Joint *et al.*, 1986) and estuarine water ($\leq 10\%$) (Iriarte, 1991). Picoplankton was the dominant size fraction during summer in Chesapeake Bay (Sampou and Kemp, 1994). In the Mediterranean Estuarine Canal System, the phytoplankton was dominated by the small sized forms, mostly under 5 μm during spring and summer periods (Puigserver *et al.*, 2002).

The predominance of the larger size category of phytoplankton along the salinity gradient can be related to the nutrient regime, especially to the supply of nitrate from freshwater. On the other hand, the increase in the relative importance of the nano and picophytoplankton during summer time is consistent with the findings of Odate and Fukuch (1995), who noted the ecological importance of the smaller phytoplankton (<5 μm) in waters low in nutrients and chlorophyll. Small size may be a competitive advantage in the nutrient depleted environment (Kiorboe, 1993). By decreasing the size, the surface / volume ratios becomes larger and smaller cells may assimilate nutrients more efficiently than the bigger phytoplankton (Herbland *et al.*, 1985). In Chesapeake

Bay (a relatively shallow turbid estuary), is characterised by phytoplankton communities dominated by large diatom cells in spring followed by nano and picoplankton dominance during summer (Malone *et al.*, 1986).

The present study results indicates that the small sized- phytoplankton rarely attain concentration of bloom levels (i.e. 10mg m^{-3}); in other words, their concentration is more tightly controlled and less frequently subject to large variation and it can be suggested, that it is mainly variations in the abundance of the larger phytoplankton that determines the size structure of phytoplankton communities.

An important, but unexplored source of temporal variability in phytoplankton abundance and variations in size-fractioned chlorophyll *a* is zooplankton grazing. The bloom of the $>20\mu\text{m}$ fraction, in the first week of June and the last week of July was likely to have been a function of reduced grazing pressure particularly from the selective grazers copepods and cirripede larvae other than a result of large phytoplankton cells outcompeting small nanoplankton cells due to physiological advantages (Kifle, 1992).

2. Variations in phytoplankton taxonomy during spring and summer periods:

a. Phytoplankton species:

The present research has shown that the species composition of phytoplankton in Southampton Water can change measurably over short time scales.

Phytoplankton analysis revealed species succession with a sequence of different species, which peaked only for short periods during the sampling period from April to August, 2003 in Southampton Water.

Diatoms (e.g. *Skeletonema costatum*, *Guinardia delicatula* and *Ditylum brightwellii* and *Thalassiosira spp*) were the most dominant species of phytoplankton community during late spring months, however, dinoflagellates and the small nano and picoplankton were dominating the summer months.

A similar pattern of phytoplankton abundance and variation along the salinity gradient was previously recorded in Southampton Water (Kifle, 1992; Howard *et al.*, 1995; Ali, 2001).

The remarkable similarity in the species composition of phytoplankton between the different salinity points (fresh, brackish and saline water) suggests that the low and high salinities are no barrier to the growth of the species observed in the present study. Several diatoms species and small flagellates found to tolerate the three salinity points in the sampling area. These results are consisted with another study conducted along the salinity gradient in Southampton Water during 1988 by Kifle (1992).

The present study results revealed that the nano-picoplankton didn't show any clear differences in their distribution along the salinity gradient, but their concentration found to increase during the summer time, as the sampling stations became depleted with nutrients. Ultraphytoplankton presence is often an indication of a stressed environment (Shapiro and Guillard, 1986).

b. Pigment diversity:

In the present study, pigment concentrations were found to be minimal during low chlorophyll *a* concentration and maximal during the peak of chlorophyll *a* (see Appendix III). Fucoxanthin reached maximum values (2.2, 5.6 and 6.2 mg m⁻³ at station 1, 2 and 3 respectively during the first week of June (during maximum chlorophyll *a* concentrations).

Other important pigments found in relatively high concentrations along the salinity gradient throughout the sampling time were 19hex, chl.*b* and 19but (signature pigments for the nanoplankton). The concentrations of the pigment showed a dramatic increase throughout the sampling period and peaked during the summer period. Another important pigment is peridinin which is a signature pigment in dinoflagellates, found to peak during summer especially at the saline water (NW Netly). These findings are consisted with another study done by Ali (2001) during spring-summer (1999) along the salinity gradient in Southampton Water.

