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PROCESSES AND CONDITIONS INFLUENCING PHYTOPLANKTON GROWTH AND BLOOM INITIATION IN A MACROTIDAL ESTUARY, SOUTHAMPTON WATER

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A thesis submitted to the faculty of science in partial fulfilment of the requirements for the degree of Doctor of Philosophy

SCHOOL OF OCEAN AND EARTH SCIENCES
"SOUTHAMPTON OCEANOGRAPHY CENTRE"
FACULTY OF SCIENCE

June 2003

Graduate School of the Southampton Oceanography Centre

University of Southampton

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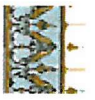

مَرَجَ الْبَحْرَيْنِ يَلْتَقِيَانِ {19} بَيْنَهُمَا بَرْزَخٌ لَّا يَبْغِيَانِ {20} فَبِأَيِّ آلَاءِ رَبِّكُمَا تُكَذِّبَانِ {21}

In The Name Of Allăh (God),
the Most Gracious, the Most Merciful

....**(19)** He has let free the two water bodies (saline and freshwater) of flowing water meeting together, **(20)** Between them is a Barrier which they do not transgress and **(21)** Then which of the blessings (favours) of your Lord will you both (jinn and men) deny ?

Al-Rahman versus; 19, 20 & 21

To ....

My Dear Husband; Islam
My Lovely Son; Omar
AND....
My Great Parents

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE
SCHOOL OF OCEAN AND EARTH SCIENCE

Doctor of Philosophy

PROCESSES AND CONDITIONS INFLUENCING PHYTOPLANKTON GROWTH AND BLOOM INITIATION IN A MACROTIDAL ESTUARY, SOUTHAMPTON WATER

By: **Elham Mahmoud Ali-Mohamed**

Southampton Water is known to be a highly dynamic, macrotidal, and hypernutrified estuary and has previously been reported to support large phytoplankton populations during the spring-summer period. However, phytoplankton blooms in the estuary have been shown to be short lived due to rapid changing conditions of irradiance and variable intensity of tidal mixing. The aim of this research was to investigate the coupling between variations in phytoplankton community and bloom development in Southampton Water and changes in environmental conditions. This was achieved through using a combination of different field sampling approaches over intensive temporal and spatial scales, as well as experimental incubation experiments.

In 1999 data from a coastal monitor (installed on the dock wall in the upper estuary) provided very frequent measurements of chlorophyll fluorescence (every 10 minutes) together with temperature, salinity and turbidity data to investigate environmental conditions causing the initiation of blooms of diatoms and dinoflagellates in the estuary. The data covered the productive period (from April to September 1999) when water samples were collected adjacent to the sensor package at frequent intervals (5-7 days). The main Spring bloom occurred during spring tides in May 1999 and was coincident with mean water column irradiance values of >100Wh $m^{-2}d^{-1}$), water temperatures of 14°C and some salinity stratification. This combination of conditions provided optimum conditions for the growth of *Guinardia delicatula* that dominated this bloom. Later secondary blooms were identified during the period of study and these were correlated with the physical, chemical and meteorological data collected. The spring diatom bloom appeared to be independent of the spring- neap tidal state, whereas a summer dinoflagellate bloom coincided with both high daily irradiance and reduced mixing during a period of reduced fresh water river flows and neap tides. A transition period dominated by flagellates and ciliates was recorded following the spring diatom bloom collapse.

A bi-weekly sampling programme was undertaken from May to September 2000 at 3 sites along the estuary to investigate the spatial distribution of surface phytoplankton in relation to variations in salinity and nutrient concentrations. These surveys indicated that phytoplankton community composition markedly changed along the estuary with diatoms being abundant at all stations whereas dinoflagellates were more abundant at the mid and upper estuary sites. The autotrophic ciliate *Mesodinium rubrum* was very abundant in the middle part of the estuary where estuarine conditions were optimal for its growth with moderate salinity stratification and intermediate nutrient concentrations. A more detailed picture of surface phytoplankton spatial distribution throughout the estuarine system was obtained from three one-day extensive sampling surveys conducted in June and July 1999 and August 2000.

The experimental laboratory work conducted in this research gave an insight into the factors controlling the species succession in three regions of the estuary; with surface water samples incubated under nonlimiting irradiance conditions for up to 2 weeks. Results from these incubation experiments indicated that phytoplankton populations are mainly light-limited rather than nutrient-limited although the nitrate-N:phosphate-P ratio can also influence phytoplankton growth and biomass yield within the estuary. At the outer estuary site, immediately following the spring bloom, nutrient uptake ratios in incubated samples indicated silicate and phosphate limitation. Results also indicated that diatoms, particularly small-celled species out-competed other organisms (eg dinoflagellates) and can grow for longer periods even under a degree of apparent P-limitation. These results using natural populations were supported by results from growth experiments conducted using two phytoplankton isolates grown in batch cultures; a diatom *Thalassiosira rotula* and a dinoflagellate *Prorocentrum micans*. In addition, it was demonstrated that the dinoflagellate had a much slower growth rate in comparison with the diatom irrespective of differences in nutrient availability and N:P supply ratio.
The HPLC pigment method was used in parallel with microscopic observations to characterise phytoplankton populations in the estuary. Results indicated that the HPLC pigment method is a useful and less-time consuming technique than microscopic enumeration and gave a reliable indication of dominant groups from the presence and ratios of indicator pigments. Results obtained throughout this research project are discussed in relation to factors controlling phytoplankton growth and bloom initiation in the Southampton Water estuarine system.

ACKNOWLEDGEMENTS

Well, coming to this moment, it is my chance then to thank everyone who has helped me during my studies in the UK, I apology in advance to any one I fail to mention. We say that a PhD is our own work but there are many who have helped and contributed to this thesis by giving their expertise, useful advice and encouraging me by their moral support and friendship.

First and above all great thanks to *"ALMIGHTY ALLAH"* for giving his blessing, support and strength to achieve this work and make it possible.

I am indebted to my supervisor Dr. Duncan Purdie for his guidance throughout this project and for his continuous help and valuable advice. My grateful thanks are extended to the Egyptian Government and the University of Suez Canal for the generous financial support and giving the chance to be among the SOC scientist team.

Enormous thank you to a number of people who have helped greatly during the initial phases in this project and my first days entering the British community. I'd like also to thank Mr. S. Hayward for his kind help that he gave to me as "an overseas". I'd specially like also to thank Russell and Alex for their help during phytoplankton identification process that make this "hard" work much easier. My thanks extend to S. Holley and Dr. D. Hydes for their help allowing access to the auto-analyser and data from TRITON and their help during the Monitor calibration. Thanks also go to Dr. C. Lucas for her help and useful discussions during the HPLC analysis. Thanks to Grame, Matt and the late Mike for their help during sample collection and to K. Davis for providing the map of the study area.

Next, a special thanks to Prof. P. Tyler and Prof. P. Holligan, members of the advisory panel, for their support and the encouraging words that always kept me going forward. Great thanks also to Prof. G. Imam, Prof. A. El-Gazzar (University of Suez Canal, Egypt) for their great help that I could not deny and to Prof. S. Shaapan (University of Mansoura, Egypt) for awakening my interest in "phytoplankton" during the MSc time. Thanks also to my PhD buds, at the SOC, of whom I've had the great fortune of knowing in the last few years and sharing lots of work, meetings, and fun. Thanks particularly to my office mate Beth, Magali, Ben and the "laboratory group", Sinhue, Silvia and Arantza and also to Ana Paula Tales. A special thank you goes to my dear friend Hala Ayad for her lovely friendship and moral support.

Last, *but not least*, my sincere and very special thank you to my beloved husband **"*Islam*"** for his constant love and for being a shoulder to lean on during the hard moments and for providing a LIGHT at the end of the tunnel. "BIG" thanks goes to my wonderful son "Omar" for his lovely smile that is always encouraging me and helps to keep myself going and also for being patient and very understandable. Very special and grateful thanks go to my affectionate PARENTS for their endless love and support and for every thing (lots) they have done for me. My gratitude is extended to my lovely sisters, Wafaa and Lamyaa, my brother, Ahmed, and my dear niece Nosa- you all have a big place in my heart. Thanks also to my dear friends in Egypt; Salwa, Ola, Sahar, Fadila, Lobna, and my cousins Khalid, Rania, Amany and Omima for their care and sincere friendship. A very special thank you goes to my youngest but dearest friend **"*TonTon*"** for her care and for being always in touch by phone calls and mobile messages- you are very special to me. Thanks also go to my dear Dr. H. Auf and Dr. M. Taha for their moral support

"At the end, but not ending, I am indebted to my late grandfather "Hajj Mahmoud A. Mahmoud" for his moral support and his believe in my scientific efficiency. I dedicate any progress in my life to his memory"

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GLOSSARY

SAMPLING SITES

Test Estuary

EL	Eling
BB	Bury
SG6	Swinging Ground No. 6
CR	Cracknore
GY	Gymp Elbow
HK	Hythe Knock

Itchen Estuary

IB	Itchen Bridge
NB	Northern Bridge
OS	Oil Spill
SW1	Swinging Ground No. 1

Southampton Water

WS	Western Shelf
NWN	NW Netley
HO	Hound
GL	Green Land
HP	Hamble point
RE	Reach
CA	Calshot

PIGMENTS

Chl *a*	chlorophyll *a*
T Chl *a*	total chlorophyll *a*
Chl *b*	chlorophyll *b*
Chl *c3*	chlorophyll *c3*
Chl *c1+c2*	chlorophyll *c1+c2*
Peri	peridinin
Fuc	fucoxanthin
Allo	alloxanthin
Zea	zeathanthin
Diad	diadinoxanthin
Viol	violaxanthin
Bras	brasinoxanthin
19-Hex	19'hexanoyloxyfucoxanthin
19-But	19'butanoyloxyfucoxanthin

EXPERIMENTS

Chapter 5

UE	culture of water samples collected from upper estuary (SG6)
ME	culture of water samples collected from mid estuary (NWN)
LE	culture of water samples collected from lower estuary (Calshot)

Chapter 6

Combination I	16 μM N:0.1 μM P
Combination II	16 μM N:1 μM P
Combination III	16 μM N:5 μM P

OTHERS

J days	Julian days
PAR	photosynthetic available radiation
SPM	suspended particulate matter

CHAPTER ONE

CHAPTER ONE

1. INTRODUCTION

1.1 GENETRAL INTRODUCTION

1.1.1 ESTUARIES AS PRODUCTIVE ECOSYSTEMS

Estuaries are considered to be the main transition zones between the fresh water of the land surface and the salt water of the oceans. Cameron & Pritchard (1963) produced the following definition "An estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage".

Estuaries have generally been regarded as the most productive of the aquatic systems supporting fisheries of commercial value. Estuarine ecosystems are of interest to marine science because of their high primary production which provides a continuous food supply and shelter for many organisms which feed and live within them. The nutrient supply from freshwater inputs is clearly important in sustaining this high rate of primary production. However, Simpson et al. (1991) argued that the biological response to the seasonal pattern of mixing, aperiodic fluctuation in river discharge, and the tidal variability seems to be difficult to predict in estuarine environments. Various authors (e.g. Smith & Hollibaugh, 1993) have estimated an average primary production in estuaries and a value of 190 g C m^{-2} yr^{-1} has been suggested intermediate between the 100 g C m^{-2} yr^{-1} reported for coastal areas and

the 300 g C m^{-2} yr^{-1} estimated for the up-welling areas (Ryther, 1963). This estimated average for estuaries excludes macrophyte production which may produce approximately the same carbon (Heip et al., 1995). These high rates of primary production in estuaries have a major influence on other features of the estuarine water column (e.g. nutrient concentrations, light attenuation) (Heip et al., 1995). Estimates of annual rates of primary production suggest Southampton Water as a reasonably productive estuary (annual of 177 g C m^{-2}; Iriarte & Purdie, 1994), although much less productive than the most productive estuaries: for example, Great South Bay and Puget Sound have annual rates of about 450 g C m^{-2} and 465 g C m^{-2}, respectively (see also table 1.1).

1.1.2 FACTORS CONTROLLING PHYTOPLANKTON PRODUCTION AND BLOOM INITIATION IN ESTUARIES:

Generally, coastal environments can be differentiated into two types of ecosystems on the basis of physical and hydrographic properties: enclosed coastal ecosystems (ECE) and open coastal ecosystems (OCE) (Cloern, 1996). In open coastal waters the extent of phytoplankton growth can be limited by the availability of both nutrients and light (Tett & Walne, 1995), however production in estuaries tends to be limited mainly by light and physical processes (Fichez et al., 1992) due to the high nutrient concentrations. In ECE tidal oscillations can generate substantial turbulence, which in turn restricts phytoplankton growth (Cloern, 1991; Monbet, 1992). The restricted depth in ECE commonly generates substantial water column turbidity which may be an important control of phytoplankton dynamics (Cloern, 1987).

Maximum phytoplankton growth, biomass and rates of production can occur in different estuaries at different times of the year depending on local environmental conditions. It appears that variations in phytoplankton growth and annual production in estuaries are mainly due to different climatological conditions (e.g. the amount of rainfall and level of surface incident irradiance), as well as various physical, chemical, and biological factors (Heip et al., 1995) (see figure 1.1).

WATER TEMPERATURE

Water temperature is considered to be one of the controlling factors influencing phytoplankton growth and production. Water temperature can reflect the changes in weather in the estuarine environment (Wright et al., 1997), however, only small ranges of temperature occur within estuaries in a given day (Dyer, 1973). Phytoplankton responses to changes in temperature vary from one local community to another, and changes in temperature can also influence the species distribution and abundance in coastal waters (M'harzi et al., 1998) and estuaries (Boynton et al., 1982)

Table 1.1. Estimates of annual production (g C m^{-2} yr^{-1}) and chlorophyll biomass for some estuaries in Europe and North America. Data adapted from Heip et al. (1995); Buzzelli et al. (1999). *Chlorophyll *a* values obtained from Monbet (1992); Boynton et al. (1982).

Estuary		Tidal Range	Annual Production	Anuual Chl-a Range	References
NORTH AMERICA					
Fourleague Bay	upper		322		Randall & Day (1987)
	inner		514		
	inner		317		
Tomales Bay	inner		70		Cole (1989)
	central		420		
	outer		460		
	average		400		
***Narragansett Bay**		<2 m		7-8	
Hudson River	freshwater		70-240	~2	Cole et al.(1992)
Hudson Estuary	outer bay		200		
Delaware Estuary	inner		105		Malone (1977)
	central		296		
	outer		344		
	average		307		
Chesapeake Bay	upper			*~25	
	average	<1 m	34-569	*10-20	
	average		185		Malone et al. (1988)
					Smith and Kemp (1995)
San Francisco Bay	Soyth Bay		27-162		Cole & Cloern (1984)
	San Paplo Bay		13-318		
	Suisan Bay		6-418		
***San Francisco Bay**	South Bay	~2 m	130	8-9	
	North Bay	~2 m	90		Jessby et al.(1993)
	San Paplo Bay		100		
	Suisan Bay		44		
Peconic Bay	inner	<1 m	213	<5	Bruno et al.(1980)
	middle		177		
Great South Bay			450		
Puget Sound			465		
EUROPE					
English channel	Bay of Somme		600		Loquet et al., (2000)
***Eastern Scheldt Estuary**		3-4 m		~8	
***Western Scheldt Estuary**		~4		6-8	
Bristol Channel	inner		7		Joint & Pomroy (1981)
	central		49		
	outer		165		
Ems-Dollard	inner		70		Colijn (1983)
	central		91		
	outer		283		
***Swansea Bay**		6.8 m		~ 2	
Westerschelde	inner		122		Van Spendonk et al. (1993)
	central		197		
	outer		212		
***Morlaix River Estuary**		5.7 m		2-7	
Westerschelde	fresh water		388		Kromkamp & Peene (1995)
	inner		122		
	central		184		
***Bay of Brest**		3-4 m		~ 3	
***Loire estuary**		3-4 m		2-4	
***Sein estuary**	outer	>5 m	230	5-50	
Oosterschelde	inner		301		Wetsteyn & Kromkamp (1994)
	central		312		
	outer		382		
Wadden Sea	eastern + western	~ 2 m		9-10	
Southampton Water	middle		177	1.5-73	Kifle & Purdie (1993)
	outer		130	0.7-17	Kifle & Purdie (1993)
	outer			<9	Iriarte & Purdie (1994)
	average of 6 stations		123		Bryan, 1979

Roden (1994) positively correlated higher averaged chlorophyll content in coastal waters in Ireland with warm water, however it was noted by Balch (1981) that chlorophyll-a was positively associated with low temperature in the Gulf of Maine. In estuaries Boynton et al. (1982) concluded that maximum phytoplankton production mostly occurs during warm periods. Also, nutrient recycling processes and consequently planktonic growth rates are strongly influenced by temperature-regulated metabolism as observed by Nixon (1981). These effects are attributed to the fact that temperature may affect the time taken for cells to adapt themselves to variations in irradiance (Jorgensen, 1968).

SALINITY

It is often assumed that phytoplankton species composition changes markedly along the estuarine gradient, mainly according to salinity changes. In general, freshwater phytoplankton dominate the upper (limnic) regions of an estuary (Heip et al., 1995). In a study conducted in three European estuaries during spring 1993 (Muylaert & Sabbe, 1999), phytoplankton were sampled from; the Elbe (Germany), the Schelde, (Belgium) and the Gironde Estuary (France), of which the later receives a high river runoff. It was noticed that phytoplankton species composition was different among the three studied estuaries: in the high salinity (polyhaline) reaches of the Elbe and Schelde phytoplankton communities were comparable, as they were mainly characterised by the diatom *Skeletonema costatum* and several *Thalassiosira* spp. (Muylaert & Sabbe, 1999) in the oligohaline regions of the Schelde Estuary however, the halophilous diatom *Cyclotella maneghiniana* dominated phytoplankton populations and the diatom *Stephanodiscus hantzschii* was dominant in the limnetic regions of the Elbe Estuary (Muylaert & Sabbe, 1999).

LIGHT

Pennock & Sharp (1986) and Van Spaendonk et al. (1993) assumed that primary production generally increases towards the mouth of an estuary and not necessary associated with the maximum availability of nutrients, indicating that the decrease in nutrients is more than compensated for by the increased water transparency. Thus, changes in productivity and spatio-temporal changes in phytoplankton biomass in estuaries reflect the modulation of light availability in the water column (Cloern, 1987).

Phytoplankton primary production was positively correlated with the depth of the euphotic zone (1% incident light) in three European microtidal estuaries; The Elb, (Germany), The Westerscelde (Belgium) and The Gironde (France) (Goosen et al., 1999). Similarly, most macrotidal nutrient-rich estuaries in Western Europe have a relatively low phytoplankton biomass because of light limitation (Nedwell et al., 1999; Middelburg & Nieuwenhuize, 2000a). It is evident that phytoplankton are

primarily light-limited in the turbid, nutrient-rich oligohaline zone of estuaries (e.g. see Boynton et al., 1982; Wofsy, 1983; Cebrian & Valiela, 1999; Nedwell et al., 2002; Kocum et al., 2002a).

Smayda (1959) reported that phytoplankton prefer to live and grow with solar energy above 0.09 g cal. cm^{-2} d^{-1} (7.38×10^{-3} E $m^{-2}d^{-1}$) at the surface. A critical mean light level of about 40 g $cal.cm^{-2}d^{-1}$ (i.e. 3.28 E $m^{-2}d^{-1}$) was firstly suggested by Riley (Riley 1957; Riley 1967) and modified as a daily mean of 20.9 W m^{-2} (Labry et al., 2001) for a pronounced increase in phytoplankton growth rate and for a bloom to begin. A threshold of 100-200 W h m^{-2} day^{-1} mean water column irradiance was previously suggested by Jahnke (1989), Peperzak (1993) and Peperzak et al. (1993) for balanced phytoplankton growth with a lower threshold of 50 W h m^{-2} day^{-1} suggested by (Riley, 1957) for diatoms.

TURBIDITY

The turbidity of the water column is known to be an important factor influencing phytoplankton biomass especially in estuarine and coastal water systems. Changes in turbidity and, in turn, light availability markedly influence the species distribution and abundance (M'harzi et al., 1998). Maximum concentrations of total suspended matter (TSM) are usually recorded in the turbidity maximum zone at the fresh water saline interface at the head of the estuary (Goosen et al., 1999). In highly turbid estuaries primary production tends to peak in summer during the time of reduced rainfall and maximum irradiance (Cole & Cloern 1984; Randall & Day, 1987).

A minimum in phytoplankton abundance and biomass, with different species composition, was observed in the Gironde Estuary (France) compared with the Elbe (Germany) and the Schelde (Belgium) estuaries by Muylaert & Sabbe (1999) during spring 1993. This finding was attributed to a combination of high turbidity and high river runoff in the Gironde Estuary (Muylaert & Sabbe, 1999). Similar findings were found in the Bristol Channel, with primary production of 7g C $m^{-2}yr^{-1}$ in inner turbid areas, where the photic zone was 0.5m (Joint & Pomroy, 1981) compared to 165 g $Cm^{-2}yr^{-1}$ in the clearer zone where the photic depth was 10 m. The development of the turbidity maximum is a permanent process controlled mainly by tidal action in macrotidal estuaries (Salomon & Allen, 1983, cited in Monbet, 1992).

RIVERINE INPUT/FLUSHING RATE

Fresh water input creates a vertically stratified water column with a shallow mixed layer, and can therefore improve the light conditions and initiate phytoplankton blooms in estuaries (Cloern, 1989).

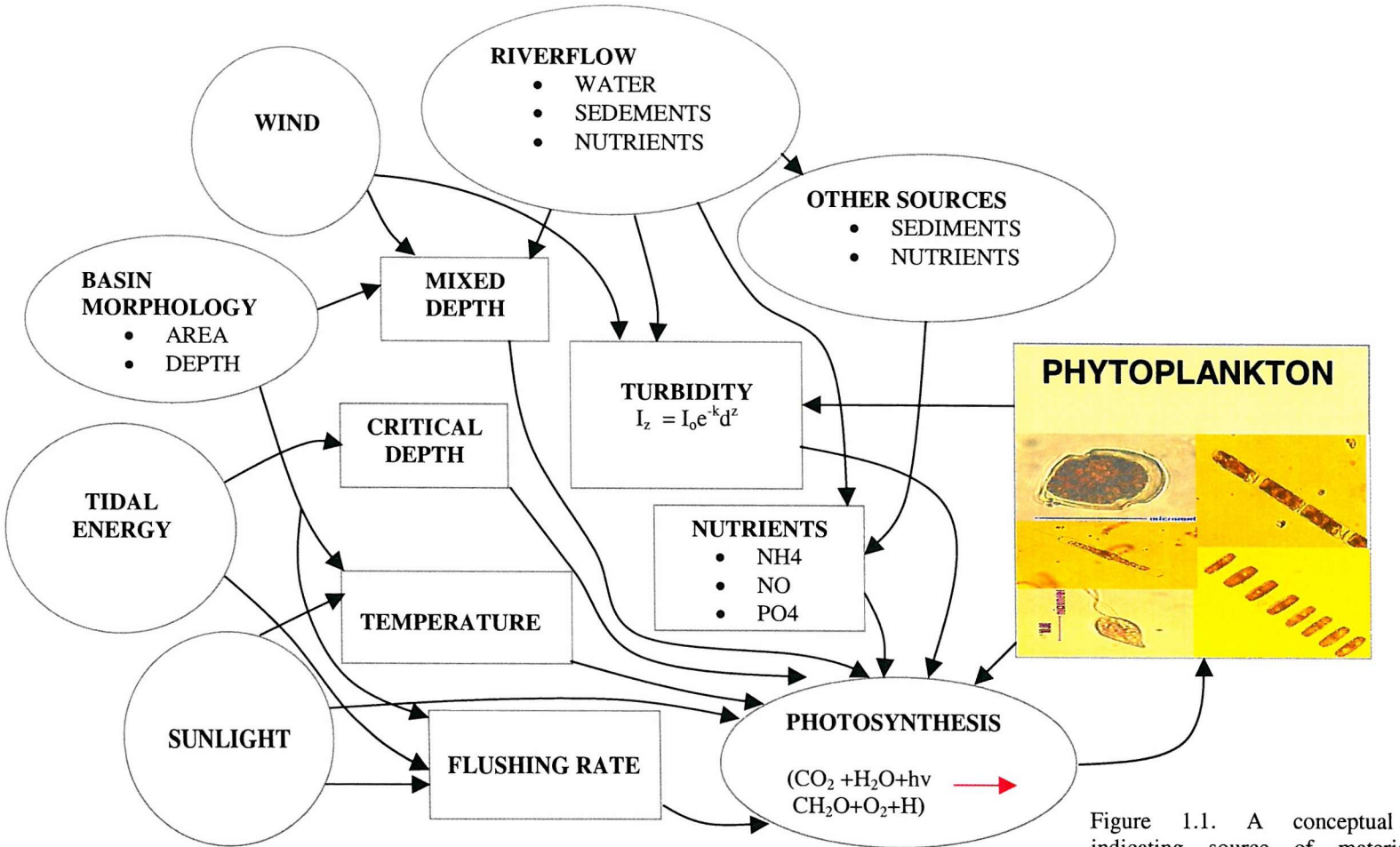


Figure 1.1. A conceptual model indicating source of materials and mechanisms influencing phytoplankton growth and production rates in estuarine ecosystems. Modified by Boynton et al. (1982).

Freshwater input can be the major source of nutrients and, as such, be responsible for stimulation of phytoplankton growth (Fisher et al., 1988; Fichez et al., 1992; Revilla et al., 2000). Despite these promoted effects, freshwater can have a negative effect on phytoplankton growth in estuaries, creating a salinity gradient causing osmotic stress (Kromkamp & Peene, 1995), affecting water residence time (Relexans et al., 1988; Nedwell et al., 1999) as well as flushing rate (Muylaert & Sabbe, 1999). In addition, riverine inputs can import large quantities of total suspended matter (TSM), thereby creating a turbid environment (Kromkamp et al., 1995).

Although, nutrient load has a great impact on an estuary, its residence time, which indicated by the fresh-water flushing time (Monbet, 1992; Nedwell et al., 1999) within the estuary, is also important. It was previously shown that phytoplankton biomass within an estuary can be related, to some extent, to its flushing time (Monbet, 1992; Nedwell et al., 1999). Even if a large nutrient load is received by an estuary, it may not have a great impact if flushed rapidly out of the estuary (Nedwell et al., 2002). A negative relationship has been estimated for example, between phytoplankton biomass (estimated as chlorophyll-a) and river flow rate in the Gamtoos estuary (South Africa) when the flow rate exceeded 1.2 $m^3 s^{-1}$ (Snow et al., 2000).

SPRING / NEAP TIDAL CYCLE

The spring-neap tidal cycle has been identified as an important variable for phytoplankton growth in estuaries by many authors, but its effect differs over time and region (Winter et al., 1975; Balch, 1981; Harris, 1986). Sylaios & Boxall (1998) observed that tidal effects appear to be much more important than river flow discharge and wind shear stress for the longitudinal-vertical distribution of physical variables. The tidal prism, or range and associated processes (e.g., tidal mixing, current velocity, light penetration and sediment re-suspension) influence phytoplankton biomass development in some estuaries. As noticed by Monbet (1992) tidal mixing of the water column appears to be one of the factors responsible for the differing responses of phytoplankton populations to nutrient input observed in estuaries. Monbet (1992) has also shown that the average concentration of Chl *a* within an estuary is a function of the tidal range.

Differences in the stability of the water column accompanying neap and spring tides greatly influence the distribution and abundance of the phytoplankton community. Timing of the bloom could be related to the spring-neap tidal cycle; which varies both seasonally and regionally. In general, bloom events coincide with the neap tides as it provides the calm physical conditions that promote the development of a bloom; however increased tidal mixing during spring tides, and consequently the

reduction in stratification are generally accompanied by a reduction in the standing crop of estuarine phytoplankton (Monbet, 1992).

Spring blooms have been found to be correlated to neap tides in coastal waters and estuaries: for example in Puget Sound (Winter et al., 1975), on the coast of Connemara (Roden, 1994) and in Southampton Water (Wright et al., 1997; Hydes & Wright, 1999) when reduced tidal currents resulted in stratification. However, phytoplankton blooms sometimes coincided with the spring tide, as recorded by Roden (1994) on the coast of Connemara in late summer (wih a tidal range of 5.2 m height) as well as in the coastal waters of Maine (Balch, 1981). (Balch, 1981) related this phenomenon to either the increased nutrients or to the upward movement of a subsurface chlorophyll layer.