The overall results show that the communities in Southampton Water were dominated by diatoms in spring-summer samplings, while the estuarine phytoplankton summer bloom was characterised by the significant contribution of both dinoflagellates and the small nano and picophytoplankton..

In general, the pigment diversity was extremely high during the sampling time (spring-summer periods).

3. Enumeration and identification of the small phytoplankton groups (ultra phytoplankton) by FISH and flow cytometry:

The important novel part of the present study was the use of the new molecular method (Fluorescent In-Situ Hybridisation) combined with flow cytometry for the enumeration and identification of the small photosynthetic eukaryotes (both nano and picophytoplankton) that were difficult to be identified in most cases using the light microscopy because of their small size and simple morphology.

Target cells were easily detected and enumerated under epifluorescence microscopy provided that the following precautions were carefully observed. First, the fixation step should not exceed one hour, beyond which there is a significant cell loss. Second, the use of paraformaldehyde (1%) to fix the ultraphytoplankton cells is highly recommended (Marie *et al.*, 2000). Third, in order to achieve an intense labelling of all target cells, the filter must always remain wet during filtration and dehydration steps (Not *et al.*, 2002).

Other parameters that might reduce the fluorescence of the target cells, in particular low rRNA content linked to low growth rate (Amann *et al.*, 1995). In this study, the best results and counting achieved at bloom periods. The cells are easily detected because they are in the exponential growth phase. Under these conditions, the relative proportion of a target taxon in a mixture can be very accurately estimated (Not *et al.*, 2002).

In the preset study samples, the general probe (EUK516) targets a high proportion of the eukaryotic cells. It should be noted that some cells might be destroyed during fixation or during the treatments associated with FISH (ethanol for example). Furthermore, some eukaryotic taxa might be present in abundance at the station sampled but not labelled by the probes used (e.g. the cells were poorly labelled at station 1 and 2 during the second bloom period. This raises another problem with FISH method; the presence of the debris particles at the high turbidity zones might weaken the fluorescence signal).

There was no clear seasonal difference between the ultraphytoplankton populations along the three stations during the sampling period (April to August).

It was very clear from the relative abundance of the different groups calculated by using the combination of FISH and flow cytometry data, that Chlorophyta and Haptophyta are generally the most important groups with significant contribution. In the present study, the division Chlorophyta and the group Haptophyta were found to dominate the sampling sites in spring-summer times. Chlorophytes ranged between 89 and 20381 cells ml⁻¹ and Haptophytes ranged between 14 and 14663 cells ml⁻¹). Haptophyta found to peak during the first week of June in all stations during the maximum chlorophyll *a* concentration. Light microscope observations revealed the presence of the *Phaeocystis* colonies during that peak time.

Only very few cells were detected with the probe targeting the Pelagophyceae. This class is assumed to be important in marine waters (Anderson *et al.*, 1996). The uncertainty of the presence of this class in the estuary is high (STD up $\pm 10.4\%$). The presence of Bolidophyceae in the estuary during the present study was unexpected since members of this class have been isolated to date only from oligotrophic marine waters of the Pacific and the Mediterranean sea (Guillou *et al.*, 1999 a, b).

It has been found that a large fraction of cells are composed of non-targeted groups such as Crptophytes, Euglenaphytes and Dinophytes or of unknown organisms.

In samples collected from the English Channel in 2000 by Not *et al.*, (2002), the ultra phytoplankton community has been found to be dominated by cells belonging to the division Chlorophyta, Haptophyta. Bolidophyceae and Pelagophyceae were also detected but at low abundance.

The FISH method combined with flow cytometry appeared during this study to be an appropriate method to directly combine identification and quantification of cells from a complex ultra phytoplankton community, it is critical to increase considerably the number of available 18s rRNA sequences in databases (Not *et al.*, 2002).

Ideally, for the general probes such as eukaryotic probes or probes targeting division taxa, the simultaneous use of two or three probes appeared to be the best way to assess the abundance of the corresponding group in a sample (Amann and Ludwig, 2000).

Phytoplankton ecology has relied heavily on approaches that were developed more than a decade ago, such as flow cytometry (Olson *et al.*, 1985) or HPLC pigment analysis

(Gieskes and Kaay, 1993). Clearly, it should extensively adopt molecular approaches such as FISH or gene cloning that have proved to be useful for the study of the diversity and the role of ocean bacteria (Beja *et al.*, 2000). An additional advantage of these methods is that they also target heterotrophic eukaryotes (have not been targeted in the present study), for which, until now, it was only possible to estimate the total abundance with stains such as DAPI and Primulin (Not *et al.*, 2002).

CONCLUSION

On the basis of the observations made in the present study the following conclusions are made.

There are clear spatial and temporal variations in community composition and abundance in Southampton Water. These variations are caused by a combination of several physical and chemical factors (i.e. spring/neap tidal cycles, solar irradiance, water temperature and inorganic nutrients).

Species composition of phytoplankton showed differences between different bloom periods that occur during either spring tides or during neap tides. The spring tidal bloom comprised mainly of diatoms, while the neap tide bloom that occurred during late summer comprised mainly of relatively high abundance of dinoflagellates and the ultra phytoplankton (nano and picophytoplankton).

The changes in chlorophyll *a* concentration as an indicator of biomass appeared to be light and temperature dependent. The chlorophyll *a* concentration was relatively high during the sampling period (spring-summer months), with three peak times.

The production of the whole phytoplankton populations and the different size fractions of chlorophyll *a* are highly correlated in most cases with the biomass and light availability.

Depletion of nutrient during the summer months would limit the phytoplankton growth, especially the microphytoplankton. At this stage there was an observable shift in the phytoplankton community structure from diatom-dominated to a flagellate- dominated one especially in the saline water.

In general (with few exceptions), there was a horizontal gradient of increasing chlorophyll *a* concentration (indicator of biomass) in a seaward direction where turbidity decreases and light availability increases.

The present research showed that the new molecular techniques are important in the enumeration and the identification of the ultra phytoplankton community of Southampton water.

From the present study, I highly recommend the use of the molecular method (FISH) combined with flow cytometry for the identification and enumeration of the different ultra phytoplankton groups that are difficult to be identified using the light microscopy. Most of the data available about these groups in Southampton Water were classifying them according to their size. Only few studies have been conducted to classify this group into the class or division levels. I also recommend the use of other group specific probes (e.g. probes for Dinophytes and Cryptophytes) as it appeared from the light microscope observations, they are an important part of the phytoplankton community in Southampton Water, beside the use of CHLO01 and PRYM02 probes that have shown a significant contribution to the ultra phytoplankton communities.

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**APPENDIX I: Nutrient concentrations (μM) at station 1, 2 and 3
during the sampling period.**

<i>Julian</i>									
<i>days</i>	<i>Station 1</i>			<i>Station 2</i>			<i>Station 3</i>		
	Si	N	P	Si	N	P	Si	N	P
91	169.2	432.6	34.2	67.4	210.6	4.8	19.3	68.3	1.4
105	135.0	519.2	6.3	47.1	195.5	4.9	21.3	95.3	1.0
122	147.1	472.9	23.2	65.4	167.7	4.3	20.8	54.0	1.1
136	144.3	493.8	8.0	68.6	195.2	5.1	10.6	20.1	0.5
154	165.3	497.4	8.8	69.7	165.2	4.0	9.8	23.3	0.2
163	180.7	427.4	6.9	106.0	278.1	9.0	13.3	33.7	0.6
183	188.2	439.4	9.9	98.0	181.4	7.2	7.7	19.5	0.5
195	152.4	426.6	9.8	71.9	140.8	6.4	15.9	19.4	2.0
205	176.4	382.3	10.3	109.1	233.3	9.7	5.9	28.3	0.5
223	163.6	444.7	14.5	50.8	120.7	7.0	8.3	20.0	0.1

APPENDIX II: The average concentrations of POC, PON (mg l^{-1}), total chlorophyll *a* in (mg m^{-3}) and POC in (mg m^{-3}) at the three stations, C:N(POC to PON ratio), C:Chl.*a* (POC to Chl.*a* ratio).