WATER MIXING

According to Ryther (1963) reduced water mixing and stratification are the main drivers for the onset of phytoplankton blooms, particularly in temperate zones. Vertical mixing, as a result of tidal force, wind stress and/or fresh water runoff, has been shown to have marked influences on phytoplankton dynamics in the macrotidal estuary, Southampton Water (Kifle & Purdie, 1993; Wright et al., 1997, Lauria, 1998). Vertical mixing indirectly affects the phytoplankton population through light fluctuation (Falkowski, 1980; Koseff et al., 1993). Therefore, when vertical mixing is persistent, as is the case in macrotidal estuaries (Allen et al., 1980), the phytoplankton cells experience the mean environmental conditions (Falkowski, 1980), which in turn influences phytoplankton biomass and species composition (Uncles & Joint, 1983).Vertical stratification is an important requirement for bloom onset, as it reduces water mixing [a prerequisite for a bloom to develop and maintain (Cloern, 1996)] and grazing pressure (by preventing or reducing benthic consumption). Vertical mixing also affects the transport of heat, salt, nutrients and plankton. The water column may be stratified for a long enough period for a bloom to develop, offsetting the effect of both tidal and wind stirring (Cloern, 1996). The timing of phytoplankton blooms in many temperate estuaries is mainly regulated by water column stability (Smayda, 1983; Pennock, 1985; Wright et al., 1997). Species composition of phytoplankton blooms varies both regionally and seasonally, in accordance with the state of water column stability and stratification. For example, flagellate species, in general, tend to dominate stratified waters but diatoms are mainly found in more turbulent waters (see figure 1.2), relying on the increased kinetic energy (KE) to prevent sinking from the photic zone during stable periods (Lauria et al., 1999). Reduced turbulence greatly increases diatom sinking rates (Titman & Kilham, 1976; Margalef, 1978) (figure 1.2). Conversely dinoflagellates aggregate in surface waters during slack periods (Lauria et al., 1999) although they are able to maintain themselves in the water column

(Lannergren, 1979) and also to migrate vertically to optimum nutrient and light conditions (Margalef, 1978).

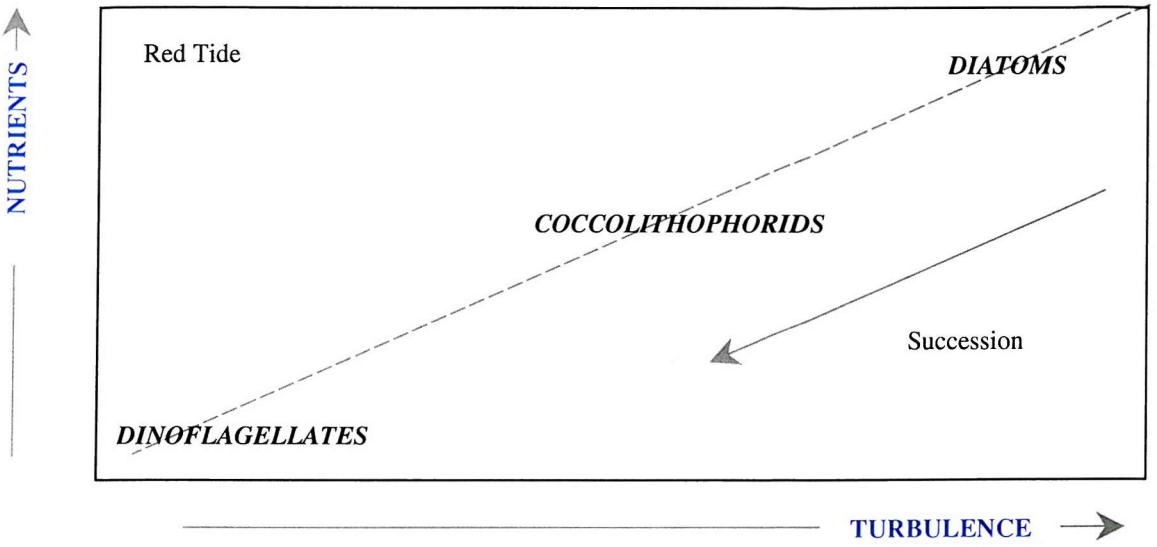


Figure 1.2. A schematic representation of the conceptual model of phytoplankton community abundance and succession on the basis of two environmental factors (nutrient supply and turbulent intensity) as proposed by Margalef (1978).

NUTRIENTS

Inorganic nutrients, such as phosphate (P), nitrate (N), and silicate (Si) are key factors influencing structure and biomass of the phytoplankton community in aquatic environments. It is often assumed that marine and estuarine phytoplankton are nitrogen-limited, whereas freshwater phytoplankton are phosphorus-limited (e.g. Hecky & Kilham, 1988). Phosphorus has been suggested to be limiting in marine, coastal (Howarth, 1988; Thingstad et al., 1993) and estuarine (Fisher et al., 1992; Pennock & Sharp, 1994; Holmboe et al., 1999; Yin et al., 2000; Labry et al., 2002; Nedwell et al., 2002) waters if large nutrient loads reach the coastal waters accompanied by a high N:P ratios. However, nitrogen (or nitrogen + phosphorus) limitation is associated with periods of low river runoff with balanced N:P ratios of sea water (Fisher et al., 1992). A higher availability of N and P in coastal waters would increase utilization of Si as recorded for the Baltic Sea for over 20 yr (Wulff & Rahm, 1988). This, in turn, would lead to decreased Si in water and increased P:Si and N:Si ratios (Carlsson & Graneli, 1999). Nitrogen (N) and phosphorus (P) loads are anthropogenically elevated in many European estuaries (Nedwell et al., 2002) and regulate the coastal phytoplankton biomass in spring before it becomes dependent on regenerated nutrients (Dugdale & Goering, 1967). Silica (Si) loads are relatively independent of anthropogenic influences (Hessen, 1999) and its seasonal variations mainly

result from variations in biological removal more than seasonal variations of fluvial loads (Fichez et al., 1992; Balls et al., 1995). Silica (Si) is always abundant in coastal waters in early spring but removed after the spring bloom by diatoms (e.g. Justic et al., 1995; Del Amo, 1997) which lead to Si-limitation (Del Amo, 1997; Underwood & Kromkamp, 1999). Thereafter, the phytoplankton community becomes dominated by microflagellates, which do not require Si (Justic et al., 1995); e.g. *Phaeocystis* (Prymnesiophyceae) (Reid et al., 1990; Peperzak et al., 1993). Potential nutrient limitation in UK estuaries suggested to be in the order P>Si>N (in decreasing order of limitation) (Nedwell, et al., 2002).

Data derived from an intensive study by Monbet (1992) included 40 estuarine systems (microtidal and macrotidal estuaries) demonstrated a correlation between both summer maximum values of chlorophyll a and primary production and the annual input of nutrients (particularly nitrogen). A similar correlation was reported by Boynton et al. (1982) for Chesapeake Bay. In experiments to study the responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments, Pinckney et al. (1999) found that the highest community growth rates occurred under high nitrate levels accompanied with calm conditions.

GRAZING

Grazing pressure is also an important factor controlling phytoplankton standing crops and community size structure in estuaries. The degree to which phytoplankton populations 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 reported to be a major process controlling phytoplankton biomass in Narragansett Bay (Deason & Smayda, 1982) and in Delaware Estuary (Pennock & Sharp, 1986). Differences in phytoplankton species composition are assumed to represent a considerable difference in the feeding conditions for zooplankton (M'harzi et al., 1998). In a study to characterise the grazing link between phytoplankton and zooplankton in the Gironde estuary, Sautour et al. (2000) estimated that, on average, 26% of the total primary production was grazed daily by mesozooplankton. However much higher pressure exerted by microzooplankton on the primary production was estimated. The role of benthic filter feeding animals on standing crop of phytoplankton in estuaries is not well documented. However, results from a model study conducted to investigate processes governing phytoplankton blooms in estuaries through the local production-loss balance (Lucas, et al., 1999) indicated that the highest rates of phytoplankton population growth are found in the shallowest regions of the estuary under conditions of high turbidity and slow benthic grazing. On the other hand, with low turbidity and rapid benthic grazing the highest growth rates occur in the deeper areas (Lucas, et al., 1999).

1.1.3 DEVELOPMENT OF PHYTOPLANKTON BLOOMS IN ESTUARIES

The exact conditions and mechanisms promoting phytoplankton bloom initiation, proliferation and persistence are poorly understood (Margalef, 1978; Glibert et al., 1995); however they are highly linked to environmental conditions (Pinckney et al., 1997). The timing and location of phytoplankton blooms and the level of biomass achieved, result from interaction of physical (e.g. aggregation, dissipation, advection, and mixing), biological (organism's behaviour, physiological state, life style and tolerance for environmental variables such as nutrients, light, temperature, salinity) and chemical factors (e.g. nutrient availability) (M'harzi et al., 1998; Revilla et al., 2000; Liu et al., 2001). Maximum phytoplankton biomass occurs in different estuaries at different times of the year depending on local environmental conditions. In a study in the Lower St. Lawrence Estuary (Zakardjian et al., 2000), the phytoplankton bloom typically did not occur until early summer (late June-July) although the environmental conditions (stratification, surface light, and nutrients) were favourable in May. Zakardjian et al. (2000) reported that the possible explanations for the lateness of the phytoplankton bloom include flushing of the surface layer due to the spring freshwater runoff, loss of phytoplankton cells from the thin euphotic layer through sinking and mixing, and also temperature limitation of phytoplankton growth rates. It is evident that phytoplankton enhanced growth (bloom formation), distribution and community structure within the Belgium coastal zone of the North Sea (M'harzi et al., 1998) and in the Urdaibai Estuary (Revilla et al., 2000) markedly changes in response to differences in environmental factors (such as salinity, temperature, tidal flushing and turbidity). Moreover, these differences could be due to a combination of the above-mentioned factors along with a number of other factors such as the level of nutrients and pollution associated with the freshwater input. Inorganic nutrient availability is one of the main drivers of phytoplankton growth and abundance. For example, the shortage in Si compared to the highly available N and P in coastal waters can lead to a succession from diatom-dominated community to non-Si- requiring taxa (e.g. flagellates) (Cadee & Hegeman, 1991; Conley & Malone 1992; Egge & Aksnes, 1992). Prymnesiophyceae (e.g. *Phaeocystis* sp.) tend to bloom after the spring diatom peak when Si is depleted (Reid et al., 1990; Peperzak et al., 1993), since these organisms cannot compete with diatoms for N and P but do not require Si. Nutrient availability and competitive ability of different phytoplankton species lead to continuous changes in community composition (Tilman, 1977; Sommer, 1983), which may be termed "succession" (Smyda, 1980). The light climate (combination of depth, turbidity and the amount of solar radiation) is thought to be the decisive factor for the timing of the spring bloom in the Dutch coastal waters (Vries et al., 1998). This bloom occurred 1-2 months later than in offshore areas of the southern Bight (Joint & Pomroy, 1992) due to reduced light conditions (Vries et al., 1998).

Moreover, the formation and spatial distribution of phytoplankton blooms in estuaries are controlled by local mechanisms (production-loss balance for the water column) which is a combination of water column depth, turbidity, grazing rates (Lucas et al., 1999a) and transport-related mechanisms (i.e. variations in spatial transport of water and plankton) which in turn affect bloom transport and patchiness (Lucas et al., 1999b)

1.1.4 CONTINUOUS MONITORING OF PHYTOPLANKTON

The logistics of frequent sampling in the spatial dimension is less demanding than those of frequent sampling in biological oceanography over long periods of time (Roden, 1994). Consequently, fine scale spatial data are more often reported than long sequences of frequent temporal measurements made at a fixed location. Harris (1986), however, has pointed out the danger of creating false trends in data series by too infrequently sampling, and has suggested that much shorter-term variability may be missed during oceanographic investigations. Being aware of this possibility has led several scientists to organize more frequent sampling programmes in different parts of the world (e.g. Balch, 1981; Roden, 1984; Sournia et al., 1987; Roden et al., 1987; Roden, 1994). Consequently, the study of plankton bloom dynamics requires sustained and frequent sampling.

Most recent studies satisfy these requirements; for example, coastal sites at Helgoland (Gillbricht, 1988; Radach et al., 1990) and the Marsdiep (Cadee & Hegemann, 1986; Cadee. 1992) have been sampled regularly and frequently for several decades. *In situ* fluorometers were moored at 2 sites in the North Sea during the spring of 1989 (Mills et al., 1994) providing daily estimates of chlorophyll-a concentration in the surface mixed layer. Similarly, during the spring phytoplankton bloom of 1996, a continuously monitoring fluorometer was deployed in the Humber plume (Allen et al., 1998). Continuously monitored data are useful to study short-time variations in environmental factors that drive phytoplankton growth and bloom initiation in the Southampton Water Estuary and also to give a qualitative and quantitative identification of phytoplankton bloom development and the time of higher growth (Holley & Hydes, 2002). Recent observations were made using a data buoy deployed in Southampton Water in 1996 (Wright et al., 1997), to study the short time changes in phytoplankton development in relation to nutrient input and changes in the spring/neap tidal cycle. These continuous data identified a correlation between the spring-neap tidal cycle and the timing of the phytoplankton blooms in Southampton Water (Wright et al., 1997; Hydes & Wright, 1999). A further monitoring system known as "The Ferry-Box" has been fixed in a mobile ferry to monitor the changes and the development of phytoplankton blooms along the estuary (Holley & Hydes, 2002).

1.1.5 PIGMENT CHEMOTAXONOMY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Due to the rapid changes occurring in environmental conditions that may have pronounced effects on phytoplankton dynamics and community structure in estuarine and coastal waters, relevant temporal and spatial scales need to be sampled (Cloern, 1996) for accurate and reliable determination of the composition of natural phytoplankton communities. Microscopic analysis is the most accurate technique to enumerate individual species in a mixed natural sample, but it is a time consuming and tedious technique if many samples are analyzed (Millie et al., 1993). In addition, an accurate identification of phytoplankton species requires a high level of expertise (Breton et al., 2000). The high performance liquid chromatography (HPLC) technique is now recognized as a powerful method in oceanography (Mantoura & Llewellyn, 1983; Wright et al., 1991; Jeffrey et al., 1997), for analysis of phytoplankton pigments and their degradation products allowing the abundance of dominant organisms in mixed assemblages to be evaluated. Chemotaxonomic analysis of pigments requires only a short time and gives reproducible results, however, the only limitation to this method is that the cell pigment content and ratios to chlorophyll-a change according to environmental conditions and species composition (Schluter et al., 2000).

Previous studies of HPLC pigment analyses in different aquatic environments, in the ocean (Higgins & Mackey, 2000; Trees et al., 2000), in lakes (Descy et al., 2000 Trees et al., 2000), estuaries and in coastal waters (Pinckney et al., 1998; Brunet et al., 1996a; Breton et al., 2000; Trees et al., 2000; Ansotegui et al., 2001) as well as in the Antarctic environment (Rodriguez et al., 2002; Garibotti et al., 2003) verify the validity of phytoplankton pigments, at a qualitative level, as reliable biomarkers for phytoplankton community composition.

Phytoplankton accessory pigments, analyzed by HPLC technique can be used as quantitative biomarkers of some classes and/or species and provide information on changing phytoplankton dynamics and natural community structure (Barlow et al., 1997, 1998; Breton et al., 2000; Pinckney et al., 2001) as well as the estimation of the decomposition and grazing processes (e.g. Quiblier-Lloberas et al., 1994). The HPLC method also allows the quantification of small phytoplankton cells (<5 μm) which may be underestimated or uncertain in the microscopic counts (Rodriguez et al., 2002) and/or cells with membranes of low visibility, for example, small picoplanktonic green algae (Breton et al., 2000). However, the interaction of pigments using HPLC is not always clear and should be carefully contrasted and validated with microscopic observations. For example, some classes have different pigment signatures, e.g. Prymnesiophyceae showing four different pigments

(Jeffrey & Wright, 1994). Moreover, some biomarker pigments are present in several classes and can lead to erroneous indications (Breton et al., 2000; Rodriguez et al., 2002). For example, chlorophyll c3 (Chl *c3*) and 19'-hexanoyloxyfucoxanthin (19-Hex), which are pigment biomarkers of *Phaeocystis* (Claustre et al., 1990; Jeffrey & Wright, 1994), are also found in some 19-Hex containing-coccolithophords (e.g. *Emiliana huxleyi*) (Jeffrey & Wright, 1994) and not all Prymnesiophyceae contain Chl *c3* (Stauber & Jeffrey, 1988). Similarly, alloxanthin, the major biomarker of Cryptophytes (Jeffrey et al., 1999) is found in the autotrophic ciliate *Mesodinium rubrum* (Hibberd, 1977, Jeffrey & Vesk, 1997) and the dinoflagellate *Dinophysis norvegica* (Meyer-harms & Pollehne, 1998). This is due to endosymbiosis (Gieskes & Kraay, 1983), which may vary the pigment signature of the host by changing the pattern of the symbiont pigment pattern (Ansotegui et al., 2001).

The biomarker pigment/ Chl *a* ratio is another indicator for phytoplankton community composition and can be used to indicate the presence and dominance of various classes and/or species, including minor pigments (Everitt et al., 1990; Letelier et al., 1993) and small-sized phytoplankton species (Rodriguez et al., 2002). For example, high fucoxanthin/ Chl *a* is frequently associated with diatom blooms (Vesk & Jeffrey, 1987; Jeffrey & Wright, 1994), and elevated peridinin/ Chl *a* ratio indicates the presence of autotrophic dinoflagellates (Jeffrey, 1974; Rodriguez et al., 2002). However these ratio(s) should be carefully applied, as the marker pigment/ Chl *a* ratio are highly influenced by environmental factors (Geider et al., 1993), such as irradiance (Falkowski & La Roche, 1991; Brunet et al., 1996a & b) nutrient limitation (Latasa & Berdalet, 1994), physiological status of the cells (Brunet et al., 1996b), variations of pigment content between members among a single class (Zapata & Garrido, 1997) and/or between strains among a single species (Bidigare et al., 1996). Moreover the concentration of the biomarker pigment may vary from one local phytoplankton community to another (Andersen et al., 1996; Breton et al., 2000).

1.2 THESIS INTRODUCTION

1.2.1 DESCRIPTION OF THE STUDY AREA

Southampton Water (Hampshire, Southern England) is an approximately 10 km long and 2 km wide north-westerly extension of the Solent (Figure 1.3). Near the entrance to Southampton Water the water depth is of the order 12 to 13 m with a deep water channel dredged to a depth of more than 10 m below chart datum extending to above Southampton Docks. Southampton Water is a partially mixed estuary bordered by intertidal mudflats with shingle and sand on the eastern side, and a salt marsh to the west (Howard et al., 1995). In such a partially–mixed estuary, the specific tidal

intermittency and the rapid tidal currents allow stratification and vertical mixing to occur over short timescale (i.e. a few hours). Southampton Water exhibits a semi–diurnal, asymmetric cycle of rapid ebb currents which can often be double the velocity of the flood (Webber, 1980).

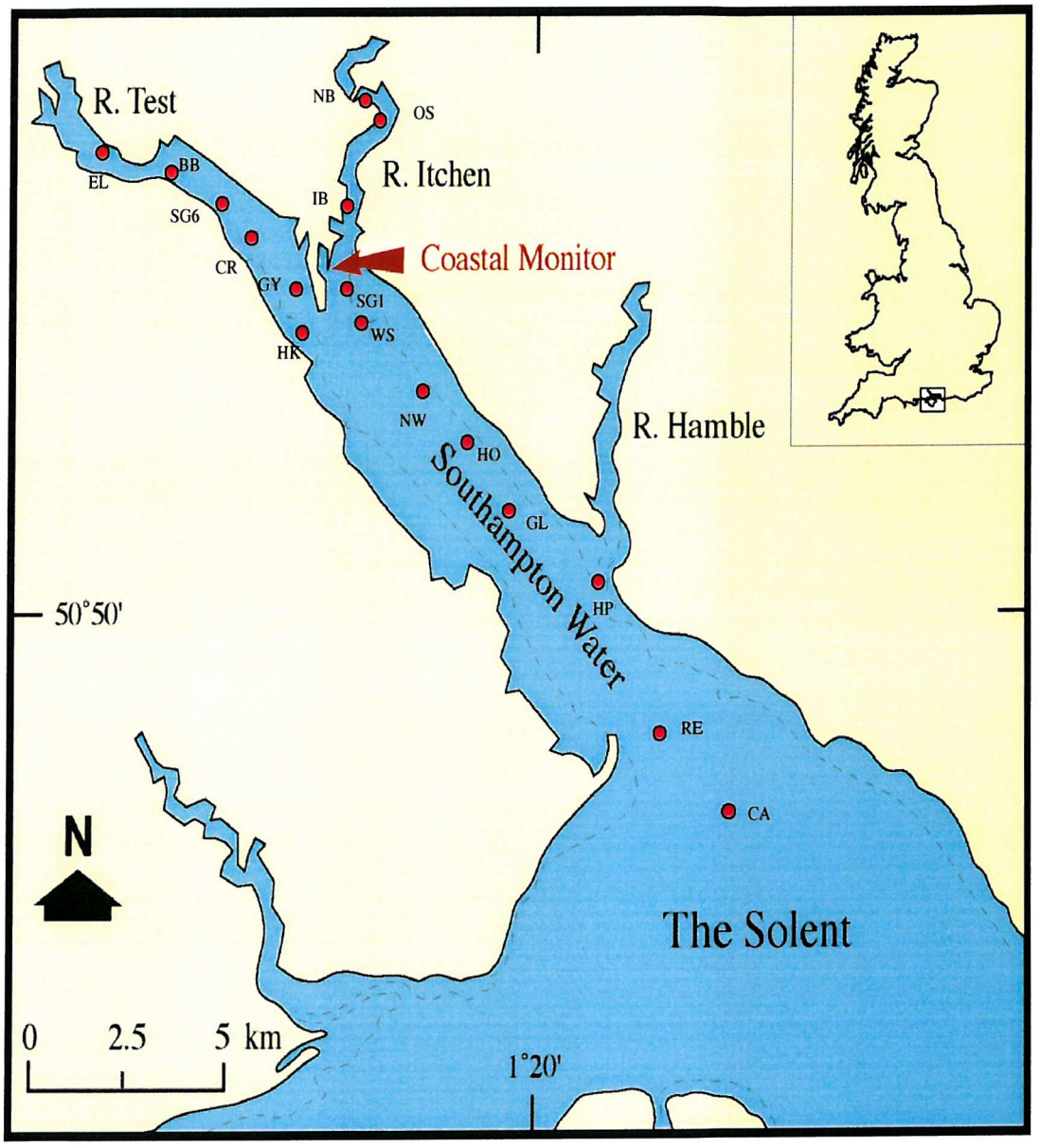


Figure 1.3 Map of the study area showing the position of the sampling sites throughout Itchen estuary, Test estuary and Southampton Water.

1- EL = Eling
2- BB = Bury
3- SG6 = Swinging ground 6
4- CR = Cracknore
5- GY = Gymp Elbow
6- HK = Hythe Knock
7- NB = Northern Bridge
8- OS = Oil Spill
9- IB = Itchen Bridge
10- SG1 = Swinging ground 1
11- WS = Western Shelf
12- NW = North West Netley
13- HO = Hound
14- GL = Greenland
15- HP = Hamble Point
16- RE = Reach
17- CA = Calshot

Though Southampton Water may be referred to as an estuary, much of it is marine in character with high salinity. According to Raymont (1972), at high water the entrance showed high salinities throughout the water column exceeding 34 and the relatively high salinities extend far up the estuary with values > 31. Macrotidal estuaries (mean tidal range >2m) generally exhibit a tolerance to pollution derived nitrogen-containing nutrients despite high loading originating from freshwater outflow. In temperate regions, climatic variations lead to large seasonal changes in biological activity in the water column (Howard & Apte, 1989).

Considering Southampton Water as a temperate macrotidal estuary, tidal activity influence phytoplankton biomass by influencing the light climate, which is less favourable in macrotidal estuaries. Because of this, macrotidal estuaries do not always show a clear dependence on nitrogen loading, but may become light-limited (Heip et al., 1995). Southampton Water has an unusual tidal pattern, first described by Airy (1843) and the phenomenon of a double high water period, 2h apart and a tidal excursion of up to 2.5 km (Webber, 1980), is well known (MaCmillan, 1964). One benefit of this tidal pattern is the fact that water samples can be taken from a large area of the estuary at a nearly constant state of the tide, i.e. close to high water.

1.2.2 SOUTHAMPTON WATER AS A SPECIAL ESTUARINE ENVIRONMENT

1.2.2.1 FLUSHING RATE AND NUTRIENT SUPPLY IN SOUTHAMPTON WATER

Southampton water is part of a complex and highly populated estuarine system, receiving water from the rivers Test and Itchen with a mean annual discharge of 8.81 and 3.26 $m^{-3}s^{-1}$ respectively (Howard et al., 1995; Sylaios & Boxall, 1998). Nitrate concentrations in both rivers are relatively similar and show similar seasonal variations with maximum levels in winter during periods of high flow (Hydes & Wright, 1999). A steady and progressive increase in nitrate has taken place over the last two decades (Hydes & Wright, 1999) to approximate values of 422 μM and 393 μM during 1990-1997 in the Test and Itchen, respectively. The other two nitrogen compounds (ammonia and nitrite) that may be assimilated by algae are similar in both rivers with relatively low concentrations of values of 7 μM and 4 μM for ammonia and nitrite, respectively. Higher concentrations of phosphate have been suggested (from 1974 to 1997) in the Itchen River (up to 20 μM) than that in the Test (less than 10 μM) with a reverse pattern, in both rivers, to that recorded for nitrate (Hydes & Wright, 1999), i.e. higher levels of phosphate tend to be associated with periods of low flow during the summer season. According to Phillips (1980) salinity structure along Southampton Water, in general, is dependent on

the seasonal cycle of fresh water flow as well as on the tidal state. All nutrients, particularly nitrate, phosphate and silicate, appear to behave conservatively within the estuary decreasing in spring and summer as the flow rate and, in turn, freshwater input decrease and re-established during winter months. Removal of nutrients within the main body of Southampton Water occurs for all nutrients but it is only detectable in spring and summer months at the time of intense bloom conditions as suggested by Hydes & Wright (1999).

1.2.2.2 TIDAL FORCING AND ASSOCIATED PROCESSES IN SOUTHAMPTON WATER

Southampton Water is assumed to have a unique current structure along the whole estuary based on the estuarine geometry (i.e. it is narrow, rectilinear and shallow). The net sedimentation rate for Southampton Water is reported to range from 2 to 6 mm yr^{-1} (Dyer, 1973). Longitudinal current velocities show large vertical and lateral variations in Southampton Water, reaching high velocities of up to100 cm s^{-1} in bottom waters (Dyer, 1973). Typical current velocities range between 0.1-0.6 cm s^{-1} (Dyer, 1973; Crawford & Purdie, 1992; Lauria, 1998). During a neap tide in Southampton Water, maximum ebb velocities can reach values of 45 cm s^{-1} and the ebb period lasts for only 3 hours (Lauria, 1998).

Variations in the neap-spring tidal cycle in Southampton Water, which affects the residence time of water in the estuary, were observed to influence the blooms of phytoplankton (Kifle & Purdie 1993) and salinity structure (Lauria, 1998). The water column stability plays an important role in the development of the summer *Mesodinium rubrum* bloom. Values of chlorophyll-a ranging from 1-100 mg m^{-3} have been reported (Crawford, 1992) in Southampton Water, stratification is only temporary and does not seem to exist as a permanent phenomenon for phytoplankton increase (Kifle & Purdie, 1993; Lauria, 1998).

Southampton Water, as an estuarine environment, is a system with high input of energy (i.e. fresh water flow, tidal currents) and is potentially subject to frequent periods of stabilisation and destabilisation of the water column. This alternation between mixed and stratified conditions is known to promote primary production in several estuarine systems (Legendre, 1981). Southampton Water has comparatively low levels of suspended particulate matter (SPM) (Xiong, 2000) with maximum value of less than 100 mg l^{-1} in the outer part (up to 84 mg l^{-1}) of the estuary. Lower levels of SPM were measured during spring/summer period increasing towards winter when the river flow is high and re-suspension of sediment caused by wind occurred (Xiong, 2000).

1.2.2.3 SEASONAL CYCLES OF PHYTOPLANKTON GROWTH AND BLOOM EVENTS IN SOUTHAMPTON WATER

Southampton Water has been documented as experiencing annual algal blooms in both spring and summer (Wright et al., 1997). Phytoplankton species in Southampton Water seem to show a unique pattern over the spring and summer months (table 1.2) as described by Kifle (1992). Southampton Water experiences annual algal blooms during the spring–summer period (Kifle & Purdie, 1993; Wright et al., 1997), with diatom blooms in spring and dinoflagellate blooms in mid to late summer (Kifle, 1992). Red-tide events are known to occur mainly due to the autotrophic ciliate, *Mesodinium rubrum,* as previously noted in Southampton Water by Soulsby et al. (1984), Kifle (1992), Crawford (1992) and Lauria (1998). This ciliate dominates red tide events within the estuary with a chlorophyll *a* maximum value of ~100 mg m^{-3} (Crawford, 1992). A bloom of the relatively small prymnesiophycean, *Phaeocystis* spp., which can form nuisance blooms (Riegman et al., 1993) was recorded towards the mouth of the estuary (Iriarte, 1991) in May-June 1990. The shift from a diatom dominated community to one dominated by non-silicated algae, particularly flagellates (eg. *Phaeocystis*) (Cadee & Hegemann, 1991) is explained by the hypothesis of decreasing Si:N and/or Si:P ratios (Smayda, 1990; Sommer, 1994). Table 1.3 summarizes the dominant phytoplankton species in Southampton Water from studies conducted from 1973 to 1996. Southampton Water shows fluctuations in algal biomass and chlorophyll *a* concentration as well as species diversity (Iriarte, 1991; Kifle, 1992, Crawford, 1992; Lauria, 1998). These fluctuations occur in response to different external factors. Higher chlorophyll biomass is generally recorded at upper (estuarine) sites and decreases towards the mouth of the estuary. The down estuary decrease in chlorophyll-a (Bryan, 1979; Kifle, 1992; Antai, 1989; Lauria, 1998) is mainly associated with the seaward increase in the rate of exchange of estuarine and Solent water (Lauria, 1998).