Station 1

<i>Julian day</i>	<i>Average PON (mg l^{-1})</i>	<i>Average POC(mg l^{-1})</i>	<i>C:N</i>	<i>Avg.total chl. a (mg m^{-3})</i>	<i>POC (mg m^{-3})</i>	<i>POC:Chl.a</i>
91	0.32	1.87	5.84	2.05	1866	910
105	0.25	0.88	3.46	5.75	875	152
122	0.14	1.29	9.26	4.80	1287	268
136	0.13	1.21	9.06	6.72	1210	180
154	0.13	1.10	8.54	5.90	1102	187
163	0.37	2.31	6.16	2.46	2310	939
183	0.12	0.86	7.25	1.90	856	451
195	0.15	1.04	6.80	2.06	1039	505
205	0.19	1.32	7.07	3.80	1320	347
223	0.29	1.60	5.60	1.50	1595	1063

Station 2

<i>Julian day</i>	<i>Average PON (mg l^{-1})</i>	<i>Average POC(mg l^{-1})</i>	<i>C:N</i>	<i>Avg.total chl. a (mg m^{-3})</i>	<i>POC (mg m^{-3})</i>	<i>POC:Chl.a</i>
91	0.17	1.20	7.10	2.90	1200	414
105	0.11	1.20	11.40	1.40	1200	857
122	0.14	1.50	10.80	2.30	1500	652
136	0.10	0.95	9.20	1.30	950	731
154	0.31	2.20	7.20	24.90	2200	88
163	0.20	1.50	7.30	3.00	1500	500
183	0.17	1.10	6.50	4.59	1100	240
195	0.25	1.60	6.40	4.50	1600	356
205	0.31	1.90	5.90	22.86	1900	83
223	0.23	1.10	5.00	6.60	1100	167

Station 3

<i>Julian day</i>	<i>Average PON (mg l^{-1})</i>	<i>Average POC(mg l^{-1})</i>	<i>C:N</i>	<i>Avg.total chl. a (mg m^{-3})</i>	<i>POC (mg m^{-3})</i>	<i>POC:Chl.a</i>
91	0.09	0.90	9.60	2.90	900	310
105	0.15	1.35	9.20	2.83	1350	477
122	0.14	1.07	7.40	5.60	1070	191
136	0.23	1.56	6.69	3.08	1560	506
154	0.39	2.28	5.80	32.93	2280	69
163	0.12	0.90	7.20	8.71	900	103
183	0.13	0.91	6.90	17.60	910	52
195	0.19	1.35	7.03	4.85	1350	278
205	0.27	1.57	5.85	31.90	1570	49
223	0.21	1.23	5.88	13.20	1230	93

**APPENDIX III/A: The concentrations of chlorophylls and carotenoid pigments in
($\mu\text{g m}^{-3}$).**

	Station 1							
Julian days	Peridinin	19'But	Fucoxanthin	19'Hex	Alloxanthin	Zeaxanthin	Chl.b	Chl.a
91	0	0	258	0	14.9	122	24.9	1362
105	0	0	902	9.1	25.8	153	113	2503
122	30.2	0	398	0	4.8	222	235	1776
136	5.4	0	884	74.7	9.5	172	445	2254
154	9.2	0	2214	55.5	73.9	281	370	4587
163	0	0	487	0	0	83.9	0	879
183	15.8	0	436	19.2	4.6	138	55.4	1011
195	0	0	414	7.7	3.3	120	62.8	932
205	0	5.0	926	466	11.7	233	547	3983
223	13.1	0	1500	9.0	31	190	127	2712

	Station 2							
Julian days	Peridinin	19'But	Fucoxanthin	19'Hex	Alloxanthin	Zeaxanthin	Chl.b	Chl.a
91	9.3	3.0	546	0	45.0	0.1	128	2118
105	0	1.4	235	0	23.0	0.8	41.5	427
122	0	7.0	379	0	18.5	2.2	226	1407
136	0	0	121	0	4.7	1.0	34.3	424
154	6.8	0	5641	36.0	225	4.9	288	11592
163	0	0	176	69.9	38.1	0	960	2461
183	111	0	1315	11.1	12.6	225	18.1	675
195	0	7.4	201	13.8	166	7.0	290	1618
205	0	0	4269	38.6	227	28.9	442	9622
223	15.4	0	1043	56.4	151	43.7	502	3221

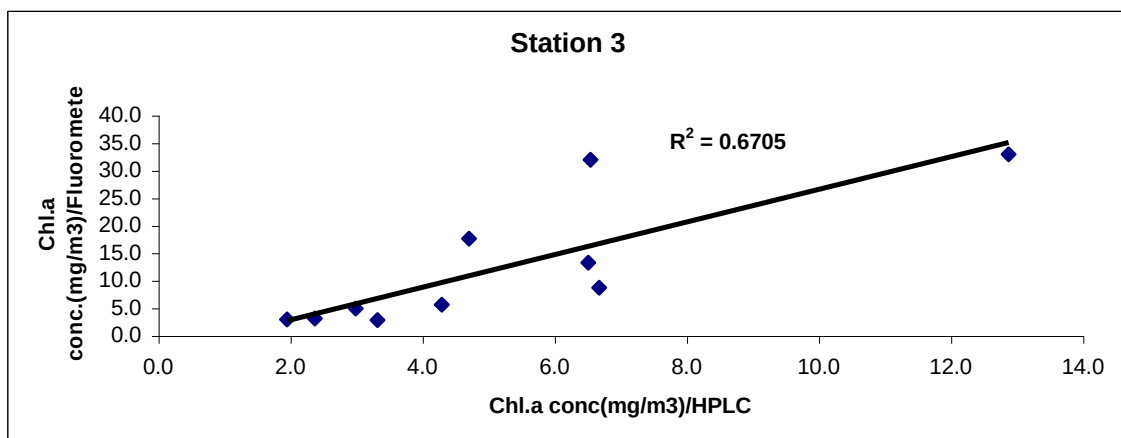
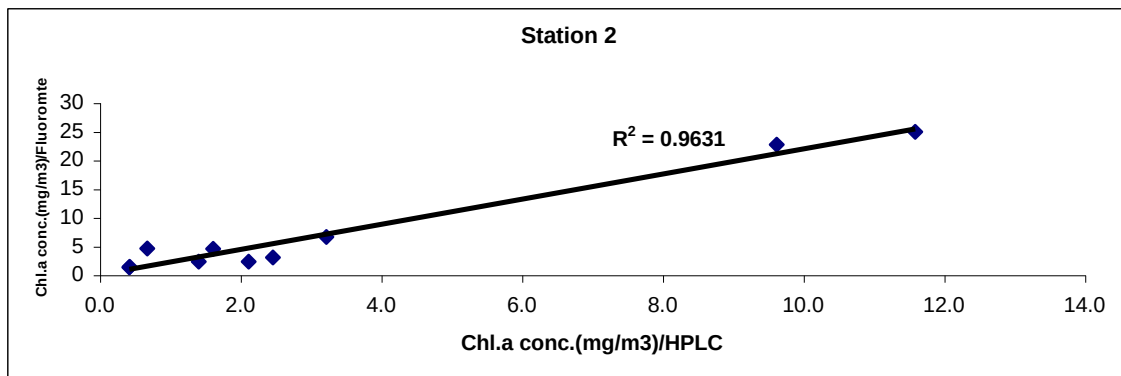
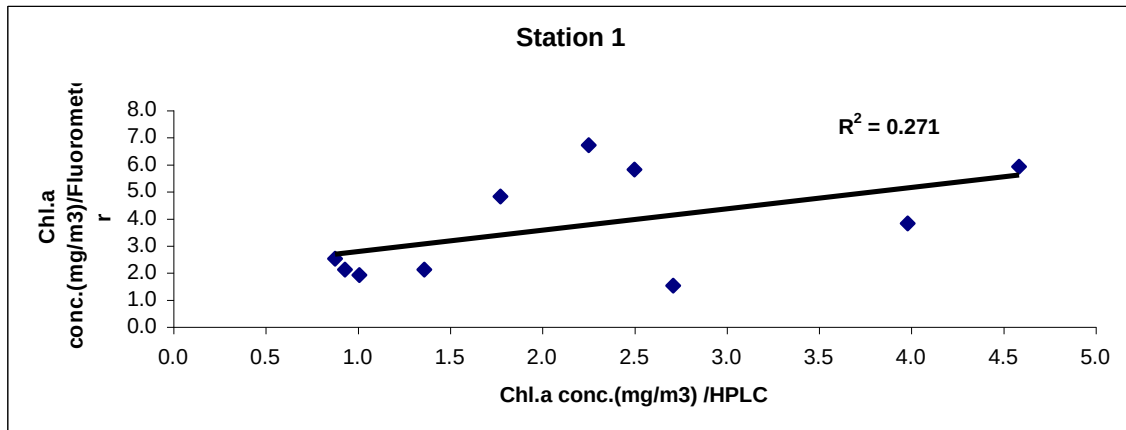
APPENDIX III/A: CONTINUED