Table 1.2. Phytoplankton species pattern in Southampton Water

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

Table 1.3. Summary of chlorophyll- a concentration, phytoplankton biomass and dominant phytoplankton species in Southampton Water from 1973 to 2002.

Time of survey	Location	Chl-a mgm^{-3}	primary Production (mg C $m^{-2}d^{-1}$)	Dominant species	Reference
June, 1973	Netley (surface)	26	ND	*Scrippsiella trochoidea*	Diwan, 1978
,,	,, (5 m)	27		*Prorocentrum* spp.	
July, 1973	Calshot (surface)	13		*Gonyaulax spinifera*	
,,	,, (5 m)	13			
February, 1974	Calshot (1m)	3	ND	*S. costatum; Navicula spp*	Burkill, 1978
May, 1974	Calshot (1m)	4		*Odontella spp; Astrionella*	
Augst, 1974	Calshot (1m)	6		*Predinium* spp; *Gonyaulax*	
				Prorocentrum spp.	
July / August 1974	Inner	44.8 / 130	499 / 2404	*ND*	Bryan, 1979
	Mid	166 / 25.6	565 / 2066		
	Outer	14.9 / 20	1025 / 318		
	Lower Test	22.4 / 596	403 / 3174		
	Solent	14.4 / 46.5	335 / 682		
May, 1985	Netley (Mean)	ND	ND	*Mesodinium rubrum*	Crawford, 1992
June, 1985	Netley (Mean)	ND			
May, 1986	Calshot (1m)	27	ND	*S. costatum; M. rubrum*	Leakey, 1986
Augst, 1986	Netley (1m)	74			
May, 1987	Netley (0 - 1m)	39	ND	*Guinardia delicatula*	Antai, 1989
Augst, 1987	Netley (0 - 1m)	50		*M. rubrum*	
May, 1988	Netley (0 - 1m)	10		*G. delicatula*	
Augst, 1988	Netley (0 - 1m)	< 5			
May 12, 1988	Netley (1m)	12	ND	*G. delicatula ; E. marina*	Kifle, 1992
June 17, 1988	Netley (1m)	50		*S. trochoidea ; M. rubrum*	
June 27, 1988	Netley (1m)	73		*Chaetoceros spp.*	
June 30, 1988	Netley (1m)	36		***S. costatum*	
5 & 12 May1988	Calshot (1m)	~17			
May, 1990	Netley (surface)	11	ND	**Phaeocystis; Chaetoceros*	Iriarte, 1991
Early Aug., 1990	Netley (surface)	~19		*M. rubrum*	
Late April, 1990	Calshot (surface)	~16			
May. 1992	Netley (surface)	22	ND	*G. delicatula ; Phaeocystis*	Anning, 1995
May, 1993	Netley (surface)	15		*G. delicatula ; Phaeocystis*	
June - July 1992	Upper Test	> 40***	ND	*S. costatum ; R. dlicatula;*	Proenca, 1994
May 1, 1992	NW Netely	> 20***		*Chaetoceros spp.*	
May 1, 1992	Calshot	> 20***			
July, 1993	Calshot	5.46	991	*ND*	Hirst, 1996
	Hamble	10.92	2144		
	NW Netely	13.26	2088		
	Cracknore	33.93	4465		
	Bury Buoy	58.9	8996		
Late August, 1994	Netley (surface)	> 50	ND	*M. rubrum*	Ryan, 1994
Early August, 1996	transect along	11		*Asterionella japonica; Gyrosigma* sp;	Lauria, 1998
	the esturay			*Prorocentrum micans; M. rubrum*	

ND = not determined
* The first year in which a bloom of *Phaeocystis* sp. was reported in Southampton Water
** Appearently dominated in early April (12 April 1988)
*** Chlorophyll a measured using HPLC method

1.3 AIMS AND OBJECTIVES

Phytoplankton play an important role in the trophic dynamics and the bio-chemical cycling of estuarine ecosystems. To understand their relative contribution to estuarine processes a detailed description of phytoplankton distribution, growth and production need to be accurately quantified in relation to the surrounding environmental variables.

The research program described in this thesis is concerned with investigating the spatial and temporal variability of the phytoplankton community in the Southampton Water Estuary, and relating the changes in the community structure and distribution to environmental variables. The factors controlling phytoplankton growth, in particular, will also be investigated to determine which factors (light or/and nutrients) influence bloom initiation in this macrotidal estuary at different times of the year. A number of previous studies of phytoplankton have been conducted in the estuary, however in this study one of the aims was to set a more frequent sampling program in order to improve our knowledge of the ecosystem dynamics in Southampton Water. The present work proposes for the first time in Southampton Water Estuary to use continuous data (every 10 minutes) obtained from a fixed coastal monitor (CLM-2) together with a frequent discrete water sampling pogramme. From April 1999 to September 2001 the Southampton Water Estuary was sampled with four different sampling programmes. Measurements of phytoplankton community structure were conducted quantitatively in terms of biomass (carbon), chlorophyll-a (fluorometrically), and accessory pigments (HPLC method). Changes in phytoplankton populations in Southampton Water was analysed using pigment signatures (HPLC method) as well as microscopic counts to determine the phytoplankton composition and species succession. Analysing the phytoplankton community composition of the samples collected over this period in relation to the environmental variables, together with some laboratory culture work, forms the central basis of this research.

The specific objectives of the research were:

(1) To describe the temporal variation in phytoplankton community structure and species succession at a fixed site using a combination of continuous data and discrete water samples.

(2) To determine the spatial distribution of phytoplankton species throughout Southampton Water, the Test and the Itchen estuaries in one-day surveys undertaken during the productive period of the year.

(3) To describe the changes in phytoplankton community composition in different regions of the estuary over the spring/summer period in relation to changes in environmental variables.

(4) To determine whether light and/or nutrients are the controlling factor that influences the growth of phytoplankton in different regions of the estuary and determine the degree to which a bloom can develop if supplied with sufficient irradiance. Also to compare changes occurring in phytoplankton biomass and community structure at these sites with that occurred in the incubated sea water.

(5) To investigate the optimal nutrient conditions for phytoplankton growth in Southampton Water for two different isolated species, a diatom (*Thalassiosira rotula)* and a dinoflagellate (*Prorocentrum micans).*

1.4 THESIS STRUCTURE

Based on the literature reviewed in **chapter 1,** a plan for this research was proposed to investigate the spatial and temporal variability of phytoplankton community in the Southampton Water Estuary, and relating the changes in their community structure and distribution to environmental variables (see flowchart, figure 1.4). Short-time changes in phytoplankton biomass and seasonal succession in relation to environmental factors are investigated in **chapter 3** through the application of a continuous coastal monitor at a fixed position in the estuary. The spatial distribution of nutrients and chlorophyll *a* along with species changes throughout the estuary are analyzed in **chapter 4** during three one-day surveys conducted in 1999 and 2000. Contrasts between nutrients and irradiance as growth promoting factors for phytoplankton growth are analyzed in **chapter 5** through a series (four) of incubation experiments using the natural phytoplankton community. In **chapter 6** the results from two parallel sets of growth experiments are presented using two isolated microalgae known to be abundant in the estuary. Spatial (4 one-day surveys in 1999 & 2000) and temporal (one site from April-September 1999 & 3 sites from May-August 2000) changes in phytoplankton community composition are investigated in **chapter 7** through the application of high performance liquid chromatography (HPLC) pigment analysis. Methods used for analysing all parameters are included in **chapter 2.**

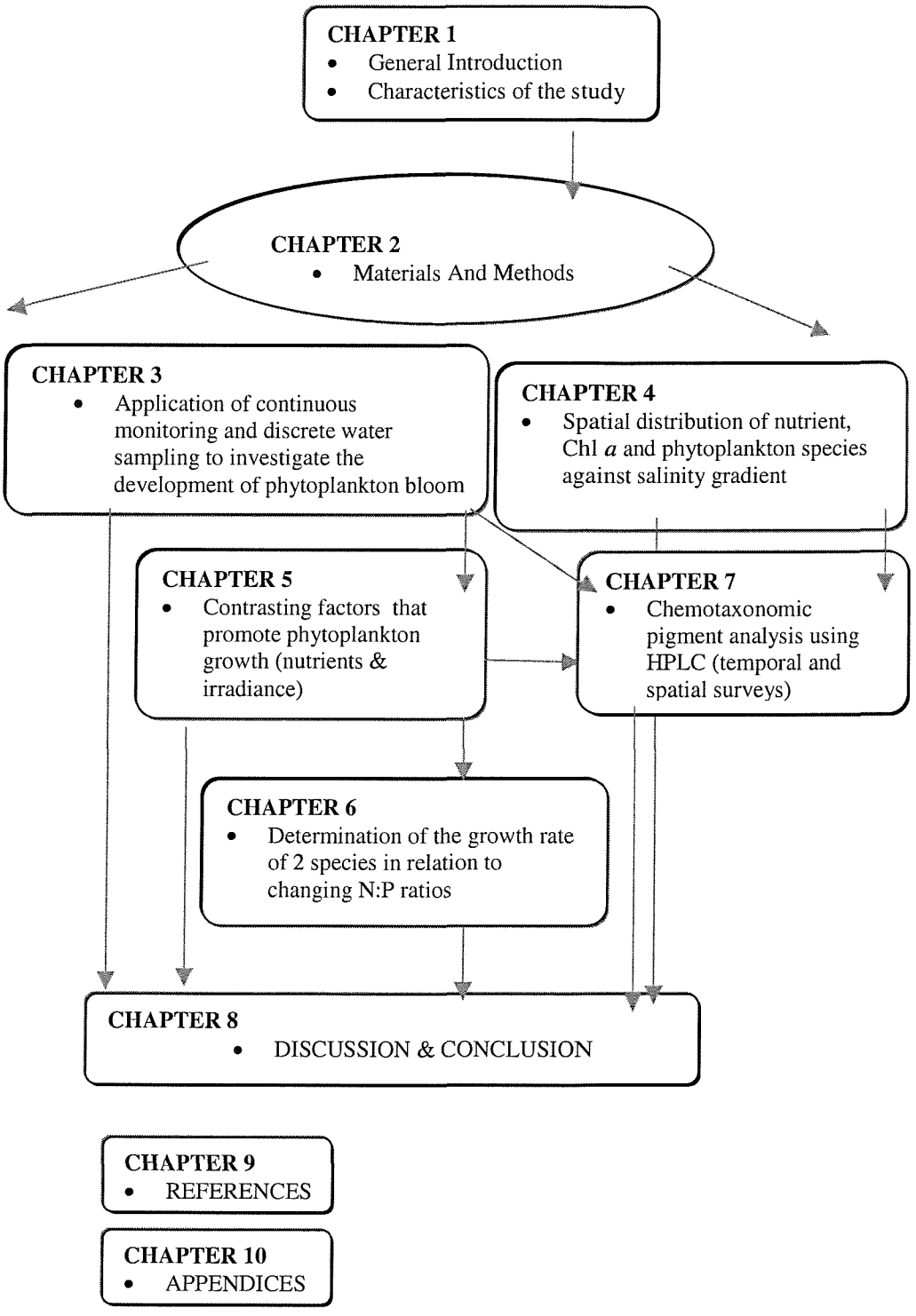


Figure 1.4.

Schematic representation of the thesis structure

CHAPTER TWO

CHAPTER TWO

2- MATERIALS AND METHODS

2.1. SAMPLING STRATEGY

The following sampling programme was undertaken in Southampton Water during the period of this study (1999 to 2001): An intensive-sampling program to monitor the short term variations in environmental variables including nutrients, irradiance as well as phytoplankton pigments and abundance at one position in the estuary was carried out during spring-summer, 1999. Surface water samples were collected at approximately weekly intervals from the dock wall at the Dock-Mooring site (close to the entrance to Empress Dock) from April to September 1999.

Four one day spatial surveys were conducted during the productive period (spring-summer) in 1999 (10^{th} June and 22^{nd} July) and 2000 (16^{th} May and 15^{th} August) covering the whole estuary (14 - 17 sites) including the lower Itchen estuary, the Test estuary and Southampton Water using the University of Southampton research vessel Bill Conway. Water samples were collected from 3 different depths (surface, middle and bottom) at each station and analysed for phytoplankton abundance, pigment analysis and nutrients to determine the spatial variability of these parameters throughout the estuary.

In 2000, surface water samples were collected from three sites; SG6 (upper estuary), NW Netley (middle estuary) and Calshot (lower estuary or coastal water) at approximately 2 weekly intervals from May to August using the Bill Conway to investigate changes in phytoplankton abundance and nutrient concentrations. Water samples (surface) were also collected from these sites on four dates for incubation experiments conducted in the laboratory to investigate the effect of non-limiting light conditions on phytoplankton development and nutrient uptake.

Surface water samples were collected from the estuary to isolate several diatom (during the diatom bloom) and dinoflagellate species (during the dinoflagellate bloom) in 2000/2001 for laboratory-based culture investigations.

2.2 FIELD MEASUREMENTS

2.2.1 CONDUCTIVITY TEMPERATURE DEPTH SENSOR (CTD)

A Falmouth CTD unit with a Chelsea Instruments Aquatracka III fluorometer and a Seatech 25 cm pathlength transmissometer was used to obtain vertical profiles of temperature, salinity, chlorophyll fluorescence and transparency of water during the estuarine surveys conducted in 1999 and 2000. The Falmouth Scientific CTD unit has an inductive conductivity sensor, a titanium pressure sensor with a range of 0-2000 psi and a pressure protected stabilised thermistor (glass) with a range of -2 to 32 ^{0}C.

Water samples were collected (from 3 depths) with 1.5 litre Niskin bottles attached to the CTD rosette. A simple basic programme was used to average the CTD data into 1 metre bins. Values of fluorescence were also obtained from the CTD as voltage values, which were then converted to calibrated chlorophyll values using an equation derived from the linear relationship (see Appendix I) between the CTD fluorometer voltage values and the fluorometric measured discrete chlorophyll values on the same sampling date. The slope of the given regression line was used to convert the CTD chlorophyll voltage output into quantitative chlorophyll values using the chlorophyll values measured for the three depths collected from each site. This conversion provides a real quantitative chlorophyll profile at each sampling site (see figure 4.3b, 4.14b & 4.26) rather than having chlorophyll concentration for just 3 depths (surface, middle and bottom).

2.2.2 SALINITY MEASUREMENTS

A high precision Guildline salinometer was used to measure the salinity of discrete surface water samples collected from the Dock Mooring site between April-September 1999. Salinity measurements were made by Susan Holley. Salinity measurements of water samples collected

during the estuarine surveys conducted between May and September 2000 were made using a WTW Salinity probe.

2.2.3 SURFACE INCIDENT LIGHT

Daily global irradiance data was obtained from the meteorological office via the British Atmosphere Data Centre web site (*www.badc.nerc.ac.uk/*). Hourly total surface irradiance data ($W.h.m^{-2}$) is available from 2 monitoring stations near the Southampton Water Estuary; Everton (50.74°, 1.57°, approximately 10 miles west of the estuary) and Thorney Island (50.81°, -0.92°, approximately 20 miles east of the estuary). Hourly values were summed for each day and converted to photosynthetically available radiation (PAR) by multiplying by 0.46 and expressed as $W.h.m^2.d^{-1}$. Mean water column daily irradiance was calculated using the equation of Riley (1957) as given in Peperzak (1993).

$$\mathbf{I_m = I_0 (1\text{-}e^{-k.z}) / k.z}$$

Where, I_m is the mean water column irradiance, $\mathbf{I_0}$ is the surface water irradiance, k is the attenuation coefficient (m^{-1}) and z is the water depth (m).

When calculating the mean water column irradiance, a 4-day running mean was used to smooth the large day to day changes in irradiance levels as recommended by Peperzak et al (1993)

2.2.4 WATER COLUMN IRRADIANCE MEASUREMENTS

Vertical irradiance profiles were obtained using a submersible Li-Cor sensor and data logger. The cosine-corrected sensor allows measurement of irradiance flux densities through a given plane. Irradiance values were recorded on the data logger from the underwater sensor (I_z) at 1 metre intervals throughout the water column and at the same time from the surface sensor (I_0). The light attenuation coefficient (k) was calculated from a plot of $\ln(I_0/I_z)$ against depth (z) and the slope of the line estimated by linear regression. The 1% light depth was calculated using the following equation:

$$\mathbf{\ln(1) = \ln(100) - kz}$$

Where k = attenuation coefficient and z = 1% light depth

2.2.5 CLM-2 - CONTINUOUS COASTAL MONITOR

A coastal monitor (CLM-2, supplied by WS Ocean System Ltd.) was placed at the entrance to the Empress Dock during 1998. This monitor has a sensor array designed to provide continuous (every 10 minutes) physical, biological and meteorological measurements. The meteorological sensors were sited on the dockside and the water sensors mounted onto a single unit, the CLM-2 monitor held at depth (fixed point) in a holding tube. Sensor data are communicated back to the

Southampton Oceanography Centre in real time. These data were saved on the TRITON server at the SOC. The coastal monitor is shown in figure 2.1.
The turbidity sensor (supplied by Seapoint Sensor Inc.) has an 880 nm light emitting diode and measures the scattered light from the presence of particulates with a range from 25 to 500 Formazin Turbidity Unit (FTU), with a noise of < 1mV and linearity of < 2% deviation.

The fluorometer sensor (supplied by Chelsea Instruments) measures red light emitted by chlorophyll and similar organic molecules when irradiated with blue light. The sensor has a high resolution for the assessment of phytoplankton biomass and monitoring primary production in fresh and marine waters.

Calibration of the CLM-2 - Coastal Monitor

A calibration procedure for the coastal monitor sensor was needed to confirm the sensor readings and to convert the voltage output from the fluorometer and transmissometer sensors into quantitative values. Between April and September 1999 a regular weekly sampling programme was conducted during which the sensor probe was removed from the water and any fouling cleaned from optical windows and the salinity sensor. A previous deployment of a similar salinity sensor on a mooring in Southampton Water had suggested drift in the salinity signal was due to fouling (Hydes and Wright, 1999). The sensor unit was then placed in a large plastic container containing surface collected estuarine water from adjacent to the sensor unit and left for 30 minutes covered in black plastic.

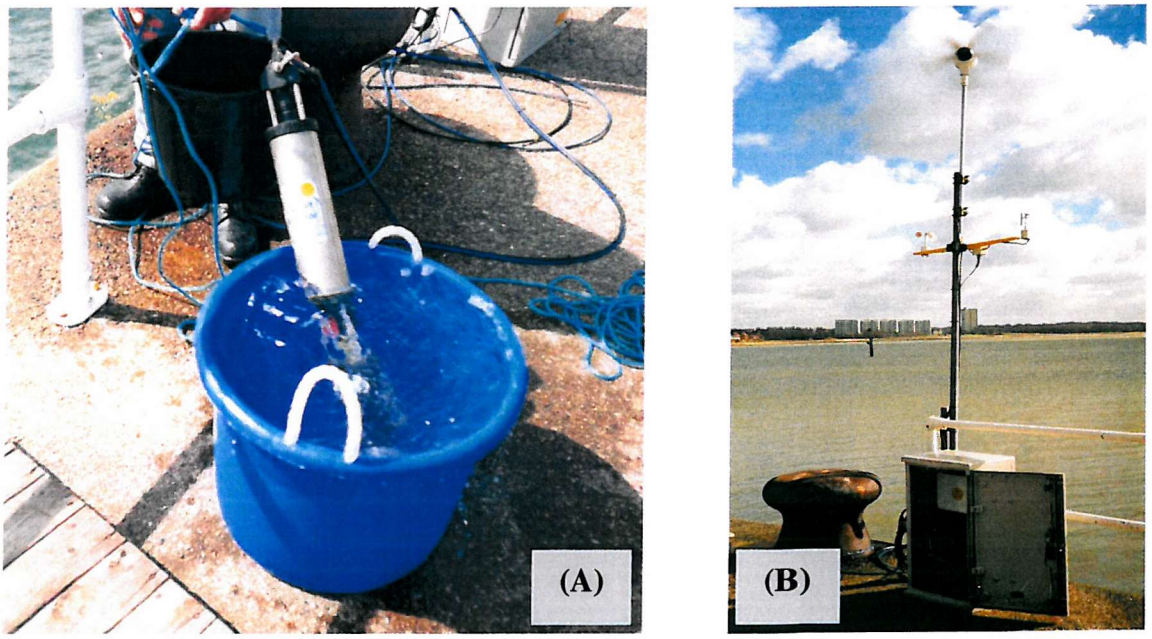


Figure 2.1. A picture of the coastal monitor (CLM-2) in Southampton Water at the entrance to Empress Dock showing: the seawater sensors (A) during the calibration procedure and the meteorological sensors (B).

SALINITY SENSOR

The salinity values given by the WS Ocean System software were compared with discrete measurements made on surface water samples collected from the plastic container using a Guildline salinometer (see above). A plot of sensor salinity values against surface samples is shown in figure 2.2 and indicates a good correlation ($r = 0.99$, $p < 0.001$) allowing conversion of all sensor data to absolute values of salinity.

CHLOROPHYLL SENSOR

The equation given below was available for converting the sensor fluorescence readings to chlorophyll *a* supplied by the WS Ocean System:

$$\textbf{Chlorophyll a} = (f_l * (5/65535)) - 1.071) * 25.46$$

Where f_l is the fluorometer reading

Using this formula, peak concentrations of chlorophyll were around 80 mg m^{-3} compared to maximum values of 20 mg m^{-3} from the weekly chlorophyll calibration procedure. These high values are unrealistic and confirm the need for a calibration procedure against weekly collected samples. A plot showing chlorophyll fluorescence as recorded by the coastal monitor against discrete measured values (surface water) during the sampling period (April-September) is presented in figure 2.3. Some scatter is seen in this relationship with reasonable correlation coefficient ($r = 0.87$, $p < 0.01$).

TRANSMISSOMETER CALIBRATION

The attenuation coefficient (k) determined from water column light profiles (section 2.2.3) were compared to mean daily turbidity values measured by the transmissometer. These variables showed a good linear fit (figure 2.4) and a regression relationship was determined ($r = 0.77$, $p < 0.01$) allowing the daily values of turbidity from the Dock Mooring sensor to be transformed into daily values of k for the period April-September 1999.

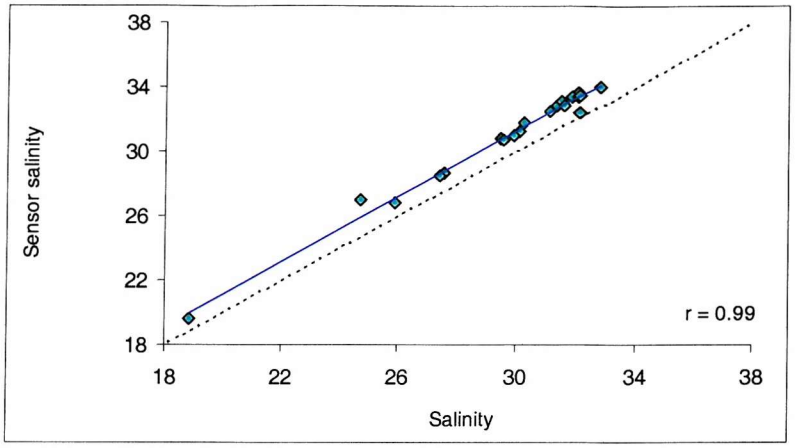


Figure 2.2. Plot of salinity values as recorded by the coastal monitor against discrete measured values (surface water) during the sampling period (April-September 1999)

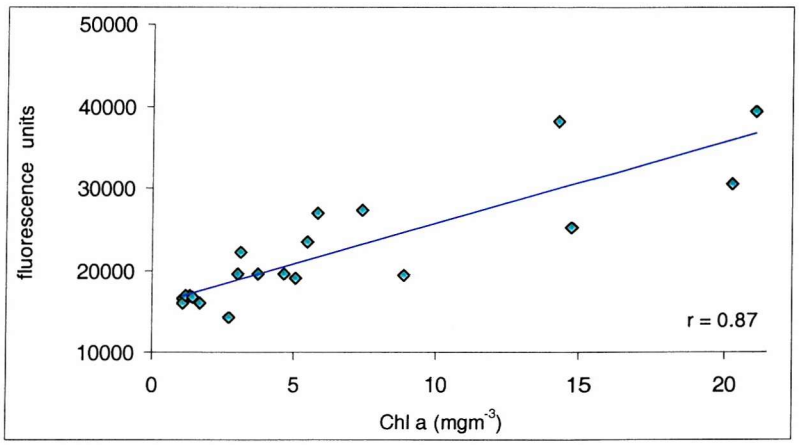


Figure 2.3. Plot of chlorophyll fluorescence as recorded by the coastal monitor against discrete measured values (surface water) during the sampling period (April-September 1999).

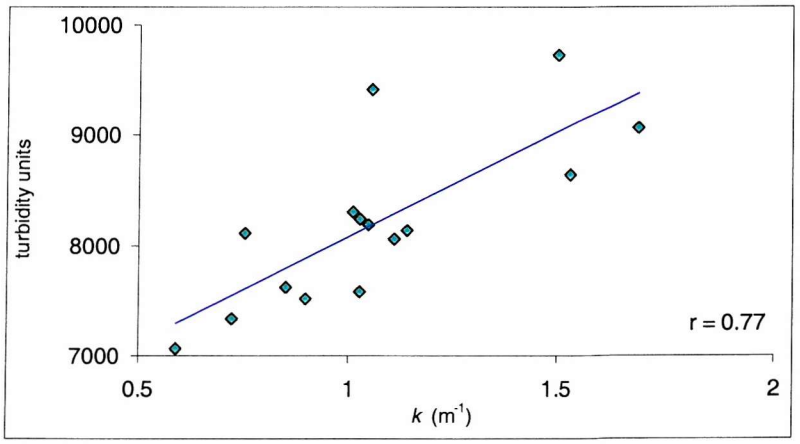


Figure 2.4. Plot of daily mean turbidity values as measured by the sensor transmissometer against water column attenuation coefficient (*k*).

Table 2.1 : Summary of calibration results listing measured values of salinity and chlorophyll in discrete surface water samples plus k values (estimated from water column light profiles) together with sensor values of temperature, salinity, chlorophyll fluorescence (at the time of sample collection) and mean daily transmissometer values.

Date	water temperature	water salinity	sensor salinity	discrete Chl-a (mgm^{-3})	sensor fluoresence	measred k	sensor turbidity
13/04/1999 08:26-08:45	10.78	32.07	33.65	1.1	16506	nd	7842.6
23/04/1999 13:03-13:29	11.63	18.82	19.60	1.4	16859	1.14	8132.9
27/04/1999 09:56-10:33	12.55	30.28	31.74	1.1	15990	1.01	8306.1
04/05/1999 13:36-14:00	13.1	29.46	30.83	1.2	16816	1.05	8192
14/05/1999 10:45-11:32	12.24	31.38	32.87	7.4	27376	nd	9257.2
19/05/1999 13:30-14:18	13.89	31.55	33.07	21.1	39458	1.53	8636.3
27/05/1999 09:56-10:33	16.6	27.57	28.70	4.6	19589	0.76	8106.4
03/06/1999 13:26-14:06	19.97	29.59	30.68	20.3	30488	1.06	9414.6
17/06/1999 14:31-14:51	17.85	31.90	33.34	8.9	19469	nd	8161.6
25/06/1999 09:14-09:34	18.37	25.88	26.81	2 7	14299	nd	7715
01/07/1999 12:45-13:38	18.46	32.09	33.34	14.8	25194	nd	7498.8
09/07/1999 05:30-06:30	19.34	27.40	28.45	5.5	23422	0.59	7061.5
14/07/1999 11:30-12:30	nd	32.72	nd	8.0	nd	nd	7735.7
21/07/1999 12:45-13:30	19.6	30.11	31.22	3.0	19477	0.72	7341.6
28/07/1999 12:25-13:05	20.9	32.13	33.43	14.3	38133	0.92	nd
12/08/1999 11:26-11:45	20.19	31.65	32.89	3.1	22141	1.51	9734.4
19/08/1999 12:21-12:41	17	29.91	31.01	1.7	16068	0.85	7617.2
25/08/1999 11:21-11:25	nd	30.36	nd	4.4	nd	0.91	nd
02/09/1999 14:45-15:03	20.05	31.17	32.47	5.8	27052	1.03	7580.2
09/09/1999 14:30	20.19	32.15	32.38	5.1	18959	1.03	8241
16/09/1999 14:43-15:03	19.06	32.79	34.02	1.4	16672	1.11	8054.1
23/09/1999 14:53-15:10	17.82	24.72	27.01	3.7	19626	0.9	7520.3
27/09/1999 12:23-12:35	nd	31.76	nd	1.6	nd	1.31	nd

2.3 LABORATORY MEASUREMENTS

2.3.1 NUTRIENT MEASUREMENTS

Water samples for nutrient analysis were filtered through Whatman GF/F filters either immediately after collection on board the research vessel or soon after returning to the laboratory. Filtered water samples for silicate determination were stored in plastic vials in the dark at room temperature prior to analysis. Samples for nitrate and phosphate determination were stored frozen in plastic vials prior to analysis.