Julian days	Station 3							
	peridinin	19'But	Fucoxanthin	19'Hex	Alloxanthin	Zeaxanthin	Chl. <i>b</i>	Chl. <i>a</i>
91	0.2	15.6	829	10.7	87.6	1.7	75.9	1953
105	7.2	0	1280	28.1	186	35.6	204	3322
122	53.4	613	1691	570	184	17.7	213	4296
136	9.8	1.2	1055	0	31.5	9.3	77.0	2374
154	260	9.5	6179	42.0	256	18.2	395	12875
163	1.3	6.0	1539	28.0	165	12.3	529	6.677
183	6.0	4.2	2244	80.3	55.9	4.9	82.2	4704
195	0	0	747	47.6	151	11.1	92.4	2993
205	11.5	35	4128	2.4	61.5	2.8	33.8	6547
223	386	0	3327	30.5	40.7	13.4	215	6514

APPENDIX III B: Summary of signature pigments useful as markers of algal groups in the sea (from Jeffrey and Veski, 1997):

<i>Pigment</i>	<i>Algal group</i>
Chl. <i>a</i>	All photosynthetic algae
Chl. <i>b</i>	“green algae”
βcarotene	Major pigment in “ green algae”
Peridinin	Photosynthetic dinoflagellates
Fucoxanthin	Major pigments in diatoms, prymnesiophytes
Alloxanthin	Major pigments in cryptomonads
Zeaxanthin	Major/minor pigment in prochlorophytes, cyanobacteria, “green algae” chrysophytes
19’Hex	Major/ minor pigment in several prymnesiophytes, some dinoflagellates
19’But	Major pigment in some prymnesiophytes, some chrysophytes dinoflagellates

APPENDIX IIIC: The regression line between chlorophyll a measured by HPLC and total chlorophyll a measured by fluorometry.



APPENDIX IV (A): The different phytoplankton groups (number of cells per ml) at station 1:

Algal group	Julian days				
Diatoms	91	122	154	183	223
<i>Amphora spp.</i>	0.2		0.1		
<i>Asterionella spp</i>					
<i>Bacillaria spp</i>					
<i>Chaetoceros spp</i>					0.2
<i>Coscinodiscus spp</i>	0.7		1.7		
<i>Dictyoneis spp</i>		0.2	0.2		
<i>Ditylum brightwellii</i>					
<i>Fragillaria spp</i>					
<i>Guinardia delicatula</i>		0.5	0.8		
<i>Leptocylindrus spp</i>					
<i>Lithodesmium spp</i>					
<i>Mastoglia spp</i>					
<i>Melosira spp</i>					
<i>Meridion spp</i>		0.5	1.4		
<i>Navicula spp</i>	3.3	17	9.5	2.7	
<i>Nitzschia spp</i>	0.9	3.1	0.6	1	
<i>Pleurosigma spp</i>	0.1	0.1	0.1	0.1	0.5
<i>Rhizosolenia spp</i>					0.3
<i>Scenedesmus quadratus</i>	0.3	0.4		0.7	0.2
<i>Skeletonema costatum</i>					8
<i>Thalassionema spp</i>		1.8			1
<i>Thalassiosira spp.</i>	0.4				10.5
<i>Thalassiothrix spp.</i>					
Total	5.9	23.6	14.4	4.5	20.7
Ciliates					
<i>Mesodinium rubrum</i>	0.8	0.7	0.2	0.2	0.7
<i>Tintinninds spp</i>					
Total	0.8	0.7	0.2	0.2	0.7
Dinoflagellates					
<i>Gyrodinium spirale</i>	0.4				
<i>Gymnodinium sp.</i>	0.3				
<i>Peridinium</i>					
<i>Prorocentrum spp</i>					8
<i>Scripsella spp.</i>			0.2	0.5	8.5
Total	0.7	0	0.2	0.5	16.5
Flagellates					
<i>Cryptomonas</i>	0.2	66.9			
<i>Eutreptiella marina</i>			0.4		0.1
<i>Phaeocystis</i>					
small flagellates(0.2-5um)	2.1	172.8	142.5	228.9	36213.6
Total	2.3	239.7	142.9	228.9	36213.7