All three nutrients were analysed using a Burkard Scientific SFA-2 Auto-analyser, as described by Hydes (1984). Nitrate was reduced to nitrite using a reduction column of copper coated cadmium wire (Nydal, 1976). Phosphate and Silicate were detected as their respective molybdate complexes as described by Parsons et al. (1984). Three mixed standards were prepared covering the range of concentrations in the analysed samples. The data were then calculated using the calibration relationship and accounting for drift through each run, using Digital-Analysis-Microstream software.

2.3.2 PHYTOPLANKTON PIGMENT MEASUREMENTS AND CELL ENUMERATION

2.3.2.1 Fluorometric Determination of Chl a

Water samples for Chl *a* analysis were filtered through Whatman GF/F filters either immediately after collection on board the research vessel or soon after returning to the laboratory and kept in the freezer prior to analysis. Chl *a* (and phaeopigment) concentrations were determined as follows by the method of Parsons et al. (1984). The frozen chlorophyll filters were each placed in a 10 ml plastic centrifuge tube and sonicated in 6-ml of 90% acetone for 30 seconds using a "Vibra Cell" sonicator. The extracts were then centrifuged at 3000 rpm for 10 minutes in a "MSE Mistral 2000" centrifuge. The fluorescence of each extract was measured using an Aminco-fluorocolorimeter both before and after acidification using 2 drops of 10% HCL (Lorenzen, 1967). The concentration of chlorophyll in seawater was calculated using the following equation:

$$\textbf{Chl } a \textbf{ (mgm}^{-3}\textbf{)} = ((\textbf{Std } R_b/\textbf{Std } R_a)/(\textbf{Std } R_b/\textbf{Std } R_a\textbf{-1}))*((\textbf{Samp } R_b\textbf{-Samp Ra})*(\textbf{v/V}))*(\textbf{Std conc/Std } R_b)$$

Where, Std = chlorophyll *a* standard, Samp = sample, R_b = fluorescence pre acidification, R_a = fluorescence after acidification, v = volume of extract (ml), V = volume of sample water (L), and Std conc = concentration of chlorophyll *a* standard.

The Aminco fluorocolourimeter was calibrated using appropriate dilutions of a known standard solution of chlorophyll *a* (Sigma). The concentration of the chlorophyll *a* standard was determined spectrophotometrically according to the following equation (Parsons et al., 1984)

$$\textbf{Chl } a \textbf{ (mg m}^{-3}\textbf{)} = 26.7 * (664_b - 667_a)$$

Where absorbance values at given wavelengths were measured in a 1cm cuvette before (b) and after (a) acidification of the extract.

For the growth rate experiment (Chapter 6), chlorophyll *a* was determined by extraction in 90% acetone and measuring the fluorescence of the sample using a Turner Designs 10-AV-fluorometer adapted for the Welschmeyer (1994) procedure. Chl *a* concentrations were calculated using the following equation:

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

Where; R = fluorometer reading, v = volume of the extract (ml) and V volume of the sample (L).

2.3.2.2. HPLC Pigment Measurements

Method Outline

The high performance liquid chromatography (HPLC) technique separates phytoplankton pigments and their degradation products, allowing the dominant species of phytoplankton to be evaluated as well as providing an indication of the decomposition and grazing processes. Pigments were separated, in this study, by ion-pairing reverse-phase HPLC as described by Mantoura & Llewellyn (1983) and modified by Barlow et al. (1993) using a Perkin Elmer C18 column and a Thermoseparation HPLC system with on line vacuum degasser, a dual solvent pump (P2000), an autosampler (AS3000), a UV detector (UV1000), a fluorometer (FL3000), integrator (SN4000) and integration software PC1000. Pigmet extracts were loaded into the autosampler which retained a temperature of 0 ^{0}C. A 100 µl filtered sample (500 µl sample mixed with 500 µl 1M ammonium acetate) was injected into the column. The mobile phase consisted of a binary eluant system with solvent A (80% methanol: 20% 1M ammonium acetate) and solvent B (60% methanol: 40% acetone). Ammonium acetate acts as an ion-pairing agent to prevent dissociation of the anionic carboxyl group, which normally dissociated at neutral pH. This anionic group gives a poor separation of the acidic compounds in the pigment mixture under normal conditions. The ion-pairing reagent, thus allows separation of pigments not possessing a phytol group (Zapata et al., 1987). A linear gradient from 0 to 100% of eluant B is created for 10 minutes, followed by an isocratic stop (for 7.5 minutes) at 100% eluant B. A second gradient of 2.5 minutes is used to return to the initial condition of 100% eluant A.

Extraction Procedure for HPLC pigment analysis

For each water sample 0.5 – 1.0 L was filtered (in duplicate), through a 47-mm GF/F filter then frozen immediately prior to pigment analysis. The frozen samples were placed in 90% HPLC grade acetone (5 ml), sonicated for 20 seconds and centrifuged for 15 minutes at 3000 rpm.

Filtered extracts were injected into the HPLC system for the estimation of chlorophylls and the main accessory carotenoid pigments using the ion-pairing reverse-phase HPLC technique.

Detection and Identification of Chlorophylls and Accessory Pigments

Carotenoid pigments were detected by absorbency at 440 nm, however chlorophylls and other degradation products were detected by absorption at 440 nm as well as by fluorescence with excitation at 410 nm and emission at wavelengths > 670 nm. Peaks of Chl *a*, Chl *b* and *β*-carotene were identified by noting the retention times for each pigment with authentic standards dissolved in acetone; Chl *a* and Chl *b* (Sigma Chemical Company) Chl *c* and carotenoids (DHI, DENMARK).

Chl *a* standards were loaded every 7 samples to monitor variations in retention times during sample analysis. At the beginning of this study, accessory pigments were identified by running filtered samples of reference algae, which contain well-documented pigment composition during sample analysis and noting the retention times. The chromatograms of these reference samples were compared to other published data (Wright et al., 1991, Barlow et al., 1993, Proenca, 1994 and Dransfeld, 1999). An inline photodiode array detector was used in the later stages of this work for more accurate identification of accessory pigments. Table (2.2) gives the common accessory pigments used as biomarkers for particular groups of phytoplankton.

Table 2.2 Distribution of major accessory pigments for some phytoplankton taxa as given by Barlow et al., 1993; Jeffrey & Vesk 1997; Jeffrey et al., 1997; Gibb et al., 2000.

Algae group	*Common pigments*
Diatoms	fucoxanthin (Fuc), diadinoxanthin (Diad), diatoxanthin (Diat)
Cryptophyceae	alloxanthin (Allo)
Blue-green algae	zeaxanthin (Zea), myxoxanthophyll, echinenone
Green algae	violaxanthin (Viol), lutein, zeaxanthin (Zea)
Dinoflagellates	peridinin (Peri), diadinoxanthin (Diad), fucoxanthin (Fuc)

2.3.2.3 Phytoplankton Counts

Aliquots of 100 ml of seawater samples were preserved with Iml of acidic Lugol's iodine solution (Parsons et al., 1984) and kept in dark bottles prior to cell counting. Phytoplankton cells were counted using a Leitz Fluovert inverted microscope according to the sedimentation technique described by (Utermohl, 1958).

Phytoplankton samples were mixed and sub-sample volumes of usually 10 ml (in duplicate), placed into a sedimentation chamber. If chlorophyll levels were low (<1 mg m^{-3}) larger volumes of preserved sample (up to 100 ml) were pre-sedimented in a 100 ml measuring cylinder. Samples were allowed to settle for ~24 h prior to microscopic enumeration. The whole floor of the

sedimentation chamber was counted at x100 magnification for larger (i.e. >10 μm in diameter) and less abundant phytoplankton cells, while two transacts were counted using x200 for identifying and counting smaller organisms (5–10 μm in diameter). For the much smaller and more frequent microplankters (e.g. *Phaeocystis* and *Cryptomonas*) transects at x400 magnification were used and compared to counts from 5 individual fields of view using an oil immersion objective (x1000). The number of cells obtained from the duplicate counted chambers was calculated with the difference between duplicates usually <10%. To convert the counted cell number to cells ml^{-1} the following approaches were used:

1- When the whole chamber was counted: (in low Chl *a* samples)

Number of cells ml^{-1} = total cell number counted /10

2- When two transects were counted the following calculations were used:

- Area of two counted transects = 20.83 mm^2 (Calculated by measuring the diameter of the whole chamber and of 1 field of view).
- Area of the whole chamber = 450.13 mm^2

Number of cells ml^{-1} = (n * 100) / (p * v)

Where; n = number of counted cells, p = % of the counted area (4.63 %) in relation to the area of the whole chamber (100 %), and v = volume of the chamber (10 ml).

3- When one (or more) fields of view (FOV) were counted: (for small species using x400)

% of area of 1 FOV (p) = r^2 * Π/total area

= $((0.44)^2$ * 3.14)/450.13)

= 0.00135

Number of cells ml^{-1} = n/ (0.00135 * 10)

Where; r = the radius of one field of view

Fresh sub-samples of unfiltered seawater were kept in the fridge and examined microscopically as soon as possible after collection to facilitate the identification of the major genera and species present. The phytoplankton community was identified to the species level, where possible using Tomas (1997) and Dodge (1982). Some smaller cells, which were difficult to identify, had to be included in more general taxonomic categories (e.g. small flagellates). Images of some of the dominant phytoplankton species are shown in Appendix II.

2.3.2.4 Biomass Estimation

Total phytoplankton biomass was estimated from microscopic enumeration of cells by estimating cell volume of individually measured cells and converting these to carbon using the cell

volume/carbon relationship given by Eppley et al. (1970) as described by Holligan et al. (1984). In each sample, the dimensions of 15-20 cells of each species were measured and converted to volume using a standard spreadsheet algorithm provided by Derek Harbour based on the algorithms given in Kovala & Larrance (1966). Carbon values for two dinflagellates (*Scrippsiella trochoidea* and *Prorocentrum micans*) were calculated according to a more recent estimate of carbon per cell volume (Menden-Deuer & Lessard, 2000). Carbon estimates of individual species identified were subsequently summed to quantify the carbon contribution (mg C m^{-3}) of major groups (diatoms, dinoflagellates, flagellates and/or ciliates) to the total phytoplankton carbon (Shaw & Purdie, 2001).

2.4 LABORATORY-BASED CULTURES

Four diatom species (*Thalassiosira rotula, Odontella sinensis, Ditylum brightwellii* and a *Cheatoceros* sp.) and two dinoflagellates (*Prorocentrum micans* and *Scrippsiella trochoidea*) were isolated from Southampton Water and maintained in culture for an extended period. Individual cells were isolated by a drawn out fine tipped Pasteur Pipette and placed in a clean sterile microtitre plate well and washed through other wells. The isolated individual cells were then incubated (at 14L: 12D light cycle, 15 ^{0}C) for 2-3 days (for diatoms) or a week (for dinoflagellates) using both sterile ASW with Kellers' additions (Keller et al., 1987) as well as supplemented seawater collected locally. Cultures were checked for growth then picked again using a sterile tipped automatic pipette and placed into 100 ml of media in a 250 ml conical flask. Stock cultures were sub-cultured, every 3-4 weeks for diatoms and every 5-6 weeks for dinoflagellates due to their relatively slower growth rate, prior to the growth experiment. Fresh sub-samples of the 6 isolates were taken for scan electron microscopy (SEM) following the method described by Faust (1991) using LEO1450 variable pressure SEM. Scanned pictures of the 5 isolates are shown in Appendix III. Two species of these isolates (*Thalassiosira rotula* and *Prorocentrum micans)* were then chosen for the laboratory-based experiments.

2.4.1 MAINTENANCE OF STOCK CULTURES

Stock cultures of the six isolates were generally maintained in artificial seawater media with Kellers additions (Keller et al., 1987). The composition of the artificial seawater used in this study was as described by Harrison et al. (1980) and the recipe is shown in Table 2.3.

2.4.2 PREPARATION OF ARTIFICIAL SEA WATER AS A GROWTH MEDIA

500 ml of milli-Q water was placed into each of two 2 litre conical flasks. Anhydrous salts were weighed out as given in the table below (table 2.3) and added to one flask (solution 1) and hydrated salts were added to the second flask (solution 2). Both solutions were autoclaved then

Solution 1- Anhydrous salts	MW	Amounts to weigh out for 1 litre (g)	mmol L^{-1}
NaCl	58.44	20.758	**362.661**
Na_2SO_4	142.04	3.447	**24.993**
KCL	74.56	0.587	**8.038**
$NaHCO_3$	84	0.17	**2.066**
KBr	119.01	0.0845	**7.249 X 10^{-1}**
H_3BO_3	61.83	0.0225	**3.715 X 10^{-1}**
NaF	41.99	0.0027	**6.570 X 10^{-2}**

Solution 2- Hydrated salts	MW	Amounts to weigh out for 1 litre (g)	mmol L^{-1}
$MgCl_2.6H_2O$	113.33	9.395	**47.176**
$CaCl_2.2H_2O$	147.03	1.316	**9.139**
$SrCl_2.6H_2O$	266.64	0.0214	**8.200 X 10^{-2}**

Table 2.3. Recipe of Solution 1 (anhydrous salts) and Solution 2 (hydrated salts) for preparation of the artificial seawater.

mixed after cooling and nutrient solutions and other supplements added according to table 2.4. To maintain the stock cultures of the isolated species for extended periods, they were regularly sub-cultured every month with freshly prepared Kellers media based in artificial seawater and incubated at about 100 µmol m^{-2} s^{-1} light at 16 ^{0}C.

Table 2.4. Composition of Keller's media as described by Keller et al. (1987).

Compound	MW	Primary stock (100ml milli-Q)	Working stock (100ml milli-Q)	Volume added to 1L ASW	Molarity in final media
$NaNO_3$	84.99		0.75 g	1 ml	**88**
$NaH_2PO_4.2H_2O$	156.01		0.0158 g	1 ml	**1**
$NaO_3Si.9H2O$				1 ml	**53.5**
H_2SeO_3	129	0.013 g	1 ml	1 ml	**0.01**
Trace metals					
$CuSO_4.5H_2O$	249.68	0.1 g			**0.04**
$ZnSO_4.7H_2O$	287.5	0.22 g			**0.075**
$CoCl_2.6H_2O$	237.93	0.11 g	1 ml	1 ml	**0.047**
$MnCl_2.4H_2O$	197.91	1.8 g			**0.91**
$Na_2MoO4.2H2O$	241.96	0.065 g			**0.026**
FeNaEDTA	367.05	0.440 g		1 ml	**12**
Na_2EDTA	372.24	0.372 g		1 ml	**10**
Vitamins					
Biotin		0.01 g	1 ml	0.5 ml	**0.002**
B12		0.01 g	1 ml		**0.0004**
Thiamine HCl			0.02 g		**0.3**

ASW = Artificial Sea Water

CHAPTER THREE

CHAPTER THREE

3- APPLICATION OF CONTINUOUS MONITORING AND DISCRETE WATER SAMPLING TO INVESTIGATE FACTORS INFLUENCING PHYTOPLANKTON GROWTH IN A MACROTIDAL ESTUARY

3.1 INTRODUCTION

Phytoplankton biomass and community structure in temperate macrotidal estuaries vary according to dynamic changes in physical and chemical gradients (Monbet, 1992). Frequent sampling is therefore required to allow short term changes in phytoplankton populations to be detected in estuarine and coastal waters (Balch, 1981; Roden, 1984 & 1994). Seasonal changes in phytoplankton species abundance and chlorophyll biomass in Southampton Water have been reported by several researchers (e.g. Iriarte, 1991; Kifle, 1992; Proenca, 1994; Kifle & Purdie, 1993). A detailed understanding of the factors that control phytoplankton growth and the bloom timing throughout the estuary was, however, not evident from these investigations due to limited sampling frequency (typically once per week).

In this chapter continuous data from a coastal monitor placed in a fixed position (for details, see section 2.2.5, chapter 2) is used to show daily changes in chlorophyll and turbidity in relation to less frequently collected phytoplankton abundance data. The continuously monitored data (recorded every 10 minutes) were used together with discrete surface water samples collected

close to the monitor at approximately weekly (5-7 days) intervals over a six-month period from April to September 1999. Short-term variations in phytoplankton chlorophyll levels derived from a calibrated fluorometer (see chapter 2 for calibration details) are compared with changes in water temperature, salinity and other meteorological parameters to investigate the factors that affect bloom initiation and termination in the estuary.

In addition to Chl *a*, water samples were collected to monitor the temporal variations in environmental variables including nutrients. Temporal variations in phytoplankton community composition and species succession in surface waters during the main productive period (April-September) in 1999 were examined using microscopic analysis of preserved samples. Surface irradiance levels were obtained from a local meteorological station and light profiles adjacent to the monitor were also measured at regular intervals (see chapter 2).

3.2 TEMPORAL VARIATIONS IN ENVIRONMENTAL FACTORS AT THE DOCK-MOORING SITE

3.2.1 VARIATIONS IN WATER TEMPERATURE AND SURFACE INCIDENT IRRADIANCE

Temporal variations in daily surface incident irradiance (PAR, W h m^{-2} d^{-1}) for the Southampton Water region, (obtained from Everton see section 2.2.3) are shown in figure 3.1a. Values ranged from 700-3570 W h m^2 d^{-1} during the sampling period (April-September 1999). The temporal variation of daily mean water temperature at the Dock-Mooring site was measured during the sampling period using the continuous data from the coastal monitor. A predictable seasonal trend in water temperature is seen with lower water temperatures (10–15 ^{0}C) measured during April and May (figure 3.1b). Temperatures increased (16-21.5 ^{0}C) from June onwards over the summer months.

3.2.2 VARIATIONS IN SALINITY

The continuous monitored daily mean salinity values during the period of study are presented in figure 3.2. These showed noticeable seasonal changes and ranged between 29.5 and 34.3 with values increasing as river flow rates decreased towards summer. Mean daily values were generally lower on peak spring tides and higher on neap tides due to the fixed positioned sensor detecting a larger range of salinity values when tidal range was maximum.

3.2.3 ATTENUATION COEFFICIENT (k) AND 1% LIGHT DEPTH

Figure 3.3 shows values of mean daily water column attenuation coefficient (*k*) derived from the monitor turbidity sensor together with values determined from vertical irradiance profiles. The derived values (for details see chapter 2) of the attenuation coefficient (*k*) averaged between 0.2 and 2.5 m^{-1} (figure 3.3) with no obvious consistent relationship with the predicted tidal cycle.

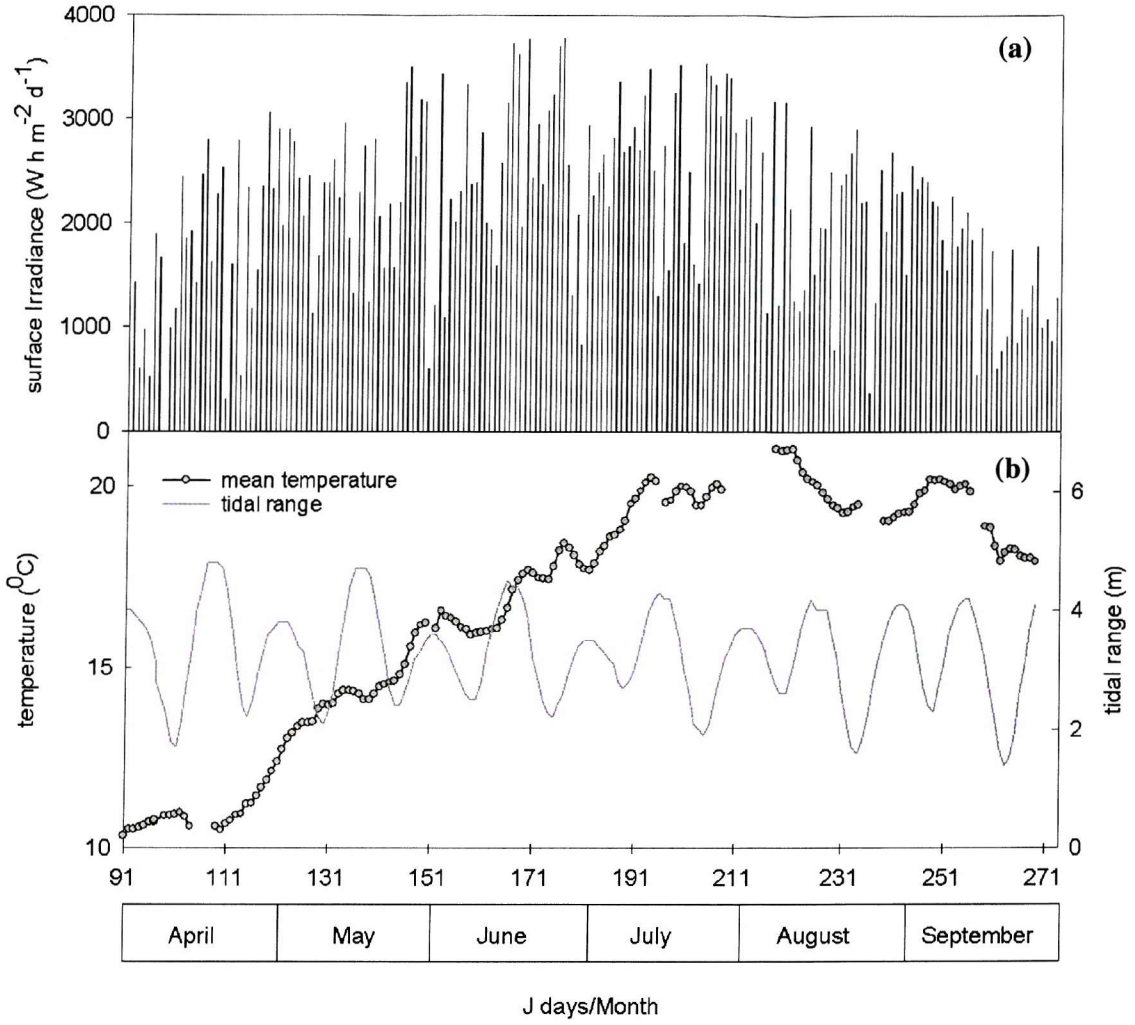


Figure 3.1.Seasonal variation in (a) daily surface incident irradiance (PAR) and (b) daily mean water temperature (monitor data at the Dock-Mooring site from April to September 1999. Predicted daily tidal range is also shown.

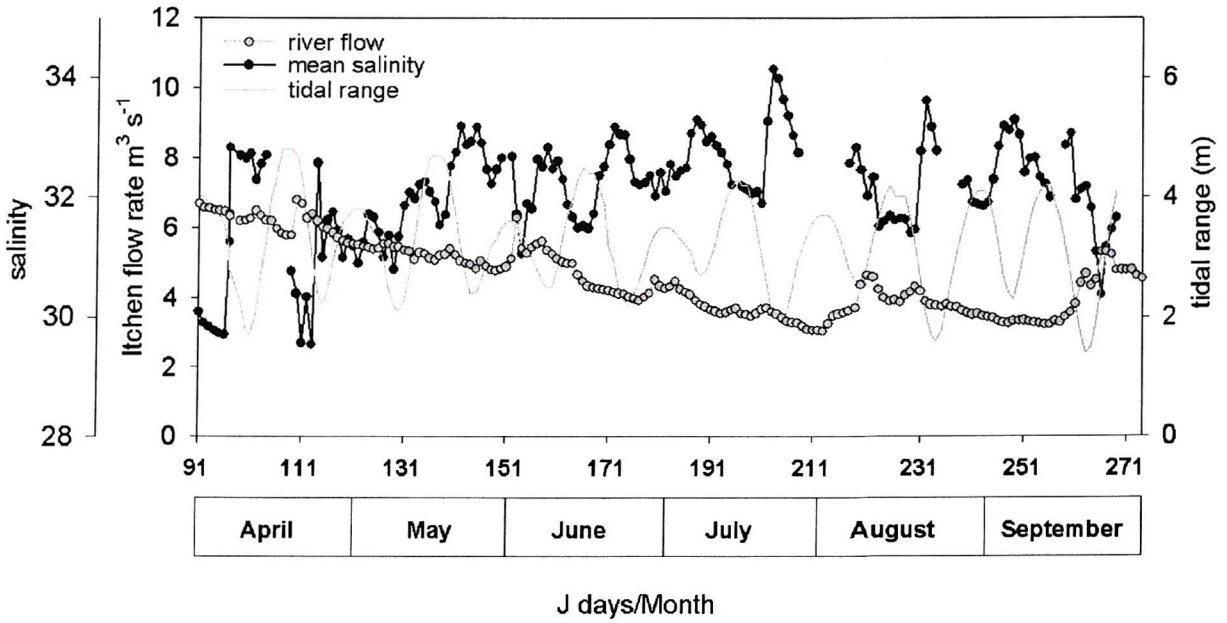


Figure 3.2. Seasonal variation in daily mean salinity (monitor data) at the Dock-Mooring site from April to September 1999. Predicted daily tidal range is also shown.

Large variations in *k* values were detected with maximum peaks in June and August. A decreasing trend in *k* values was seen between mid June and mid July. The contour plot (figure 3.4) shows the temporal variations in the percentage irradiance at the sampling site from April-September 1999 calculated from the sensor derived *k* values shown in figure 3.3. The 1% irradiance contour (often referred to as the photic depth) fluctuated between approximately 2 and >10 meters during the period from 7th April to 27th September (figure 3.4).

No clear relationship (data not presented) was found between *k* values and Chl *a* concentration, however some evidence of an inverse relationship, particularly during bloom periods, was noticed between the photic zone depth and Chl *a*. This indicates that turbidity is a limiting factor for phytoplankton growth and that the mean water column irradiance declined (figure 3.6) due to phytoplankton biomass increase during blooms.

3.3. VARIATIONS IN TOTAL CHLOROPHYLL *A*

Concentration of Chl *a* was measured in surface water samples during the sampling period (April–September 1999) and showed considerable variations between sampling dates (figure 3.5 & 3.7). Lower values of surface Chl *a* were measured in early spring followed by two peaks in mid May and early June of about 21 mg m^{-3} and 20 mg m^{-3}, respectively (figure 3.5a). A vertical profile of Chl *a* from discrete water samples was measured during the main diatom bloom in spring (19th May) and showed a decline in Chl *a* concentration (~9 mg m^{-3}) in the sub-surface water layer followed by a chlorophyll maxima in deeper water at 4 and 6 meters, with values of about 25 and 33 mg m^{-3}, respectively (figure 3.5b).

Daily changes in mean water column Chl *a* determined from the calibrated Dock-Mooring fluorometer, in relation to the discrete surface Chl *a* values are presented in figure 3.5a. Values of Chl *a* from discrete water samples do not always coincided with sensor fluorometer values of the daily mean water column. This is particularly obvious in June and end of July when chlorophyll distribution throughout the water column was apparently not homogeneous. Surface discrete Chl *a* values often tend to be higher than daily mean water column derived values suggesting phytoplankton aggregation in near surface waters.

The main spring peak in chlorophyll recorded on 19th May (figure 3.5a) increased over a spring tide (from day 131-139). On 19th May the phytoplankton community was dominated by the relatively large-celled chain-forming diatom *Guinardia delicatula* (figure 3.11 & 3.13). A further smaller peak in Chl *a* occurred on 3 June and was coincident with an increase in other chain-forming diatoms *Thalassiosira rotula* (figure 3.11 & 3.13) and *Rhizosolenia styliformis*.

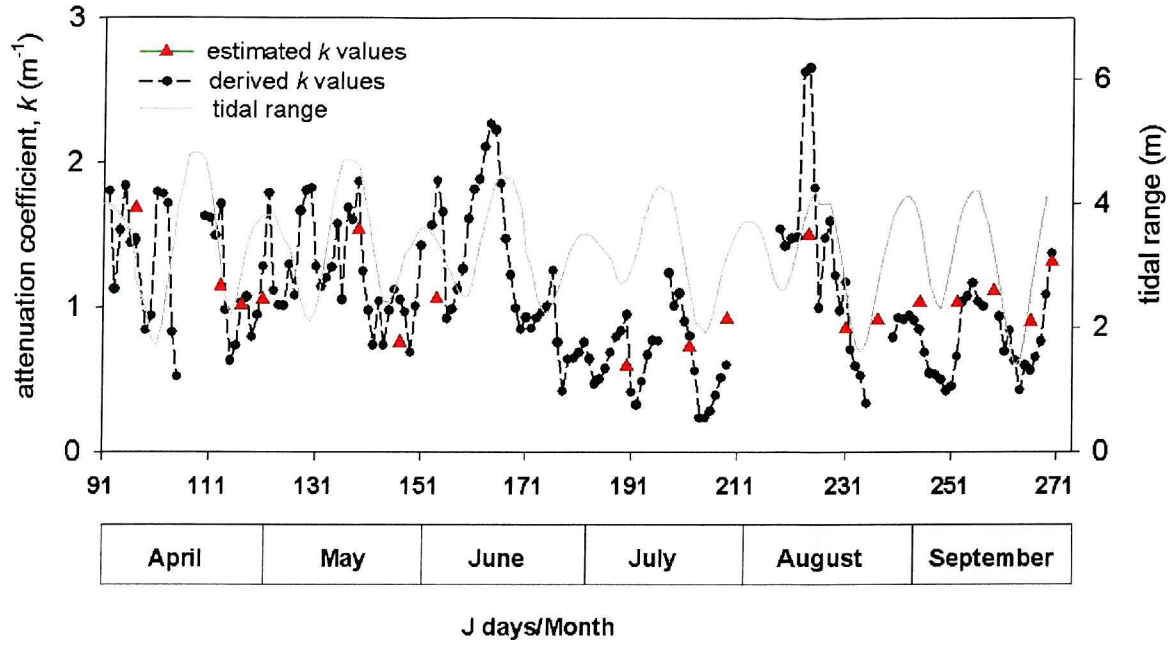


Figure 3.3. Seasonal variation in water column attenuation coefficient (k) measured adjacent to the Dock-Mooring site (red symbol) and derived from daily mean values of water turbidity (black dotted line) from April-September 1999. Breaks in data are due to sensor mal function. Predicted daily tidal range is also shown.

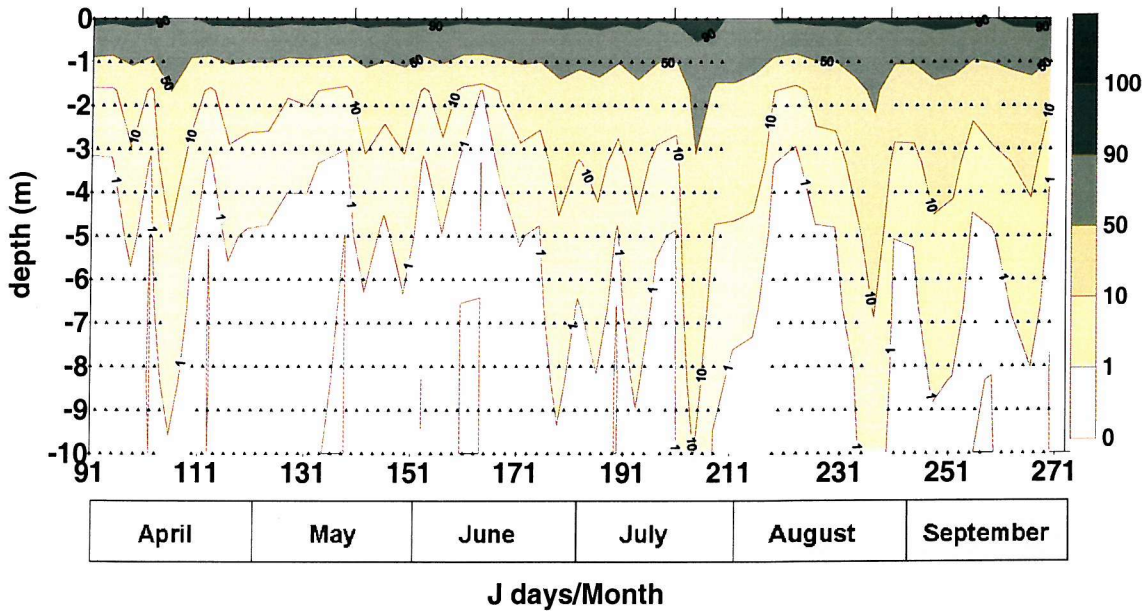


Figure 3.4. Contour plot of % irradiance at the Dock-Mooring site during the sampling period (April-September 1999) derived from mean daily values of attenuation coefficient. Surface incident irradiance = 100%.

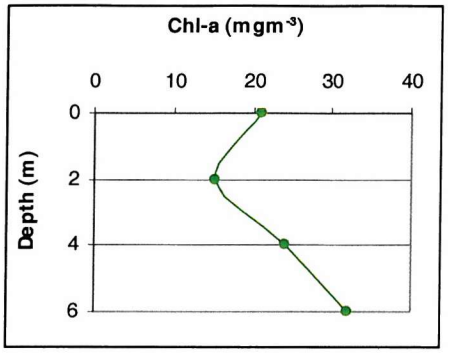


Figure 3.5b. Vertical distribution of Chl *a* (mg m^{-3}) at the Dock-Mooring site on the day of the peak spring bloom (19th May) at depths 0, 2, 4 and 6 meters.

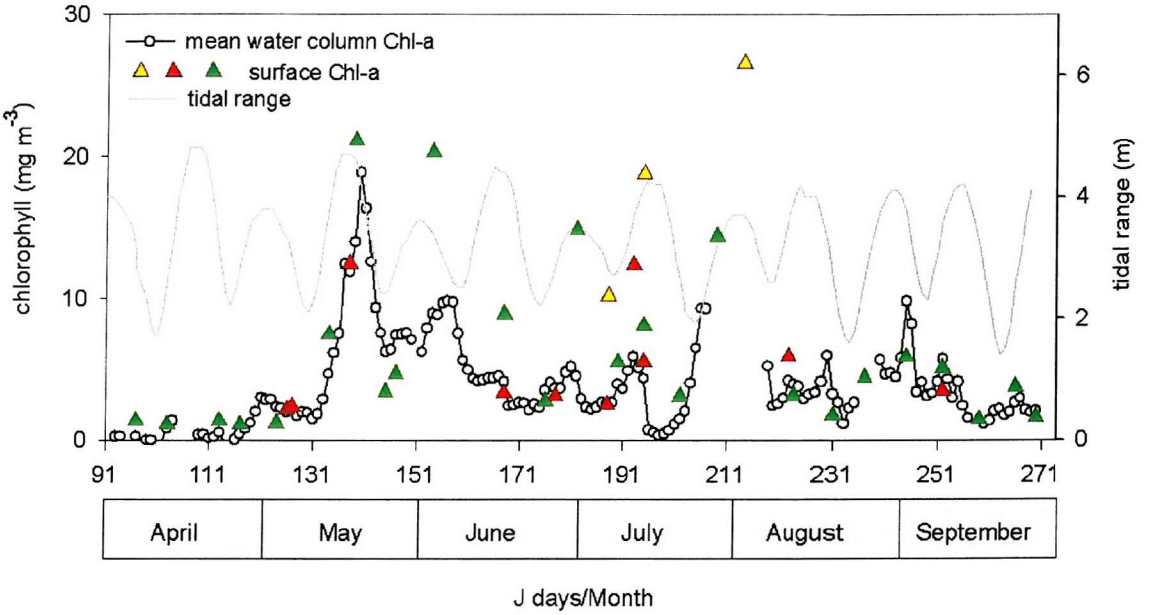


Figure 3.5a. Seasonal variation in surface Chl *a* (green symbols) and the daily mean Chl *a* concentration (mg m^{-3}) derived from calibrated fluorometer data (black line) at the Dock-Mooring site in relation to the spring-neap tidal cycle from April-September 1999. Red symbols indicate surface Chl *a* values at the Dock-mooring site obtained from the Environmental Agency and yellow symbols are surface Chl *a* values at the Western Shelf site obtained from Dubois (1999).

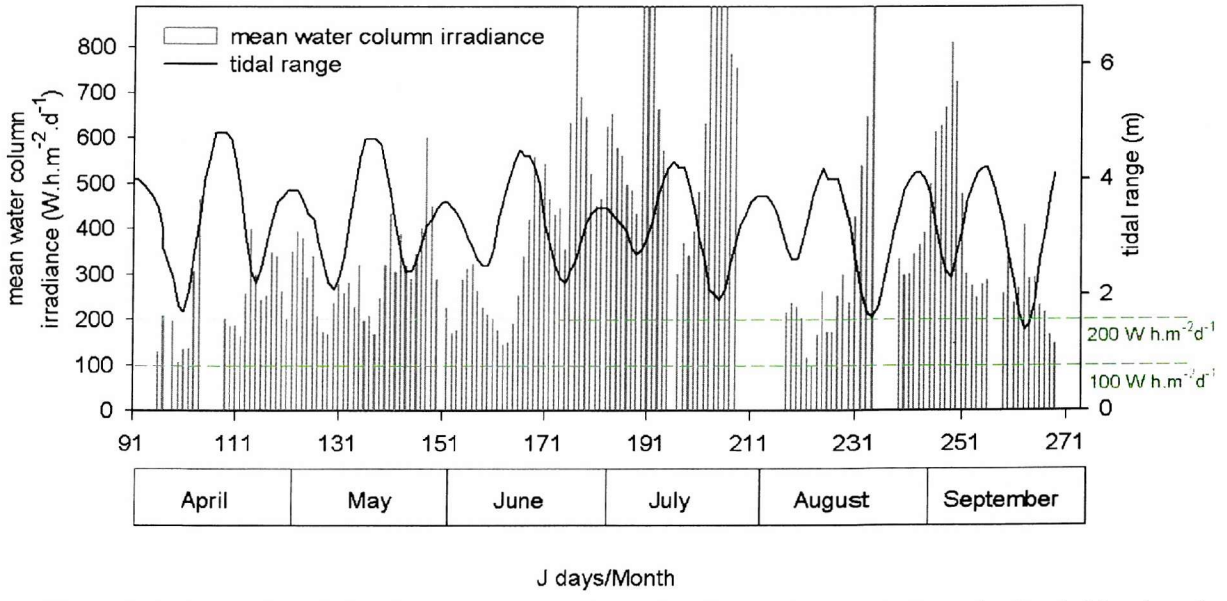


Figure 3.6. Seasonal variation in mean water column irradiance (bar symbol) at the Dock-Mooring site calculated as 4-day running mean in relation to spring neap tidal cycle (black line) from April-September 1999. Green lines indicate the irradiance threshold (100-200 W h m^{-2} d^{-1}) as previously suggested for phytoplankton growth by Jahnke (1989); Peperzak (1993) and Peperzak et al. (1993).

In addition, on this date high numbers (~2.5 x 10^2 cell ml^{-1}) of the flagellate *Eutreptiella marina* were shown to be present in the surface collected water samples (figure 3.13). This organism is known to preferentially grow in near surface waters (Kifle, 1992) and its presence may account for the large difference between the discrete surface chlorophyll concentration and daily mean water column values shown for this date (figure 3.5a). The summer chlorophyll peaks in surface waters at the Dock-Mooring site during July/August was mostly dominated by dinoflagellates with maximum peak in early July, which coincided with a large increase in the abundance of the dinoflagellate *Scrippsiella trochoidea* (about 90% of total dinoflagellate biomass) (figure 3.11 & 3.13). Relatively smaller peaks in Chl *a* recorded on 9th, 14th and 21st July (mainly *Scrippsiella trochoidea* and *Prorocentrum micans*) followed by a higher peak of approximately 14 mg m^{-3} mainly coincided with an increase in the biomass of *Scrippsiella trochoidea* (57% of total biomass), *Prorocentrum micans* (11% of total biomass) and *Odontella sinensis* (19% of total biomass). Although Chl *a* concentration recorded in early July (1st July) was similar to that recorded in late July (on 28th July), the latest was however not coincided with phytoplankton biomass. This may indicate that some organisms missed during microscopic analysis, for example, the small flagellate *Cryptomonas* sp. which found (15% of total cell number) on that day. The sensor system stopped recording data between day 209 and 217 in late July/early August. A surface water sample was however collected by Dubois (1999) from a position in the lower Itchen estuary at Western Shelf in close proximity to the dock mooring position (< 2 km) on 2nd August. Chlorophyll concentration in this sample was 26.4 mg m^{-3} indicative of a bloom of phytoplankton which was dominated by the photosynthetic ciliate *Mesodinium rubrum* (Dubois 1999). This ciliate, however, contributed less (< 3%) to total phytoplankton biomass on 28th July.

3.4 NUTRIENT DATA

The seasonal variation in surface water nitrate NO_3^-, phosphate PO_4^-, and silicate $Si(OH)_4$ concentration in relation to surface Chl *a* concentration are presented in figure 3.7. NO_3^-, PO_4^- and $Si(OH)_4$ showed strong temporal variations during the sampling period (figure 3.7). Maximum nutrient concentrations were generally measured during periods of low Chl *a* and declined to lowest values when Chl *a* was at a maximum. Minimum concentration in phosphate (below detection limit) was measured in the third week of May (19th May) during the bloom of the centric diatom *Guinardia delicatula.* Similarly, the concentration of both nitrate and reactive silicate showed a sharp decline at the same time, although the minimum values for these two nutrients were also measured on 14th July. Relatively low concentrations of both silicate and nitrate were measured during the first week of June, during a bloom of the diatoms *Thalassiosira rotula* and *Rhizosolenia styliformis.* Although the small diatom *Skeletonema costatum* showed high peaks in cell numbers (up to ~2.2 x 10^2 cell ml^{-1}) in early May (figure 3.13), its effect on

silicate concentration was not as dramatic as that of the larger-celled (> 50 μm) diatoms (e.g. *Guinardia delicatula & Thalassiosira rotula).* Figure 3.8 shows a close relationship between individual nutrient concentration and salinity, indicating the main source of all three nutrients to be riverine derived fresh waters at the Dock-Mooring site in the lower Itchen estuary (from April to September 1999).

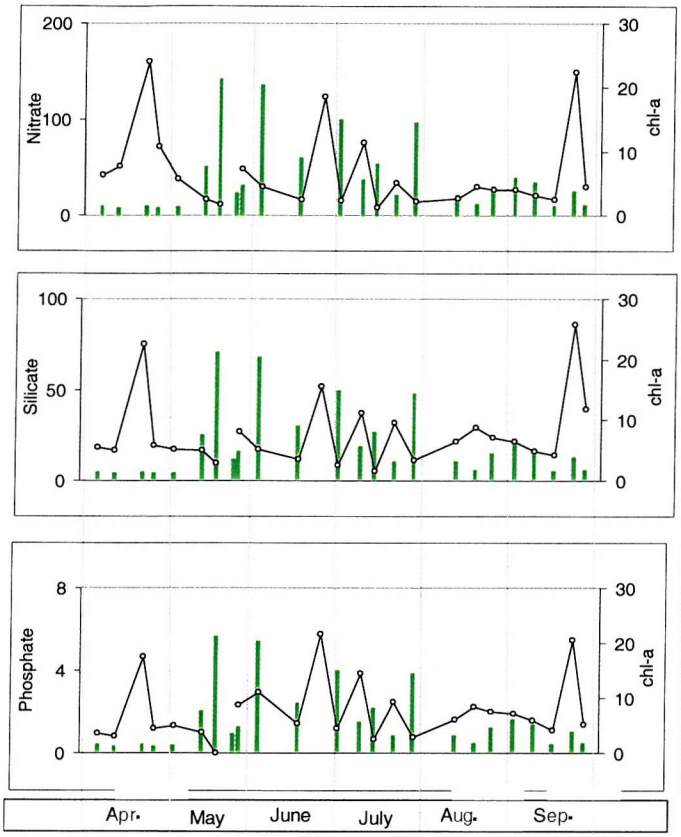


Figure 3.7. Temporal changes in surface nitrate, silicate, phosphate (black lines) concentration (μM) and chlorophyll-a (green bars) concentration (mg m^{-3}) at the Dock-Mooring site from April-September 1999.

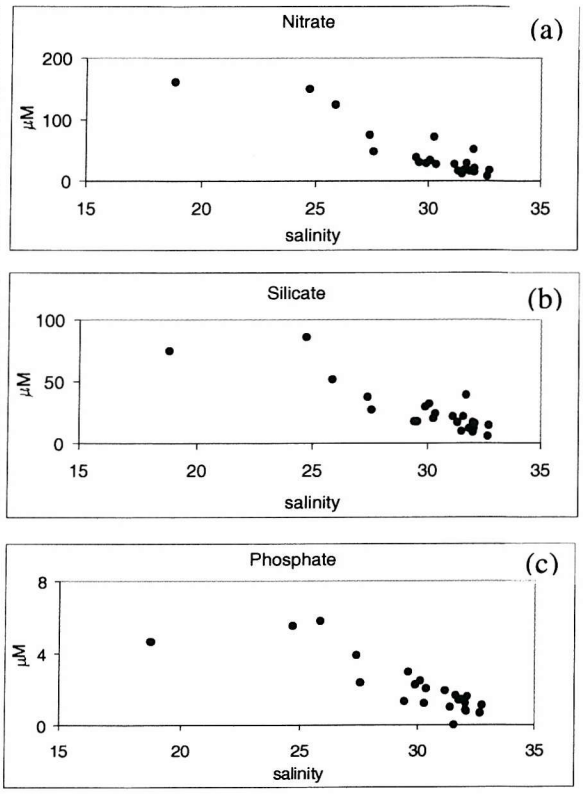


Figure 3.8. Variation in surface water nitrate, silicate, and phosphate concentrations (μM) in relation to salinity at the Dock-Mooring site from April-September 1999.

3.5. VARIATIONS IN PHYTOPLANKTON ASSEMBLAGES

3.5.1 MICROSCOPIC ENUMERATION AND CELL BIOMASS

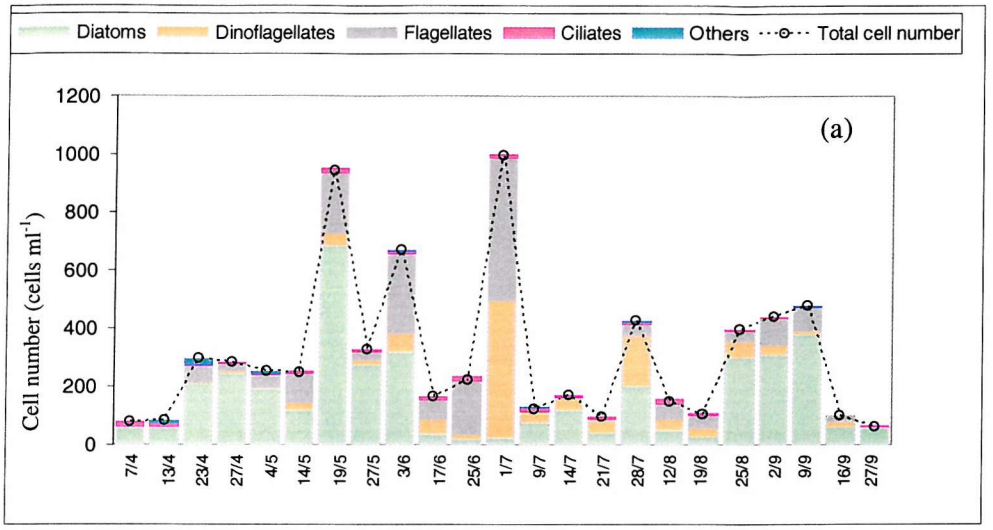
A diverse range of phytoplankton species was found in surface water samples at the Dock-Mooring site (adjacent to the coastal monitor) from microscopic examination of preserved samples. Changes in total phytoplankton cell number (cells ml^{-1}) and biomass (as mg C m^{-3}) over the sampling period is presented in figure 3.9 a & b. Total phytoplankton biomass ranged between 35-1050 mg C m^{-3} with maximum values recorded in early July coincident with the dinoflagellate bloom. Diatoms were dominant during the spring bloom (May), however the late summer bloom mainly consisted of dinoflagellates. This pattern was also mirrored by the % carbon contribution of each class to the total species biomass (figure 3.10), with diatoms dominating the spring

phytoplankton community (up to 70% of total biomass in mid May) and dinoflagellates dominating the summer community (up to 92% of total biomass in early July). Other classes, flagellates & microflagellates (up to 35% of the total biomass on 25th June) and photosynthetic ciliates, mainly *Mesodinium rubrum* (up to 58% of the total biomass on 7th April) represent transition stages in the species succession (figure 3.10) over the spring/summer period in 1999.

In spring a mixed diatom community occurred with the diatom genus *Guinardia* (*G. delicatula and G. flaccida*) being the most abundant in May (figure 3.11). The peak growth of *Guinardia* was coincident with the main spring diatom bloom on 19th May (figure 3.11), with *G. delicatula*, contributing 75% of the total diatom biomass on this day. When the diatom genus *Guinardia* declined the centric diatom *Thalassiosira rotula*, which contributed only low biomass (12.0-34.0 mg C m^{-3}) in early spring, gradually increased from mid May to a maximum peak of about 260 mg C m^{-3} in the first week of June (3rd June) comprising 75% of total diatom biomass on this day (figure 3.11).

Microscopic analysis of phytoplankton samples revealed a sequence of different diatom species that became numerically dominant only for short periods (i.e. < 7 days) during the six-month survey. Figure 3.13 shows the numerical (cell ml^{-1}) succession of the dominant species at the Empress Dock site in relation to the tidal state during the sampling period (April – September 1999). This shows that some relatively small-celled diatoms significantly (2-Tailed T-test, $p < 0.01$) contributed to the total cell numbers. For example *Skeletonema costatum* peaked (216 cell ml^{-1}) on 27th April (figure 3.13) being the most numerous diatom and comprising about 89% of the total diatom cell number on this day but only contributed 48% to total biomass. When *Skeletonema costatum* declined from its spring peak, the relatively large-celled diatom *Guinardia delicatula* increased (figure 3.13). The concentration of *Skeletonema costatum* then remained below 0.2 x 10^2 cells ml^{-1} until the end of July, however it increased again from the end of August, to reach a maximum peak in mid September when cell numbers were about 3 x 10^2 cells ml^{-1} (figure 3.13) comprising 80% of total diatom cell number on this sampling day, although it only contributed 16% of total phytoplankton biomass.

Other relatively large-celled diatom species were recorded with secondary importance to the total cell count during the spring-summer period. For example, the relatively large–celled *Ditylum brightwellii*, occurred in low cell concentration at the Dock-Mooring site with maximum cell concentration being about 1.5 x 10^2 cells ml^{-1} recorded in the last week of May (figure 3.13), comprising about 8% of the total diatom cell number and 17% of the total diatom biomass on 27th May. A peak in cell number of the chain-forming centric diatom *Thalassiosira rotula* followed the peak of *Ditylum brightwellii* and appeared in relatively lower cell number (> 0.2 x 10^2 cells



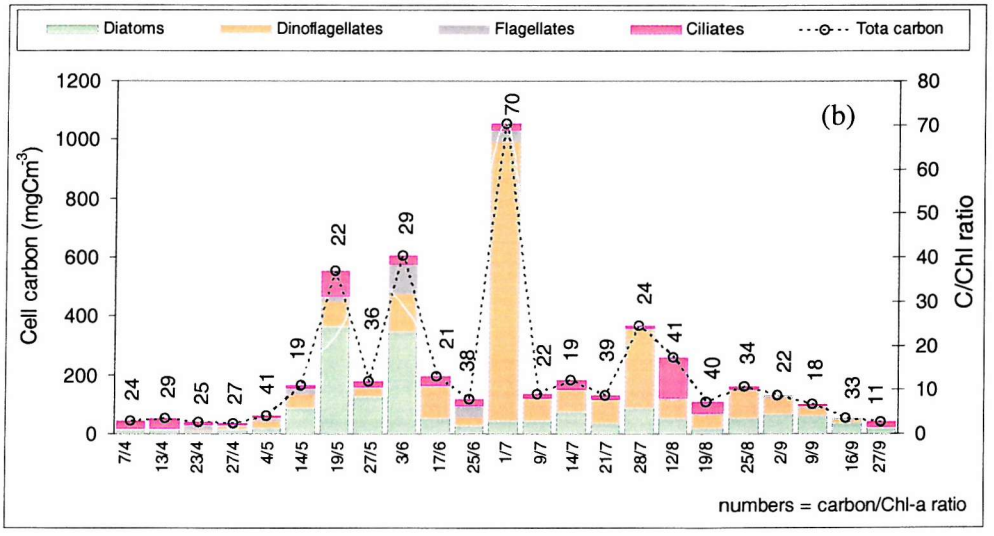


Figure 3.9. Seasonal changes in total phytoplankton cell counts, as cells ml^{-1} (a) and cell biomass, as mg C m^{-3} (b) in surface water samples at Dock-Mooring site. Phytoplankton species classified as"Others" are not included in figure (b) as they contributed only a minor component of total cell biomass.

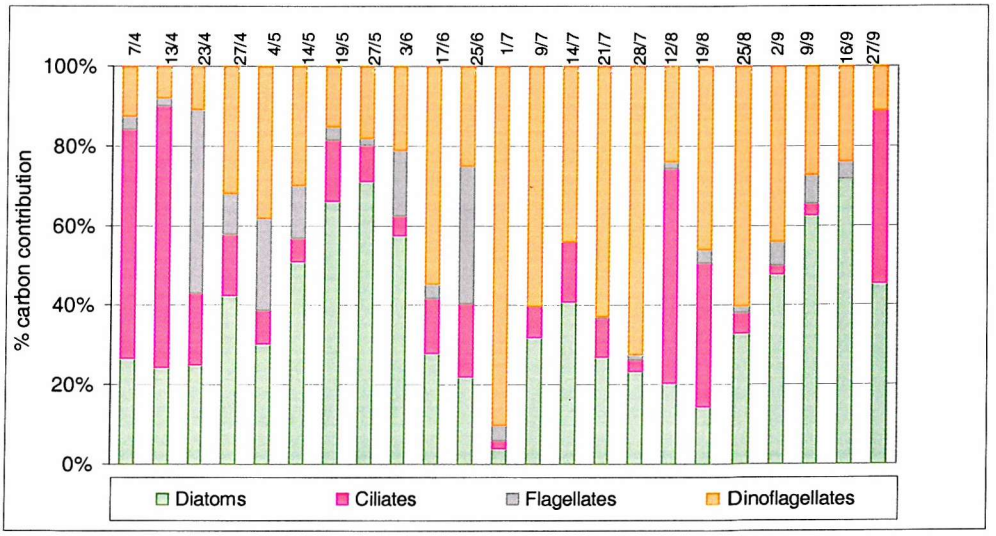


Figure 3.10. A plot summarizing % contribution to total phytoplankton biomass of diatoms, flagellates, photosynthetic ciliates and dinoflagellates.

ml^{-1}) during early spring with a maximal annual concentration of 2.5 x 10^2 cells ml^{-1} (figure 3.13) at the time of the secondary spring bloom (3rd June).

After the main spring diatom bloom (May), a noticeable decline in diatom biomass (figure 3.11) occurred followed by a smaller peak (86 mg C m^{-3}) in late July which included a peak in cell biomass of *Odontella sinensis* (= *Biddulphia sinensis*) (figure 3.11) with a maximum cell biomass of about 65 mg C m^{-3} (75% of the total diatom carbon on 28th July).

The spring bloom of diatoms was followed by a large increase in cell numbers (figure 3.13) of the relatively small-euglenoid species, *Eutreptiella marina* (up to about 2.5 x 10^2 cell ml^{-1}); although they contributed between 0.3 and 27% of total cell biomass on most sampling dates (figure 3.11 & 3.13). *E. marina* showed temporal changes during the sampling period with a noticeable increase in cell concentrations from the end of April to end of June, reaching its highest peak (2.5 x 10^2 cell ml^{-1}; 97 $mgCm^{-3}$) on 3rd June, comprising 37% of the total cell number and 16% of total species biomass. A smaller peak of about 0.81 x 10^2 cells ml^{-1} and 32 mg C m^{-3} occurred on 25th June (figure 3.11 & 3.13). Maximum % contribution (42%) of the flagellate *E. marina* to the total cell carbon was recorded during the last week of April (figure 3.11).

Dinoflagellates were an abundant component of the phytoplankton community at the Dock-Mooring site only during the summer bloom (figure 3.9, 3.10 & 3.13) with *Scrippsiella trochoidea* and *Prorocentrum micans* most abundant (figure 3.11). *Scrippsiella trochoidea* exclusively dominated the early dinoflagellate bloom in summer (1st July) with a maximum cell biomass of about 940 mg C m^{-3}, comprising ~98% of the total dinoflagellate biomass (figure 3.11) and ~90% of the total phytoplankton biomass on this day. *Prorocentrum micans* contributed less to total dinoflagellate biomass compared to *Scrippsiella trochoidea* with the highest contribution recorded at the end of July (28th July) with about 38 mg C m^{-3} (~14% of the total dinoflagellate carbon and ~11% of the total phytoplankton carbon). A mixture of other organisms (e.g. *Odontella sinensis*, *Mesodinium rubrum*) also contributed to phytoplankton total biomass in late July.