APPENDIX IV (B): The different phytoplankton groups (number of cells per ml)

at station 2:

Algal group	Julian days				
Diatoms	91	122	154	183	223
<i>Amphora spp.</i>			0.6	0.8	0.8
<i>Asterionella spp</i>	3.4				
<i>Bacillaria spp</i>					
<i>Chaetoceros spp</i>					
<i>Coscinodiscus spp</i>	0.5	0.1	1		
<i>Dictyoneis spp</i>		0.1	0.2		
<i>Ditylum brightwellii</i>	0.1			0.1	
<i>Fragillaria spp</i>					
<i>Guinardia delicatula</i>		9			
<i>Leptocylindrus spp</i>	1.5				
<i>Lithodesmium spp</i>				2.5	0.5
<i>Mastoglia spp</i>					
<i>Melosira spp</i>					0.2
<i>Meridion spp</i>		0.3	0.1		
<i>Navicula spp</i>	1.7	6	2.8	2	3
<i>Nitzschia spp</i>	2	0.5		2	1.8
<i>Pleurosigma spp</i>		0.1	0.1		0.3
<i>Rhizosolenia spp</i>					
<i>Scenedesmus quadratus</i>	0.4		0.2	0.3	0.1
<i>Skeletonema costatum</i>	3.6				5
<i>Thalassionema spp</i>		0.6			
<i>Thalassiosira spp.</i>	0.4		6	1	3
<i>Thalassiothrix spp.</i>				0.3	
Total	13.6	16.7	11	9	14.7
Ciliates					
<i>Mesodinium rubrum</i>	1.2	0.9	4.5	7	7
<i>Tintinnids spp</i>					3
Total	1.2	0.9	4.5	7	10
Dinoflagellates					
<i>Gyrodinium spirale</i>					
<i>Gymnodinium sp.</i>					
<i>Peridinium</i>					0.5
<i>Prorocentrum spp</i>					0.7
<i>Scrippsiella spp.</i>	0.2		25	1.3	
Total	0.2	0	25	1.3	1.2
Flagellates					
<i>Cryptomonas</i>	27.7	23.7	60	1367.2	1400
<i>Eutreptiella marina</i>	5.7	66.9	2006	4181.4	4555
<i>Phaeocystis</i>					
<i>small flagellates(0.2-5um)</i>	1.5	84.2	803.4	1356.4	39708
Total	34.9	174.8	2809.4	6905	45663

APPENDIX IV (C): The different phytoplankton groups (number of cells per ml) at station 3:

Algal group	Julian days				
Diatoms	91	122	154	183	223
<i>Amphora spp.</i>					
<i>Asterionella spp</i>					
<i>Bacillaria spp</i>	0.3				
<i>Chaetoceros spp</i>			0.4	25.4	1.5
<i>Coscinodiscus spp</i>	0.9	0.3	37.9	3.5	
<i>Dictyoneis spp</i>					
<i>Ditylum brightwellii</i>	0.4			3	1.8
<i>Fragillaria spp</i>	1.1				
<i>Guinardia delicatula</i>	0.1	23.5	15.1		3.4
<i>Leptocylindrus spp</i>	0.1				
<i>Lithodesmium spp</i>				1	0.4
<i>Mastoglia spp</i>	0.1				
<i>Melosira spp</i>	0.1				
<i>Meridion spp</i>					
<i>Navicula spp</i>	1.6				0.5
<i>Nitzschia spp</i>	0.7	0.1	4.9	7	6
<i>Pleurosigma spp</i>	0.1				0.1
<i>Rhizosolenia spp</i>				0.4	0.4
<i>Scenedesmus quadratus</i>					
<i>Skeletonema costatum</i>	4.6	1.8			0.9
<i>Thalassionema spp</i>	0.6		3.1		
<i>Thalassiosira spp.</i>	0.8		39	8	1
<i>Thalassiothrix spp.</i>					
Total	11.5	25.7	100.4	48.3	16
Ciliates					
<i>Mesodinium rubrum</i>	2.5	5.8	29.3	13.5	1.7
<i>Tintinninds spp</i>					
Total	2.5	5.8	29.3	13.5	1.7
Dinoflagellates					
<i>Gyrodinium spirale</i>					
<i>Gymnodinium sp.</i>		32.4			
<i>Peridinium</i>	0.1				15
<i>Prorocentrum spp</i>				0.4	0.7
<i>Scripsella spp.</i>			6.9	4.6	1.3
Total	0.1	32.4	6.9	5	17
Flagellates					
<i>Cryptomonas</i>	8.4	64.8		779.7	950
<i>Eutreptiella marina</i>	2.3	8.2	3.4	205.2	650
<i>Phaeocystis</i>			1012		
<i>small flagellates</i>	0.1	86.4	328.3	15259.2	34174.8
Total	10.8	159.4	1343.7	16244.1	35774.8

APPENDIX V: Fractions of the eukaryotic probe and the group specific probes, using the fluorescent in –situ hybridization. (Average percentages and standard deviations $\pm$)

JULIAN DAY	STATION 1	EUK516	CHLO01	PRYM02	PELA01	BOLI02
91	AVERAGE	0.2	22	15	6	1
	STDEV	7.5	2.3	5.7	5	1.4
122	AVERAGE	97.9	29.4	9.3	0	0
	STDEV	1.6	6.2	7.1	0	0
154	AVERAGE	95.9	24.2	65.2	23.8	0
	STDEV	3.6	24.9	27.9	6.3	0
183	AVERAGE	97.9	6.8	1.1	2.6	0.43
	STDEV	0.95	4.3	0.38	2.2	0.38
223	AVERAGE	95.7	37.1	4.5	38.1	0.81
	STDEV	3.8	14.9	5.4	10.4	1.2

JULIAN DAY	STATION 2	EUK516	CHLO01	PRYM02	PELA01	BOLI02
91	AVERAGE	84.1	44.4	9.8	3.4	0.97
	STDEV	1.7	25.8	3	4.8	1.4
122	AVERAGE	96.7	15.9	35.7	5.1	1.11
	STDEV	2	5.2	31.1	3.2	0.55
154	AVERAGE	96.3	4.3	62.8	5.8	0
	STDEV	3.9	0.68	14.8	1.2	0
183	AVERAGE	91.8	4.6	12.4	1	3.5
	STDEV	0.53	2.4	6.3	0.8	2.5
223	AVERAGE	96.2	64.3	3.2	5.2	1.3
	STDEV	0.64	0.37	2.3	6.8	1.9

JULIAN DAY	STATION 3	EUK516	CHLO01	PRYM02	PELA01	BOLI02
91	AVERAGE	74.98	10.95	7.9	3.45	0
	STDEV	13.12	8.85	5.87	4.9	0
122	AVERAGE	96	9.3	26.6	3.74	1.05
	STDEV	5.3	2.6	9.37	3.1	0.72
154	AVERAGE	82.65	18.69	81.25	6.67	0
	STDEV	16.05	3.2	13.26	2.36	0
183	AVERAGE	91.16	9.47	39.61	7.78	2.3
	STDEV	2.8	2.67	24.71	2.7	0.63
223	AVERAGE	90.69	41.01	2.11	0.94	0.66
	STDEV	2.5	24.02	2.62	0.44	0.93