Although their contribution was low to the total biomass (< 3%), microflagellates were always very abundant (figure 3.9) and usually exceeded (up to 50% of the total cell number) other dominant large–celled species of both diatoms and flagellates (dinoflagellates and euglenoids) (figure 3.10). A small cryptomonad species, also reported by Dunn (1987), was present with high numbers (up to 4.9 x 10^2 cells ml^{-1}) throughout the sampling period (figure 3.13). This species gradually increased in cell number from mid May until early July reaching its peak (4.9 x 10^2 cells ml^{-1}) on 1st July. The rest of July was characterised by a temporary decline in cell concentration (< 0.4 x 10^2 cells ml^{-1}), however the cryptomonad species gradually increased again in cell number to relatively high values of 0.91 x 10^2 and 0.8 x 10^2 cells ml^{-1} on 2nd September

and 9th September, respectively (figure 3.13). Photosynthetic ciliates, particularly *Mesodinium rubrum*, appeared in surface water samples on most sampling dates (figure 3.9b & 3.10), particularly at the time when diatoms dominated the community, with maximum biomass (22 mgm^{-3}) in mid June comprising 11% of the total phytoplankton biomass. The maximum contribution of *M. rubrum* (47%) to the total cell carbon was recorded in early spring (7th April) before diatoms started to flourish late in April (figure 3.9b & 3.10).

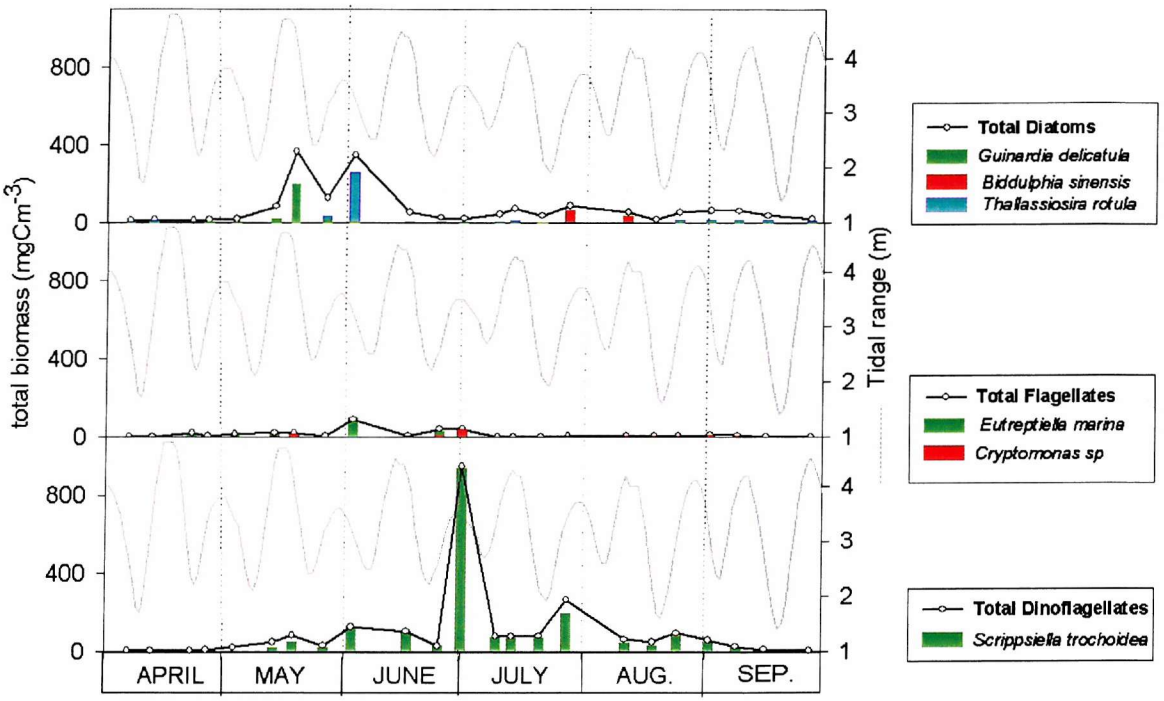


Figure 3.11. Time series plot showing the seasonal variation in total biomass of diatoms, flagellates and dinoflagellates, mg m^{-3} (black line) in relation to the spring-neap tidal cycle during the sampling period. Bars indicate carbon content of the dominant species for each group.

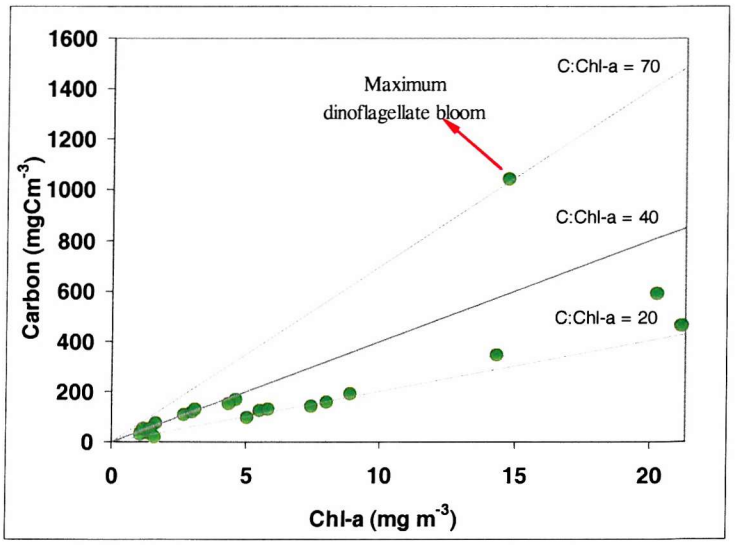


Figure 3.12. Phytoplankton total biomass (mg C m^{-3}) versus chlorophyll *a* (mg m^{-3}) for water samples collected from the Dock-Mooring site from April-September 1999 in comparison to different theoretical C: Chl *a* ratios (C: Chl *a* = 20, 40, 70).

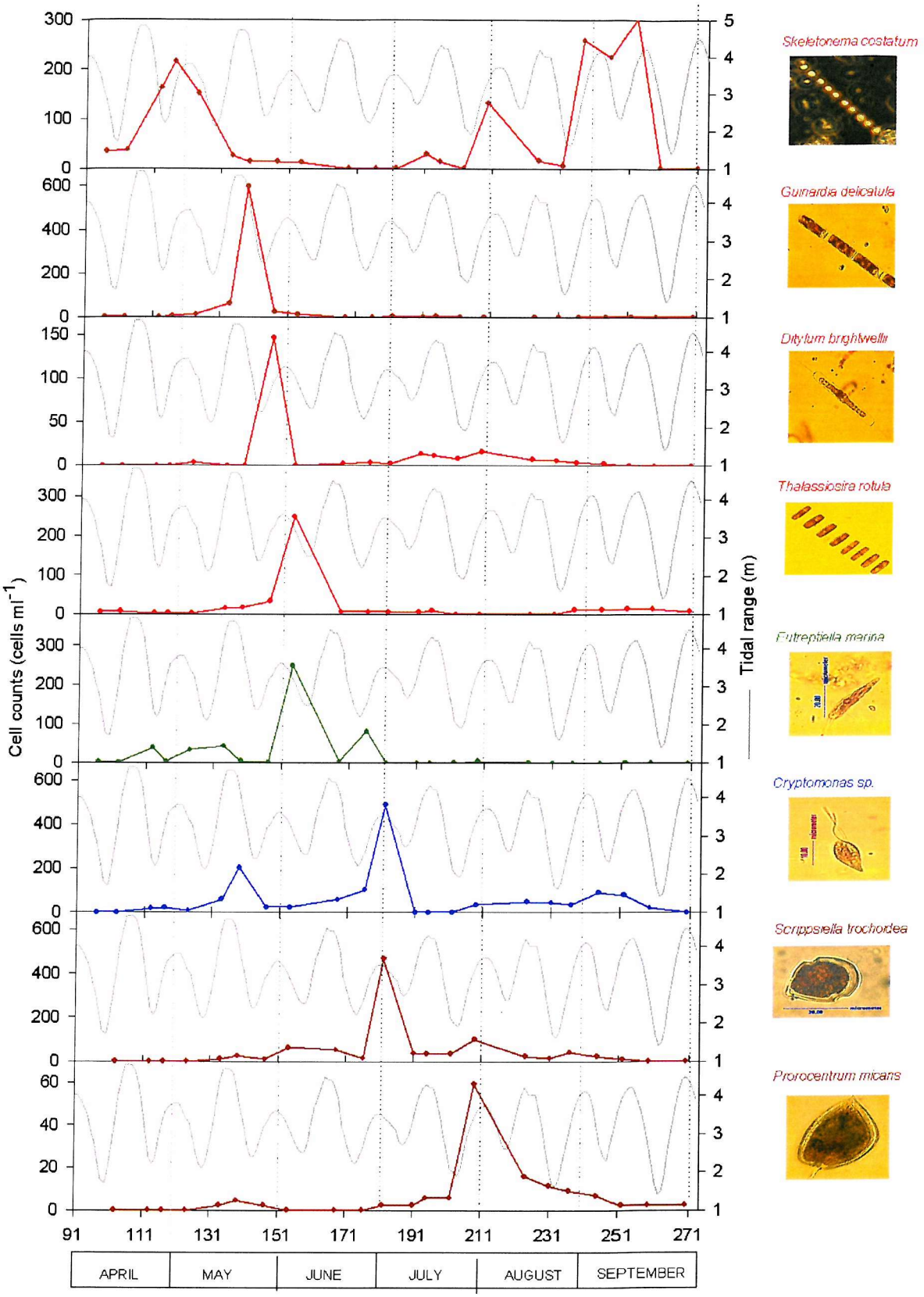


Figure 3.13. Time series plot of numerically dominant phytoplankton succession of diatoms, flagellates and dinoflagellates (expressed as number of cells ml^{-1}) at the Dock-Mooring site from April-September 1999.

3.5.2 CARBON: CHLOROPHYLL A RATIO

Figure 3.12 shows the variations in phytoplankton biomass (determined from cell counts as mg C m^{-3}) in relation to total Chl *a* (as mg Chl *a* m^{-3}) for surface water samples collected from the Dock-Mooring site in 1999. C: Chl *a* ratio ranged between 11 and 70 (from April-September, see figure 3.9b). At the time diatoms dominated the phytoplankton community, C: Chl *a* ratio varied from 18 to 22. On some sample dates (for example, 13th April, 4th May and 27th May) when a mixture of diatoms, flagellates and/or photosynthetic ciliates occurred (see figure 3.9b & 3.10) values of C/ Chl *a* ranged from 29 to 42 (figure 3.12). Highest C: Chl *a* ratios (35-70) were estimated when dinoflagellates were the most dominant organisms with a maximum ratio (70) at the time of the bloom of *Scrippsiella trochoidea* in early July (figure 3.12).

3.6. DISCUSSION

3.6.1 CONTINUOUS MONITOR IN SOUTHAMPTON WATER

A high frequency sampling programme is required for accurate and reliable studies of phytoplankton population dynamics in estuaries, which are known to be highly variable environments. However, frequent sampling programmes for phytoplankton have rarely been used in coastal waters (e.g. Winter et al., 1975; Roden, 1984; Sournia et al., 1987; Roden et al., 1987 & Roden, 1994) and estuaries (Sinclair, 1978; Rendell et al., 1997; Allen et al., 1998). Continuous measurements of Chl *a* were previously made in Southampton Water (Wright et al., 1997) during 1996 and 1997 using a data buoy deployed in the lower estuary at Hamble Oil Jetty; the floating buoy system included a fluorometer and other sensors whereas the current study made use of continuous monitoring of phytoplankton chlorophyll using a fluorometer attached to a coastal monitor, at a fixed position in Southampton Water (at the entrance to the Empress Dock, close to the confluence of the Itchen and Test estuaries). The continuously monitored data (from the CLM-2 coastal monitor) in comparison to discrete water samples (current study) provided information on short-term variations in environmental factors that influenced phytoplankton growth and bloom initiation in Southampton Water in spring/summer 1999 and also give a quantitative identification of phytoplankton bloom development and the time of maximum growth (Holley & Hydes, 2002).

Despite the fact that absolute measurements of Chl *a* are quiet difficult to achieve (Jeffery et al., 1997), calibration results of the continuous data compared with the discrete measurements (figure 3.15) showed a good correlation ($r = 0.87$; $p < 0.01$) over the study period. Despite this agreement between both methods, the calibrated chlorophyll fluorescence data does not always show a close comparison with the discrete surface chlorophyll measurements (figure 3.15). For example, in

June (day 154) and early July (day 181) the chlorophyll fluorescence peaks do not coincide with the peaks of discrete surface chlorophyll due to high concentration of flagellates in surface waters of the estuary. These peaks were dominated by the euglenoid *Eutreptiella marina* (in June), the dinoflagellate *Scrippsiella trochoidea* and the flagellate *Cryptomonas* (in July). This could be explained by the fact that the sensor, which is at a fixed height, 1 meter above the bottom of the estuary, will not reflect the chlorophyll level in surface waters if the phytoplankton are not well mixed. The euglenoid *E. marina* is known to show maximum cell density in surface water layers, even during periods of relatively stormy weather and strong tidal currents (Kifle, 1992). In 1988, *E. marina* and *S. trochoidea* showed well-marked stratification with near-surface- maxima in mid estuary (NW Netley) during May-June (Kifle, 1992).

A good agreement is also obtained by comparing the Chl *a* readings from the Dock-Mooring monitor and that obtained from the 'ferry box', a further coastal monitor placed on the Red Funnel ferry (Holley & Hydes, 2002). Results from the Ferry-Box fluorometer confirmed that the chlorophyll fluorescence was high throughout Southampton Water around the time of the main diatom bloom from day 131 (around 11th May) as recorded by the Dock-Mooring monitor as well as the discrete surface water samples. It was also helpful in predicting the summer bloom, as the Dock-Mooring sonde had to be removed for repair between 28th July and 6th August, during which time the Ferry-Box sensors clearly detected an increase in fluorescence values (Holley & Hydes, 2002) around day 208 (27th July). The Ferry-Box data showed the summer dinoflagellate blooms peaked around 27th July. This bloom, of mainly of dinoflagellates, was then confirmed in the discrete water samples with a maximum Chl *a* of about 14 mg m^{-3} on 28th July.

A consistent pattern was recorded between salinity (calibrated values) and water depth (pressure sensor) over the daily tidal cycle (figure 3.14a). Figure 3.14b shows the changes in tidal height with calibrated Chl *a* concentration. Three peaks of Chl *a* were clearly recognized in each tidal cycle (figure 3.14b) with repeatable increase/decrease in Chl *a* levels during the maximum and minimum tidal movement. Maximum values coincided with the period before the young flood stand, the period between young flood stand and first high water and period over second high water. It is likely these increases in chlorophyll which occur during periods of maximum tidal flow are due to the diatoms being re-suspended in the water column with lower values detected by the sensor during periods of slack water when cells will sink i.e. during young flood stand and between first and second high water.

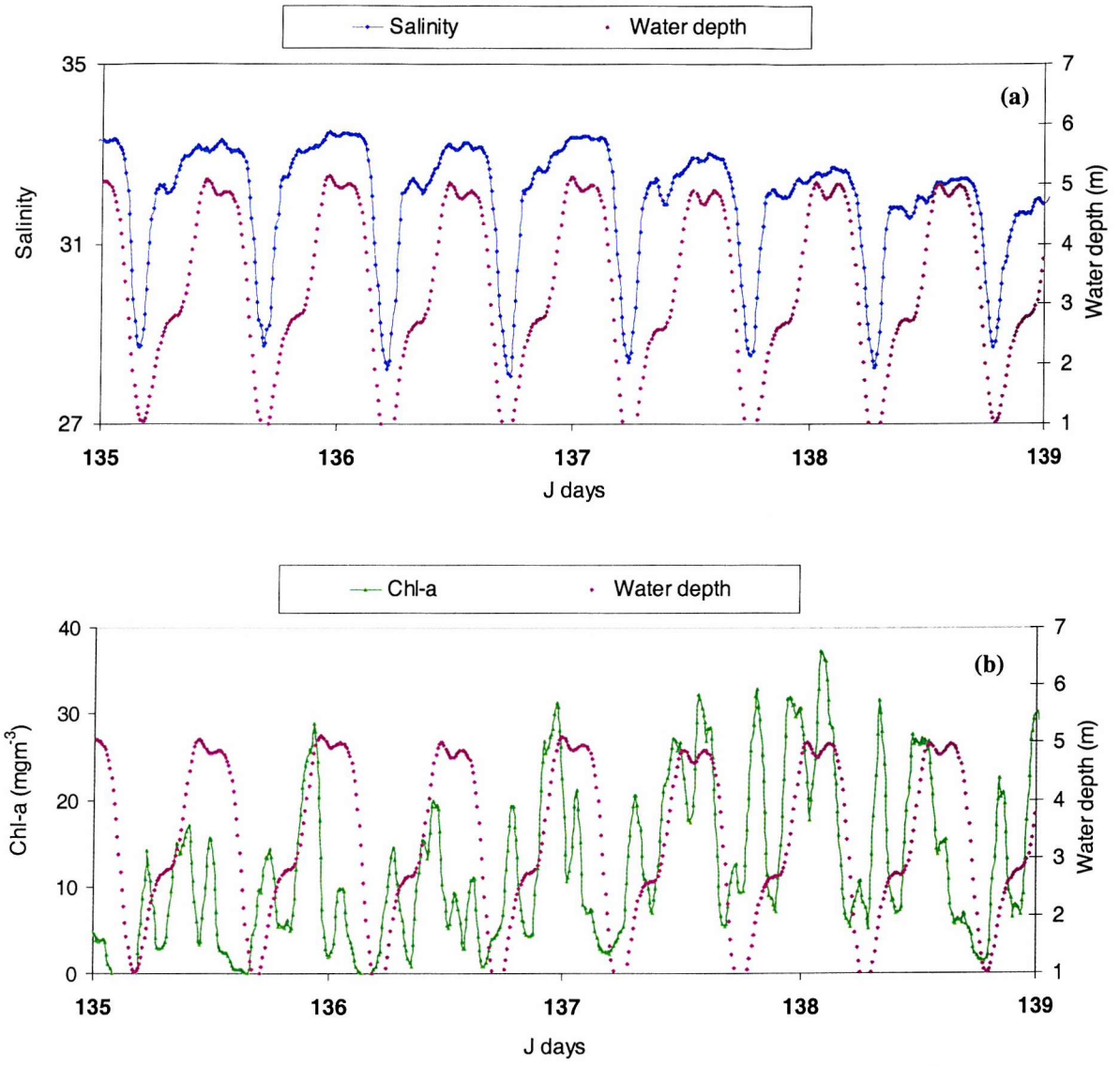


Figure 3.14. Plot showing changes in calibrated salinity values (a) and Chl *a* as mg m^{-3} (b) in relation to tidal signals (obtained from pressure sensor) during the period of the main spring bloom from day 135-139 (15^{th} - 19^{th} May 1999).

3.6.2 SEASONAL DISTRIBUTION OF NUTRIENTS AND CHL A

Several peaks in Chl *a* were recorded at the Empress Dock site through spring and summer in 1999 with smaller peaks in April and early May followed by the highest Chl *a* concentration around mid May (figure 3.15). Sequences of small peaks followed the main May bloom until the end of September, with relatively higher peaks in early June and end of July. Nutrient data (figure 3.7) indicated that surface water phytoplankton in the Southampton Water estuary are not nitrate or silicate limiting during the sampling period (April-September 1999), while phosphate was undetectable during the main spring bloom. Phosphate, like all nutrients, showed conservative like behaviour in Southampton Water and may decrease to undetectable levels at salinity ranges >34 (Hydes & Wright, 1999).

The "Southern Nutrients Study" (SONUS) conducted in Southampton Water between 1995 and 1997 (Hydes & Wright, 1999) showed that nutrient concentrations were high enough to support phytoplankton growth within the estuary throughout the whole year while, during periods of depleted nutrients due to either reduced river input in summer (Holmes et al., 2000) or increased biological activity (Kifle, 1992), additional nitrogen can be derived from benthic regeneration (Holmes et al., 2000). Lowest phosphate values in surface water at the Dock-Mooring in the lower Itchen estuary were measured during the third week in May 1999 immediately after the diatom bloom, however both nitrate and silicate dramatically declined again in mid July during the dinoflagellate bloom. This might have also resulted from the dilution effect of the low nutrient saline water (salinity was 32.7) on 14th July.

Relatively low concentrations of both silicate and nitrate were measured during the first week of June during the bloom period of the diatom *Ditylum brightwellii and Thalassiosira* sp. Despite the noticeable increase in cell numbers of the small-celled diatom *Skeletonema costatum* in early spring, its effect on silicate concentration was not as dramatic as that of the relatively large-celled *G. delicatula*. The seasonal trend (figure 3.8a, b and c) recorded between nutrient concentration (nitrate, silicate and phosphate) and salinity (as an indicator of the extent of the freshwater dilution) reflects that riverine nutrient-rich freshwater is the main source of these nutrients, with a degree of biological removal due to the growth of phytoplankton. It was previously suggested by Hydes & Wright (1997) that all nutrients particularly nitrate, phosphate and silicate behave conservatively in Southampton Water, however in spring and summer at the time of intense bloom conditions (i.e. nutrient removal) concentration of nutrients decreased and re-established during winter months (Kifle & Purdie, 1993; Hydes & Wright, 1999). A similar trend of seasonal variations in nutrient and Chl *a* concentrations were previously observed in Southampton Water in earlier studies (e.g. Antai, 1989; Kifle & Purdie, 1993; Iriarte & Purdie, 1994; Proenca, 1994; Hydes & Wright, 1999).

3.6.3 DEVELOPMENT OF PHYTOPLANKTON BLOOMS AND SPECIES SUCCSESSION

The time series of mean daily Chl *a* values (figure 3.15) and phytoplankton analysis (figure 3.12 & 3.13) show that a series of phytoplankton blooms developed in the lower Itchen estuary with different amplitude and different species during spring/summer in 1999. Smaller growth peaks (<3 mg Chl *a* m^{-3} & < 200 mg C m^{-3}) occurred in April and early May, followed by the main spring bloom (21 mg m^{-3} & ~600 mg C m^{-3}) around 17th-19th May 1999 (day 137-139). The early spring blooms (April-early May) were numerically dominated by the relatively small-sized diatom *Skeletonema costatum* and, to a lesser extent, the flagellate *Eutreptiella marina*. The numerical increase in *Skeletonema costatum* cell numbers (up to 0.2 x 10^3 cells ml^{-1}) occurred during the transition from neap-to-spring period. *S. costatum* was previously recorded in Southampton Water (April-May) with much higher (up to ~4x 10^3) cell concentration during

winter-early spring months (Kifle, 1992). *Skeletonema costatum* is known to have a high growth rate and short lag phase (Kifle, 1992) which helps it to survive and develop into a bloom, out-competing other species particularly during periods of short residence time. The diatom *Guinardia delicatula* followed the *Skeletonema costatum* bloom and peaked around day 137-139 with a maximum cell number reaching $6x10^4$ cells ml^{-1} (current study) compared to $4.3x10^3$ cell ml^{-1} in 1995 (Lauria, 1998). This main spring chlorophyll peak dominated by *Guinardia delicatula* was maximal over a spring tide (figure 3.15) in contrast to the previous findings of Wright et al. (1997) and Hydes & Wright (1999) where peaks in chlorophyll fluorescence were shown to occur during neap tides in Southampton Water. This species has been previously recorded in Southampton Water forming blooms during May in 1988 (Kifle, 1992), in 1992, 1993 (Anning, 1995) and in 1995 (Lauria, 1998) and is known to form large populations, particularly in spring (Sournia et al., 1987; Peperzak et al., 1993) and sometimes in winter, e.g. Vineyard Sound (Glibert et al., 1985), off Roscoff in the English channel (Sournia et al., 1987), and in the Dutch coastal zone of the North Sea (Peperzak et al., 1993).

The fact that *G. delicatula* prefers relatively higher water temperatures compared to that for *Skeletonema costatum* (Grall, 1972 cited in Kifle, 1992; Furnas, 1990; Kifle, 1992) might explain its dominance of the late spring phytoplankton bloom. Laboratory studies have confirmed that *G. delicatula* grows optimally at a temperature range of 13-14^{0}C and at a salinity value of 34-35 (Grall, 1972, cited in Kifle, 1992; Kifle, 1992). The temperature and salinity (although, it was relatively lower than that previously recommended value of ~34) conditions at this time of the year were at optimum levels for this diatom to grow and for a bloom to develop (figure 3.17).

Light data (see figure 3.15, 3.16) indicated that the mean water column irradiation level in Southampton water was frequently greater than the critical level (~100 W h m^{-2} d^{-1}) required by phytoplankton for balanced growth (Peperzak, 1993) during the period of this study; however, it was not at a maximum level immediately prior to the May bloom compared to values recorded latter in the summer months. From day 131 to day 139 a sunny period (figure 3.1) followed a short period of relatively low incident light (around days 126-130) and resulted in a persistent increase in mean water column irradiance (figure 3.15) of over 200 W h m^{-2} day^{-1} from day 130-135 (figure 3.17). On day 133 the main spring diatom bloom started as recorded by the continuous fluorometer data and peaked around days 137-139. An optimum irradiance level of 180-200 μE m^{-2} s^{-1} was recorded during laboratory studies (Grall, 1972) for maximum growth of *G. delicatula.* Vertical irradiance profiles (figure 3.4) indicated a maximum photic layer of ~3–6m measured in May before the main spring diatom bloom. This range of photic layer depth was suitable enough for phytoplankton to grow and develop into a bloom. A similar finding was reported in a study using a continuously monitoring fluorometer in the Humber plume by Allen et al. (1998). Allen et al. (1998) noted that the onset of net phytoplankton growth occurred when the

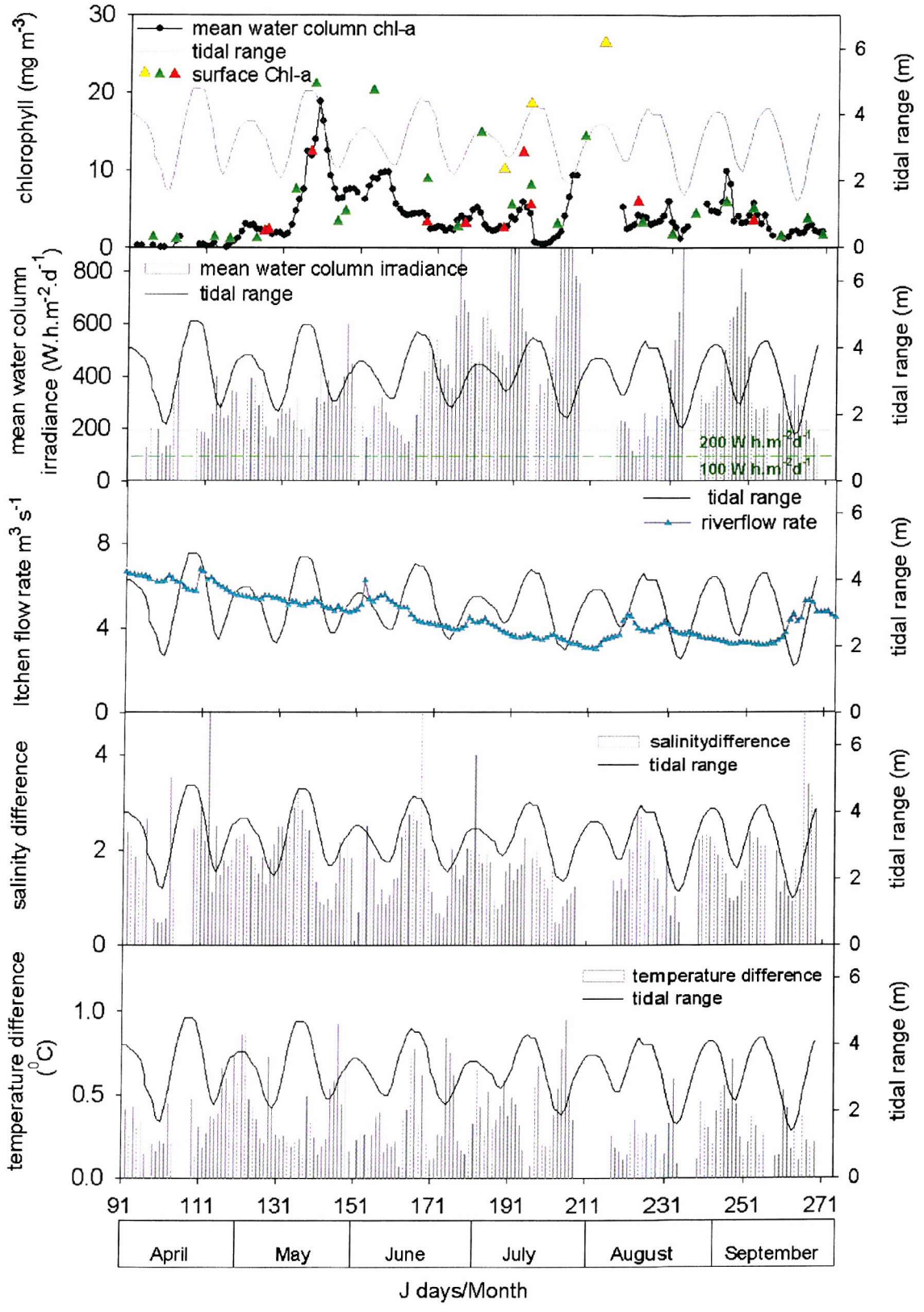


Figure 3.15. Seasonal variation in surface Chl-a concentrations in relation to variations in daily mean water column irradiance, river flow, salinity difference and temperature difference at the Dock-Mooring site from April-September 1999.

euphotic layer was > 15% of the water depth.

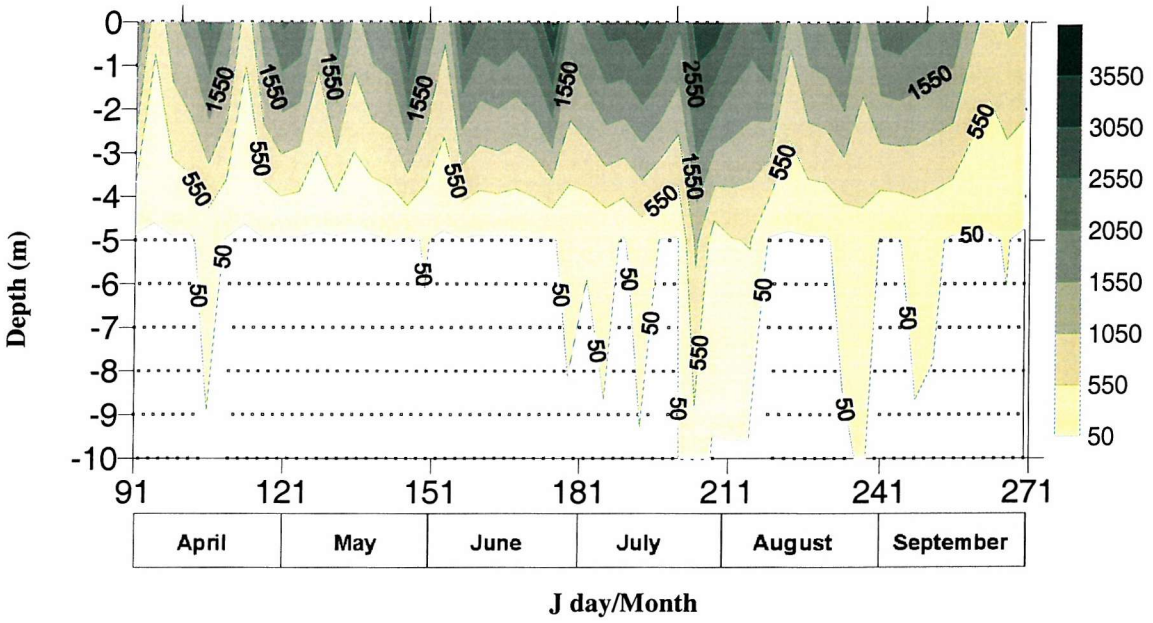


Figure 3.16. Contour plot of vertical irradiance showing seasonal variations in light conditions throughout water column at the Dock-Mooring site. Values calculated from surface incident light values (PAR) and the attenuation coefficient (*k*).

Stratification, which can be a pre-request of bloom initiation (Cloern, 1996), could be another explanation for the exceptional diatom bloom that developed in mid May. A degree of stratification indicated from the within day salinity differences was observed (figure 3.17d) resulting from the slight increase in fresh water flow of the River Itchen (figure 3.17b) during the period of the May bloom (136-139). The water column may have been stratified for a period long enough for a bloom to develop offsetting the effect of both tidal and wind stirring (Cloern, 1996). Moreover, the meteorological data, obtained from the coastal monitor showed relatively good weather with high barometric pressure (more than 1020) and low wind speed (below 5 ms^{-1}) and warm water (13-14 ^0C) at the time of the main spring bloom (Holly & Hydes, 2002).

Phytoplankton blooms are often coincident with neap tides, during which calm physical conditions and reduced tidal exchange promote the development of a bloom. For example, Winter et al. (1975) found that spring blooms in Puget Sound (USA) occurred on neap tides, when reduced tidal currents resulted in stratification. Observations collected at two stations on the coast of Connemara (Roden, 1994) showed that the highest chlorophyll *a* was measured at neap tides during spring. However, similar exceptional diatom blooms, also coincided with a spring tide of 5.2 m height, was observed in late summer on the coast of Connemara (Roden, 1994). Observations made by Balch (1981) in the coastal waters (off the coast of Maine) supported the hypothesis of diatom blooms peaking during spring tides. Balch (1981) showed that the summer

diatoms bloom, occurred at major spring tides and related this phenomenon to the increased nutrients or to the upward movement of a subsurface chlorophyll layer. Balch (1981) also noted that chlorophyll values were positively associated with low temperatures measured at the time of the bloom.

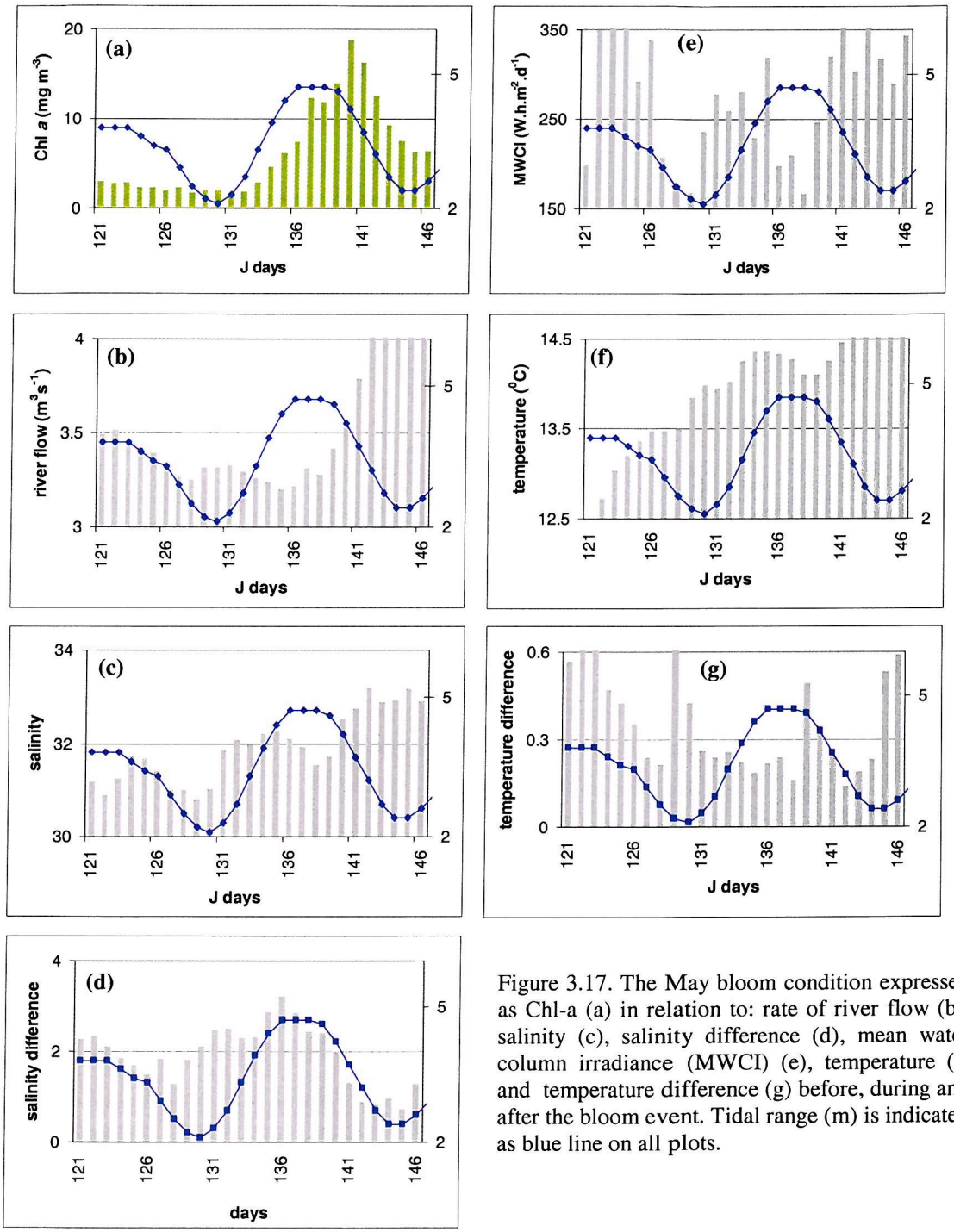


Figure 3.17. The May bloom condition expressed as Chl-a (a) in relation to: rate of river flow (b), salinity (c), salinity difference (d), mean water column irradiance (MWCI) (e), temperature (f) and temperature difference (g) before, during and after the bloom event. Tidal range (m) is indicated as blue line on all plots.

Many of the species identified in the discrete water samples collected throughout the spring/summer period at the Dock-Mooring site showed increasing cell abundance from neap to spring tides (figure 3.13) with subsequent reduction detected in cell number immediately after the peak spring tide. This observation is also reported by Kifle (1992).

Between days 139-147, at the time of the maximum diatom peak (figure 3.15), a noticeable decline in daily surface irradiance (figure 3.1a) as well as the mean water column irradiance (figure 3.15, 3.16 & 3.17) occurred. Accordingly, the attenuation coefficient, *k*, increased to a value of ~1.9 m^{-1} followed by reduction in the depth of the photic layer to < 2 m (figure 3.16). These unfavourable irradiance conditions may have consolidated to the termination of the diatom bloom (dominated by *Guinardia delicatula).* Similar changes in irradiance levels were observed in the Dutch coastal zone of the North Sea by Peperzak et al. (1993) after the spring bloom (diatoms and *Phaeocystis*) in 1992.

Following the decline of the diatom *G. delicatula* an increase in cell numbers of the diatom *Ditylum brightwellii* (up to ~150 cells ml^{-1}) was recorded (figure 3.13), which lasted only for a short period (less than one week). The noticeable reduction in nutrients, particularly silicate, and irradiance following the *Guinardia delicatula* bloom could be a reason of the short-period of growth of the *D. brightwellii.* Some evidence of an inverse relationship between Chl *a* concentrations and light attenuation values (*k)* have been found (figure 3.3 & 3.5a), indicating turbidity, in part, as a limiting factor, particularly during bloom periods, as previously recorded by Kifle (1992). The May peak in Chl *a* was followed by sequence of relatively smaller phytoplankton blooms at the Dock-Mooring with a mixture of different phytoplankton species. The flagellate *Eutreptiella marina* peaked on day 154 and co-occurred with the centric diatom *Thalassiosira rotula* (with relatively small-sized cells, 20-30 μm). *T. rotula* is known to be a shade adapted species and prefers low irradiance (Bonin & Maestrini, 1981) compared to *G. delicatula* (Kifle, 1992). Si shortage, which normally occurs after diatom blooms, can lead to a succession from a diatom-dominated community to non-Si-requiring species (e.g. flagellates) (Cadee & Hegemann, 1991; Conley & Malone, 1992; Egge & Aksnes, 1992). For example, Prymnesiopyceae, (e.g. *Phaeocystis* sp.), which do not require Si tend to bloom after the spring diatom peak when $Si(OH)_4$ has been depleted (Reid et al., 1990; Peperzak et al., 1993) since these organisms cannot compete with diatoms for N and P. *E. marina,* which is a non-Si-requiring organism, started to peak around day 154 for a short period (less than a week). It is known to be a short-lived organism (Kifle, 1992) although it has attained cell concentrations in Southampton Water as high as ~1600 cell ml^{-1} (Kifle, 1992) and ~550 cell ml^{-1} (current study). Kifle (1992) speculated that the increase in *E. marina* cell numbers at this time is due to the increased concentration of organic matter following the decline of the diatom *G. delicatula.* Increased chlorophyll fluorescence readings were detected by the fluormeter sensor during this bloom (in early June), however much higher Chl *a* measurements were made in discrete surface water samples of lower salinity (figure 3.15) resulting from the increase in river flow, which occurred around the bloom period. Chl *a* reached its highest value 4 days after the weak spring tide (on 1st June) during the period of decreasing dispersion that normally occurs by tidal forcing (Holley &

Hydes, 2002). This peak (3rd June) only lasted for a short period (less than one week) probably due to changes in weather. River flow increases are indicative of increased rainfall after day 151 (figure 3.2) with lower daily incident irradiance levels. Figure 3.1 shows signals of clear decline in water temperature, which confirms poor weather conditions at the time of the June bloom termination. Wright et al. (1997) also noted that the water temperature recorded from the SONUS data buoy could reflect the changes in weather. Phytoplankton growth responds differently to temperature according to the community structure and the growth season. Roden (1994) positively correlated higher averaged chlorophyll content with warm water, however it has also been noted that Chl *a* can be positively associated with low temperatures (Balch, 1981).

In early summer a succession of Chl *a* peaks was recorded by the fluorometer sensor at the coastal monitor. Between day 167 and 180 the dinoflagellate *Scrippsiella trochoidea*, the small flagellate *Cryptomonas* sp., and the ciliate *M. rubrum* were the most dominant species, forming small peaks of surface chlorophyll biomass of < 8 mg m^{-3} (mean water column chlorophyll of < 5 mg m^{-3}) and carbon biomass ranged between 120-250 mg C m^{-3} in surface waters during June. In summer months, the sunny weather and long day light hours resulted in an increase in the surface incident light and a consequent increase in mean water column irradiance (~350 up to 1230 W h m^{-2} d^{-1}) as well as in the photic layer depth (5->10 m), particularly immediately before the main summer blooms. The increased irradiance conditions are preferable for dinoflagellate species to grow and for a bloom to develop. Discrete water samples indicated the presence of a relatively high peak in Chl *a* of approximately 14 mg m^{-3} on 1st July (day 182). The dinoflagellate *Scrippsiella trochoidea* (> 900 mg C m^{-3}) was the main component of this bloom, comprising > 90% of the total dinoflagellate biomass and > 75% of total phytoplankton biomass on this sampling date. However, when the Coastal Monitor had to be removed for repair with no data available between 28th July and 6th August, the Ferry-Box data (Holley & Hydes, 2002) helped to identify a summer bloom which peak around 27th July. Tidal stirring in Southampton water is too great to allow development of a thermocline even during summer (Holley & Hydes, 2002), however a degree of stratification was indicated by the higher within day temperature differences recorded on neap tides, particularly during the period from the end of June to end of July (figure 3.15). These slightly more stratified conditions will have helped the summer dinoflagellate bloom initiation (figure 3.15). At the time of the maximum dinoflagellate peak the mean water column irradiance decreased to a value of < 400 W h m^{-2} d^{-1} and was followed by a remarkable decline in carbon biomass (up to 14% of the peak value) and chlorophyll from day 198 to day 202. Dinoflagellates proliferated again to a secondary bloom around day 209 when the mean water column irradiance reached values of >700 W h m^{-2} d^{-1}. This peak recorded by both discrete chlorophyll measurements and from the Dock-Mooring fluorometer was maximal between neap and spring tides (figure 3.15). Dinoflagellates in Southampton Water appeared to bloom in

summer during periods of calm water accompanied by a reduced tidal range i.e. neap tide (Lauria, 1998) and high irradiance levels (Kifle, 1992). Microscopic analysis revealed that *Scrippsiella trochoidea* and *Prorocentrum micans* dominated this peak, however the former species contributed more to the total phytoplankton biomass (>95%) in early July.

Because of the low growth rate of dinoflagellates, in general (White, 1976) and *S. trochoidea* in particular, (Kifle, 1992), they can not compete with other fast growing species; mainly diatoms that dominate the winter and earlier spring bloom. During summer the water column becomes more stable as the river flow declines and rainfall is reduced, this will also cause increased sedimentation of diatoms out of the photic zone, where light becomes a limiting factor. Moreover, increasing light levels in summer is another cause of increasing sinking rate of diatom cells (Titman & Kilham, 1976 cited in Kifle, 1992; Margalef, 1978). In contrast, dinoflagellates are able to regulate their position within the water column and can move up and down to optimize light and nutrient conditions (Lauria, 1998). Most of the laboratory studies involving dinoflagellates (White, 1976; Pollinger & Zemel, 1981; Thomas & Gibson, 1990; Berdalet, 1992; Berdalet & Estrada, 1993; Thomas et al., 1995) have concluded that dinoflagellates bloom in calm water conditions and demonstrated a direct effect of turbulence on cell growth, cell division and physiological behavior.

To conclude, the spring diatom bloom coincided with increased levels of daily irradiance and reduced water column turbidity but appeared to be independent of the spring- neap tidal state; whereas the summer dinoflagellate bloom coincided with both high daily irradiance and reduced mixing accompanied with the neap tide condition. In the light of Margalef's conceptual model (Margalef, 1978, figure 1.2), phytoplankton species succession in Southampton Water appears to follow a "typical" trend from high-turbulence–high-nutrient conditions in spring favoring diatoms to reduced-turbulence–low-nutrient conditions favoring dinoflagellates. A similar pattern of phytoplankton species succession has been previously recorded in Southampton Water (Kifle, 1992; Kifle & Purdie, 1993; Howard et al., 1995) and in other similar macrotidal estuaries, e.g. Peconic Bay (Bruno et al., 1980), in the lower Westerschelde (Tripos, 1991) and inner Oosterschelde (Bakker et al., 1994). Transition or intermediate conditions of low-turbulence–high-nutrients may also exist; and these appear to be preferable for organisms like *Mesodinium rubrum* and *Eutreptiella marina*. The planktonic phototrophic ciliate *Mesodinium rubrum* was recorded in low cell concentrations at the Dock-Mooring site (3.5-10 cells ml^{-1}) during this study in summer 1999 but was detected in high cell numbers by Dubois (1999) at WS in end of July. The same ciliate has formed dense blooms in summer throughout Southampton Water during 1984–1996 (Soulsby et al., 1984; Crawford & Purdie, 1992; Iriarte, 1991; Crawford & Lindholm, 1997; Crawford et al., 1997; Lauria, 1998).

CHAPTER FOUR

CHAPTER FOUR

4- SPATIAL DISTRIBUTION OF PHYTOPLANKTON BIOMASS, ABUNDANCE AND SPECIES COMPOSITION ALONG A SALINITY GRADIENT OF THE ESTUARY

4.1 INTRODUCTION

Few studies have been previously conducted to investigate the distribution of phytoplankton biomass and species composition relative to the salinity gradient in Southampton Water. Most of these studies were only confined to a part of the estuary, e.g. two stations (Iriarte, 1991 & Kifle, 1992), three stations (Proenca, 1994) or five stations (Lauria, 1998). In the present research, an intensive spatial sampling programme has been undertaken to determine variability in phytoplankton distribution, abundance and community structure throughout Southampton Water to investigate their possible impact on nutrient concentrations throughout the estuary.

An intensive spatial (one-day) sampling program (13-17 sites) was carried out throughout the estuary from more estuarine sites in the lower Itchen (Itchen Bridge to SG1) and Test estuaries (from Eling to Hythe Knock) to more coastal stations in Southampton water (from Western Shelf down to Calshot). These sites were sampled on 10th June & 22nd July 1999 and on 15th August 2000 with the aim of sampling during phytoplankton bloom conditions. The sampling locations of the study area are shown in figure (1.3).

4.2 TIDAL CYCLE

The predicted tidal range for Southampton Water, taken from Admiralty Tide Tables, during the productive months (May-August) in 1999 and 2000 is given in figure 4.1a & 4.1b, respectively. Tidal range varied from 4.1-4.7 m and 4.2-4.5 m during peak spring tides in 1999 and 2000 respectively to the lowest neap values of 1.9-2.2 m and 1.8-2.3 m in both years respectively.

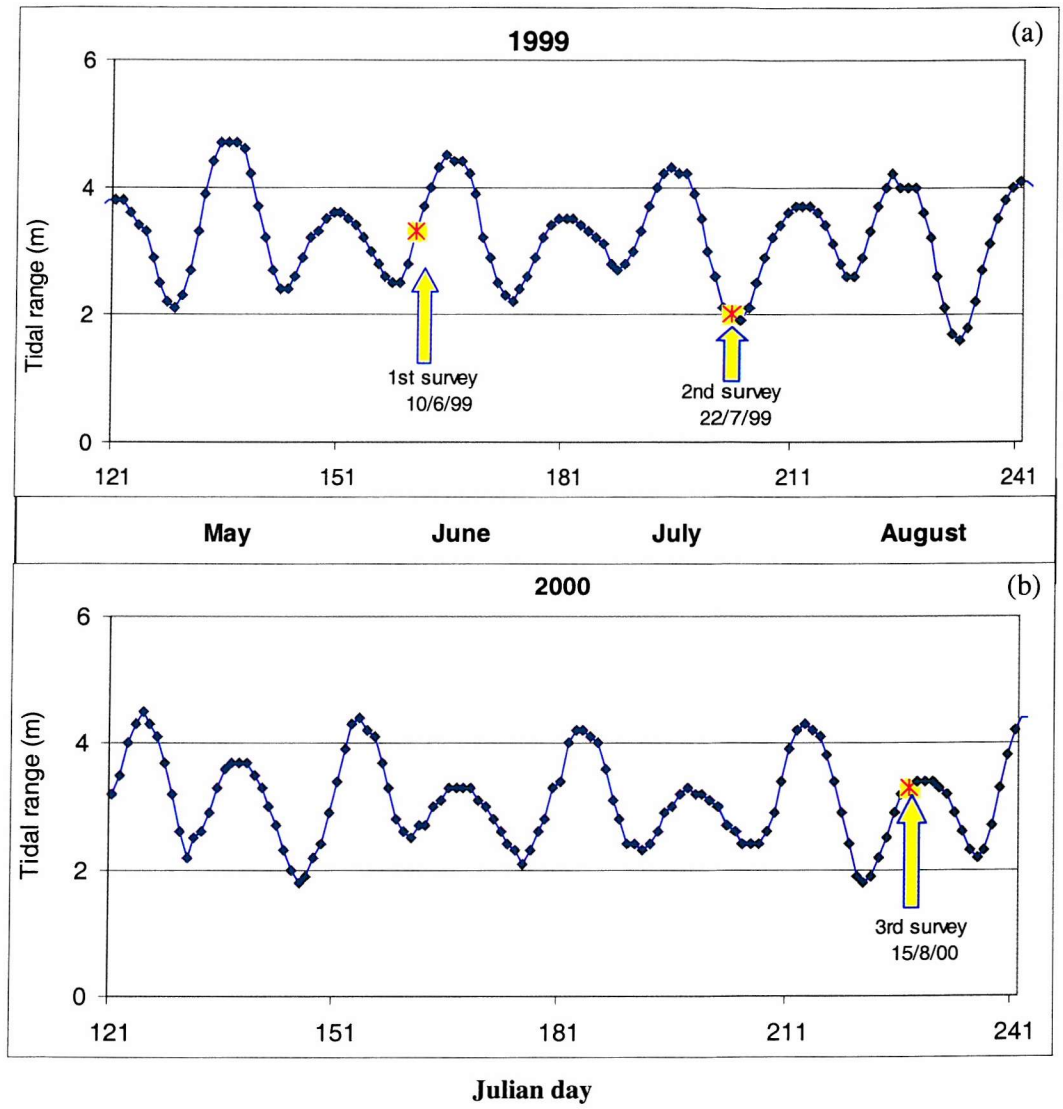


Figure 4.1. Predicted tidal range data in Southampton water during the main productive period (May-August) in 1999 (a) and 2000 (b). Yellow symbol indicates tidal state during the day of each survey.

4.3 10th JUNE 1999

4.3.1 CTD PROFILES

Data derived from CTD profiles, temperature (figure 4.2a), salinity (figure 4.2b), density (figure 4.2c), attenuance and chlorophyll (4.3a & b) were combined to produce a longitudinal view of the estuary with the spatial changes of these variables throughout the Test and Itchen estuaries and Southampton Water on one day (10/6/99).

Vertical profiles of temperature were similar at most of the sampling sites during the survey on 10th June 1999. Water temperature ranged from 15.8 – 16.3 °C with no obvious thermal stratification (figure 4.2a). Vertical temperature gradient was however reduced from 0.6 °C at BB to 0.2 and 0.3 °C at IB and EL, respectively. Maximum water temperatures (16-16.3 °C) were recorded in surface waters at CR, BB and HP with warmer water extending to 7 m depth at the later station.

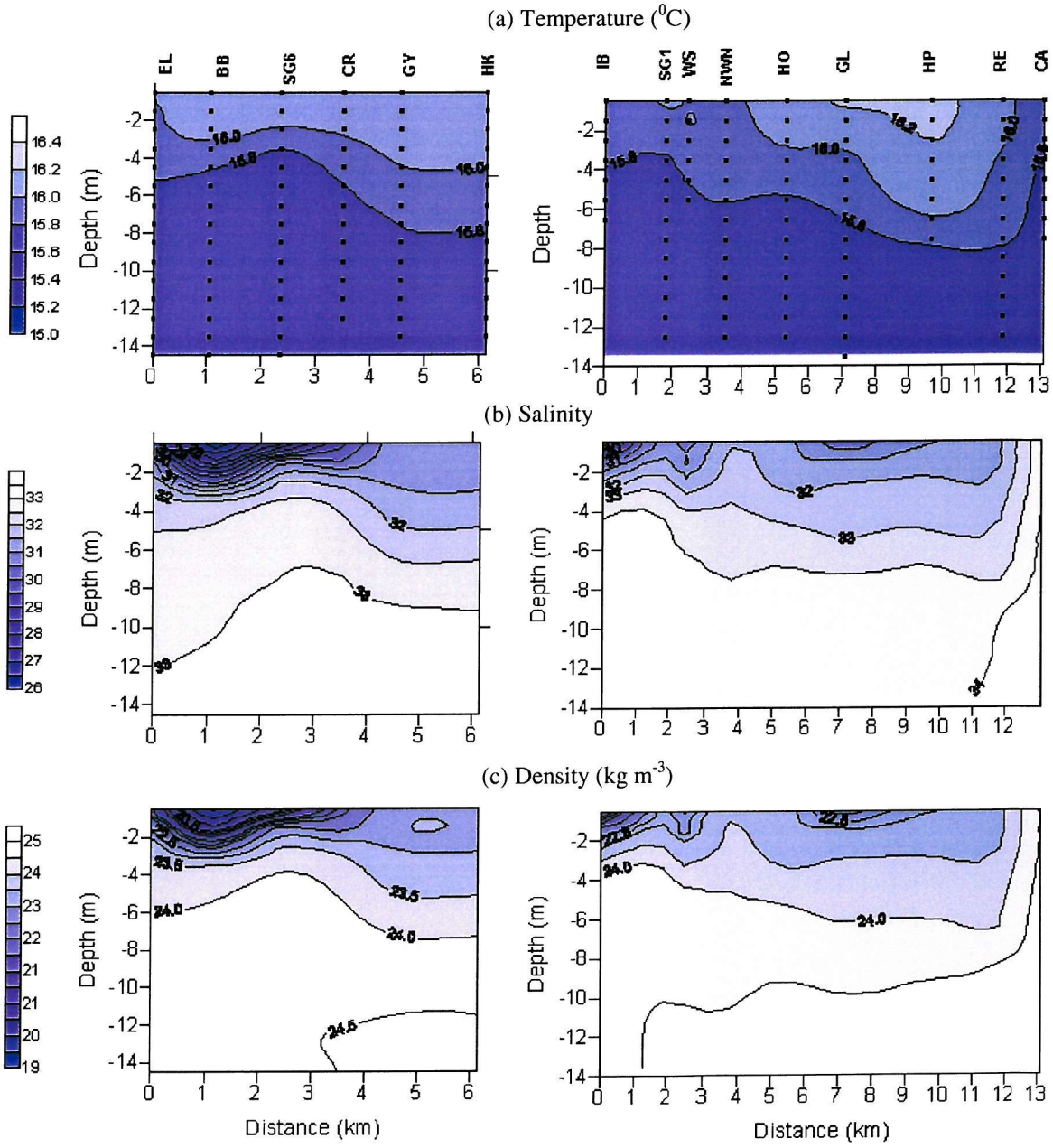


Figure 4.2 Vertical profiles of: (a) temperature, (b) salinity and (c) density throughout Southampton Water on 10th June 1999. See figure 1.3 for station identification.

Salinity values varied from 25.9 to 33.7 (figure 4.2b), with lower salinity values measured at the head of the estuary (stations of both lower Itchen and Test estuary) due to the fresh water input. Vertical salinity gradient reduced from 7.2 at BB to 0.3 at CA; this may be attributed to increased turbulent mixing at the mouth of the estuary. A degree of salinity stratification existed, particularly at stations influenced by fresh water input in the upper part of the estuary (i.e. estuarine sites).

Figure 4.2c is a Contour plot showing the horizontal variations of density for the sampling sites. Water of lower density values was restricted to the surface layer characterized by warmer waters of low salinity.

Horizontal variations of both transmission and chlorophyll *a* at the sampling sites are presented in figure 4.3a & b. Figure 4.3a shows low transmission values in deeper waters throughout the sampling sites are indicative of increased suspended particulate matter (SPM). A patch of turbid water was also recorded at the upper part of the estuary (mainly at EL).

Low chlorophyll surface water was recorded (figure 4.3b) in the lower Itchen estuary and Southampton Water with a patch of high chlorophyll concentration in deep water (4-10 m depth) at GL. However, lower chlorophyll concentrations existed in bottom water along the Test estuary with a peak in the surface layer at CR (figure 4.3b).

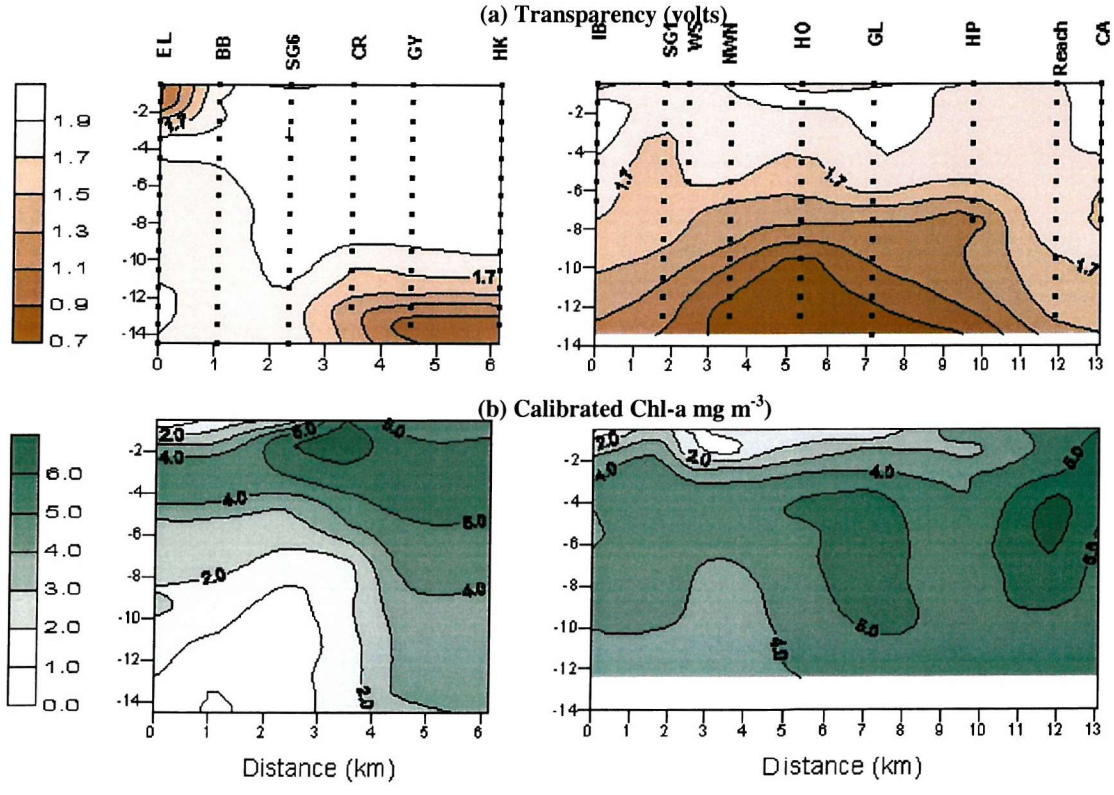


Figure 4.3. Vertical distribution of: (a) transparency and (b) calibrated Chl *a* on 10th June 1999

4.3.2 ATTENUATION COEFFICIENTS

Values of attenuation coefficient (k) were, relatively low and almost similar at all the sites (see table 4.1). The spatial distribution of light throughout Southampton water estuary is illustrated in figure 4.4. K values ranged, from 0.46-0.55 m^{-1} at sites in the Test Estuary (figure 4.4), from 0.62-0.71 m^{-1} at Itchen sites and from 0.57-0.77 m^{-1} in Southampton Water (figure 4.4).

Table 4.1. Values of attenuation coefficients (k) and 1% irradiance depth (m) throughout the estuary on 10th June 1999.

		Test Estuary					Itchen Estuary				Southampton Water						
Site	EL	BB	SG6	CR	GY	HK	NB	IB	OS	SG1	WS	NWN	HO	GL	HP	RE	CA
k (m^{-1})	0.46	0.6	0.55	0.5	0.5	0.54	0.71	nd	0.62	0.62	0.57	0.73	0.77	0.58	0.73	0.57	0.64
photic depth (m)	10.1	7.7	8.4	9.2	9.2	8.6	6.5	nd	7.5	7.5	8.1	6.3	5.5	7.9	6.3	8.0	7.1

* nd = no data

High values of attenuation coefficients were generally observed at sites of high algal biomass (represented as Chl *a*) with maximum k value (0.77 m^{-1}) measured at HO, while the lowest value (0.46 m^{-1}) was recorded at EL. The contour plot (figure 4.4) shows the % irradiation profile at the sampling sites on 10/6/99 and the depth of the 1 % light level. Data indicated that, the horizontal distribution of the % irradiation on the sampling day was almost similar throughout the Itchen estuary and Southampton Water with a photic zone (1% irradiance) ranged from 5.5 m to 8.0 m. A deeper photic layer was however recorded throughout the Test estuary with photic depth ranging from 7.7-10.1 m.

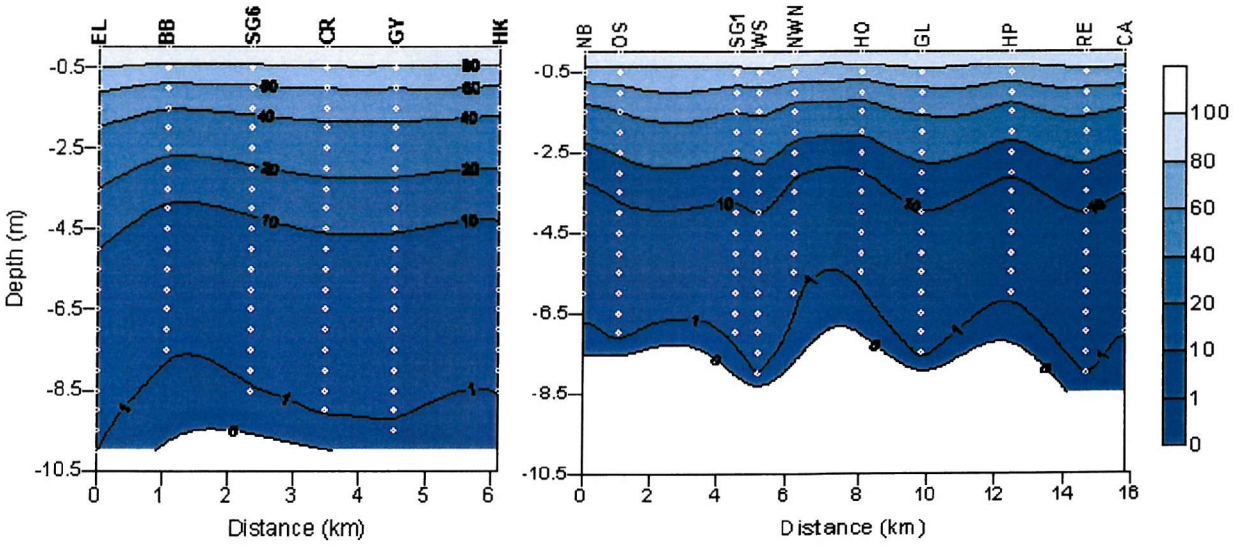


Figure 4.4. Spatial variations in % irradiation throughout Southampton Water on 10th June 1999. See figure 1.3 for station identification.

4.3.3 NUTRIENT CONCENTRATIONS

Distribution of phosphate (0-9.5 μM), nitrate (0-226 μM) and silicate (0-105 μM) concentration along the estuary for the three depths (surface, middle, and bottom) at each of the sampling sites are presented in figure 4.5. Maximum nutrient concentrations were generally measured in surface layers throughout the estuary with high concentrations in the upper Itchen estuary.

The plots of nutrient concentration and salinity measured on 10th June (figure 4.6) indicated that the highest concentrations were measured at low salinity sites (estuarine sites) due to the effect of the nutrient-rich freshwater input.

Figure 4.6 also suggests some evidence for nutrient removal, particularly nitrate and silicate at higher salinities, with more scattered data points for phosphate versus salinity due to the different phosphate sources (mainly along the Test estuary).

4.3.4 CHLOROPHYLL A

Chlorophyll a concentration measured on 10th June for the 3 depths at all sampling sites are shown in figure 4.5. Chl *a* concentrations ranged between 2.24 and 6 mg m^{-3} throughout the estuary (figure 4.5) with maximum concentrations recorded along the Test estuary (CR, GY and HK). Higher surface Chl *a* was recorded at these sites than that in deep waters opposing sites along the Itchen at which deeper waters were higher in Chl *a* concentration than surface layers (figure 4.5). With the exception of HP, all sites throughout Southampton Water were relatively well mixed with respect to chlorophyll *a* concentration. Values of Chl *a* concentration measured on 10/6/99 (surface, middle, and bottom) are plotted against the salinity of the same depths in figure 4.6 and suggesting a degree of noticeable production of phytoplankton at high salinity sites.

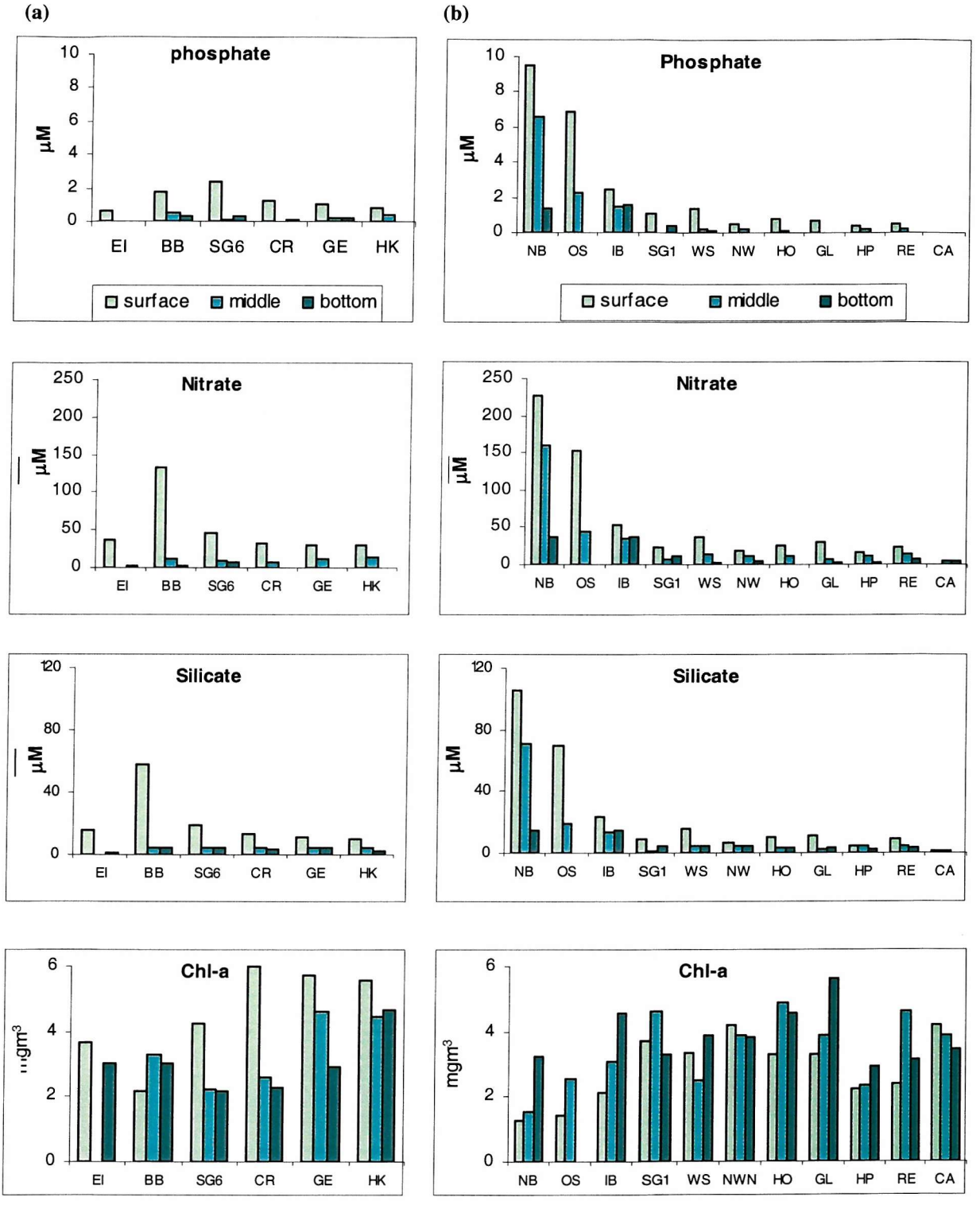
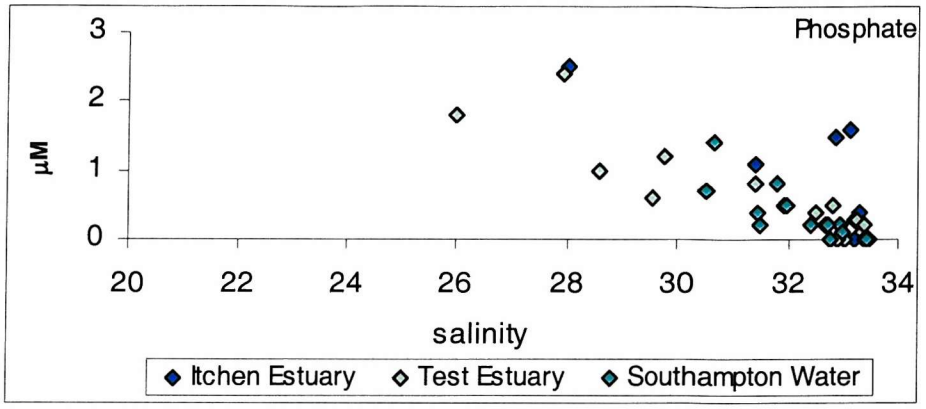
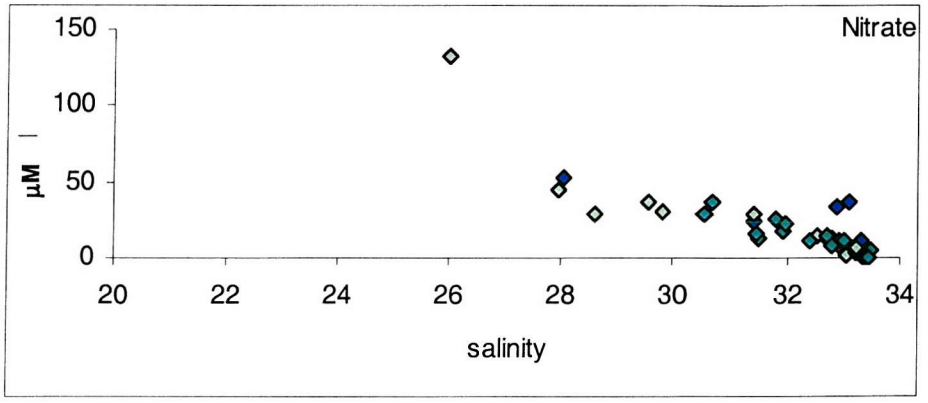
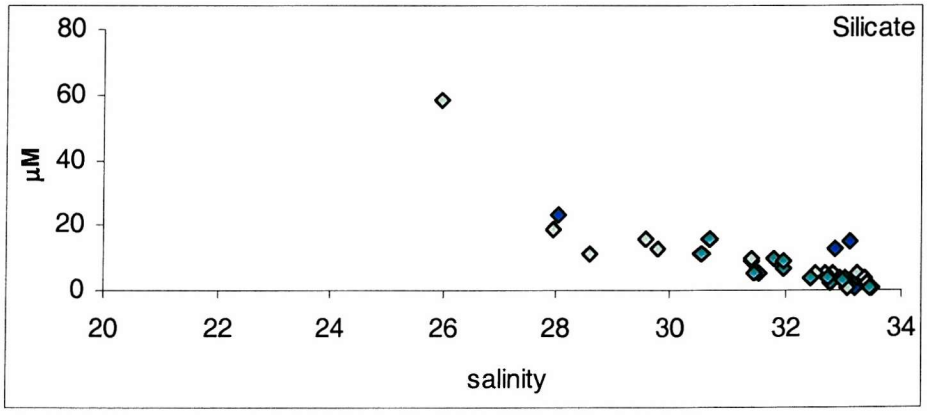


Figure 4.5. Spatial distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* throughout (a) Test estuary, (b) Itchen estuary and Southampton Water on 10th June 1999. No middle and bottom water samples for EL and OS, respectively.







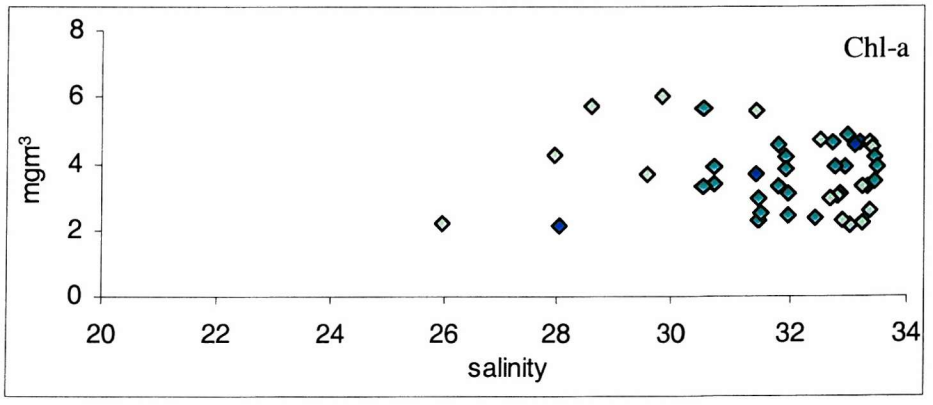


Figure 4.6. Distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* concentration against salinity along the estuary on 10th June 1999.

4.3.5 PHYTOPLANKTON DISTRIBUTION ALONG THE ESTUARY ON 10th JUNE 1999

A mixture of different phytoplankton groups were recorded in surface water samples collected along the estuary during the sampling day, of which some dominant species are shown in Appendix II. Distribution of dominant phytoplankton groups on 10th June 1999, expressed as % cell density (cells ml^{-1}) as well as % carbon biomass (mg C m^{-3}) are presented in figure 4.7a & b.

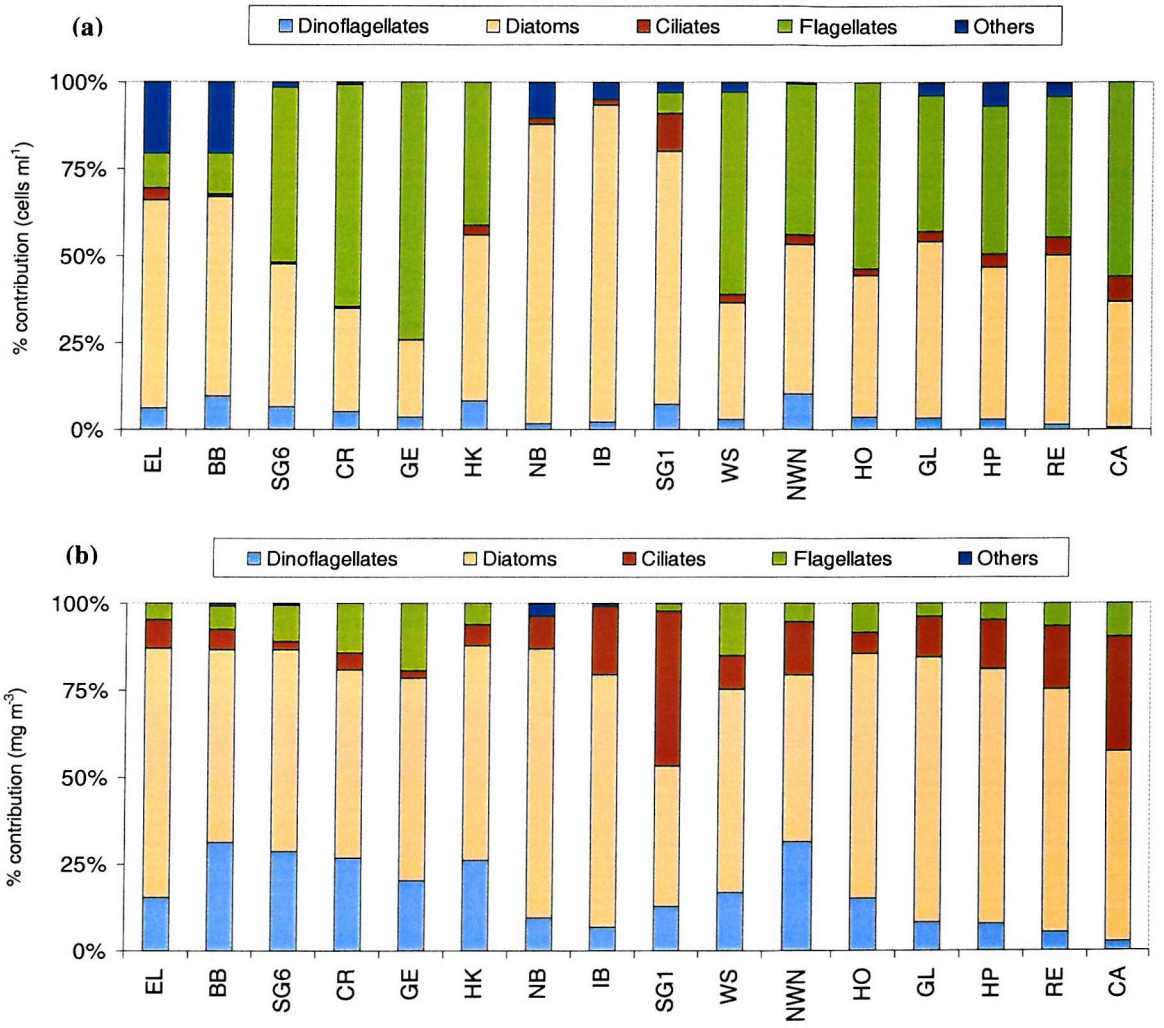


Figure 4.7. Horizontal distribution of dominant phytoplankton groups expressed as (a) % cell density (cells ml^{-1}) and (b) % cell biomass (mg C m^{-3}) on 10th June 1999.

Diatoms were the most abundant species throughout the estuary (along the Test & Itchen estuaries and Southampton Water) contributing 55%-78% of the total phytoplankton biomass at some sites (figure 4.7b). Maximum diatom biomass (107-160 mg C m^{-3}) were recorded at the coastal sites in June 1999 (figure 4.8), from HO down to CA at which the relatively large-celled diatom, *Guinardia* (*G. flaccida and G. delicatula*) accounted for 17%-53% (figure 4.8) of the total diatom biomass. None of the *Guinardia* species were recorded in the more estuarine waters during the sampling day. However, these sites were generally dominated by the diatom *Ditylum brightwellii* and *Rhizosolenia shrubsolei,* both of which were widely distributed along the

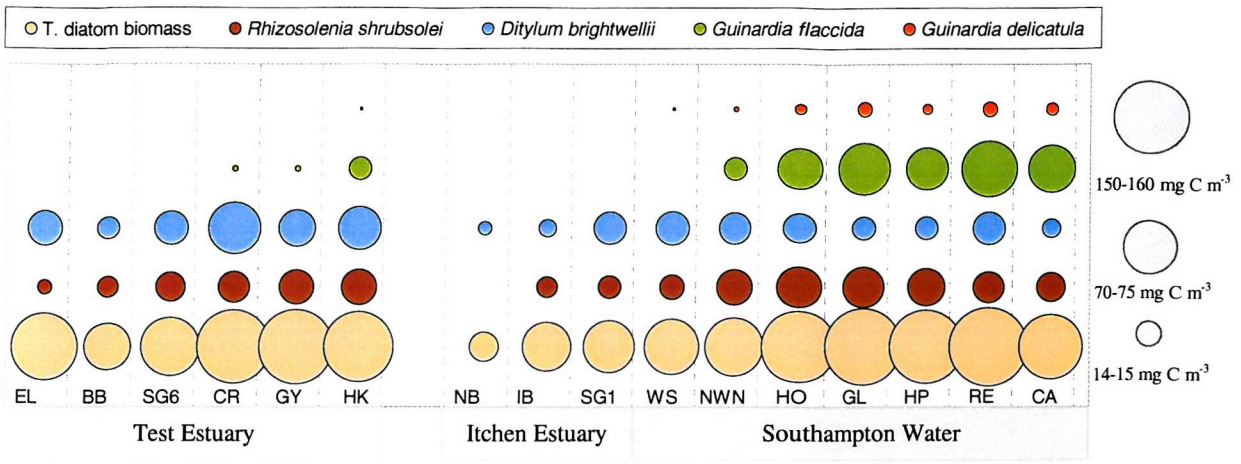


Figure 4.8. Horizontal distribution of total diatom biomass (mg C m^{-3}) and dominant diatom species (mg C m^{-3}) throughout the estuary on 10th June 1999.

whole estuary (figure 4.8). These species contributed less to the total phytoplankton biomass, however with some diatom species being numerically very abundant (see figure 4.9), of which *Skeletonema costatum* (8-88 cells ml^{-1}, up to 52% of total diatom cell number) and *Chaetoceros* spp. (5-35 cells ml^{-1}, up to 29% of total diatom cell number) were the most abundant species. A mixture of *Nitzschia* species (16-51 cells ml^{-1}, up to 38% of total diatom cell number) were record on 10th June (figure 4.9) being very abundant in estuarine waters, particularly at upper estuary sites (i.e. EL, BB, NB and IB).

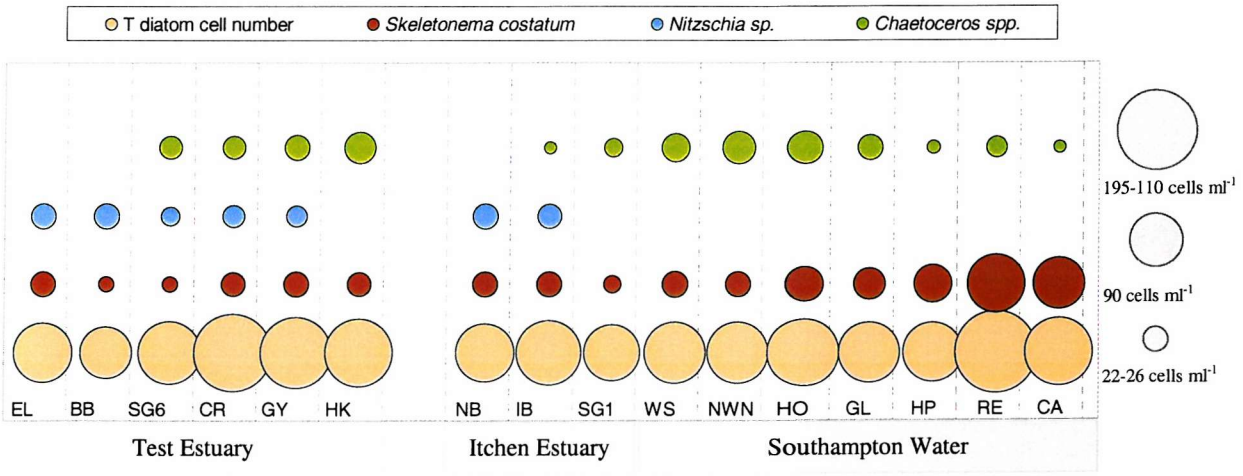


Figure 4.9. Horizontal distribution of total diatom density (cells ml^{-1}) and the numerically dominant diatom species (cells ml^{-1}) throughout the estuary on 10th June 1999.

Both dinoflagellates (3-70 mg C m^{-3}) and ciliates (3-65 mg C m^{-3}) were less abundant along the estuary on 10th June compared to diatoms (figure 4.7b). Highest dinoflagellate biomass were recorded at sites along the Test estuary (24.6-70 mg C m^{-3}) as well as the intermediate sites in Southampton Water, particularly at NWN (~60 mg C m^{-3}). Two dinoflagellate species, *Scrippsiella trochoidea* (up to 55% of total dinoflagellate biomass at some sites) and *Protoperidinium minutum* (up to 58% of total dinoflagellate biomass at some sites) exclusively dominated the dinoflagellate community (figure 4.10a) along the estuary on 10th June. Both

dinoflagellates, particularly *S. trochoidea* were clearly less abundant in coastal waters (figure 4.10a). In contrast, high ciliate biomass (15-77 mg C m^{-3}) were recorded in coastal waters (figure 4.10b) with the autotrophic ciliate *Mesodinium rubrum* (3-40 mg C m^{-3}) being the most abundant species, comprising 93% and 53% of total ciliate biomass at RE and CA, respectively. Some heterotrophic ciliates, mainly Tintinnids and *Strombidium* spp. (1.5-65.7 mg C m^{-3}) were recorded along the Itchen estuary and Southampton Water (figure 4.7b) with maximum biomass (65.7 and 16.4 mg C m^{-3}) at SG1 and NWN.

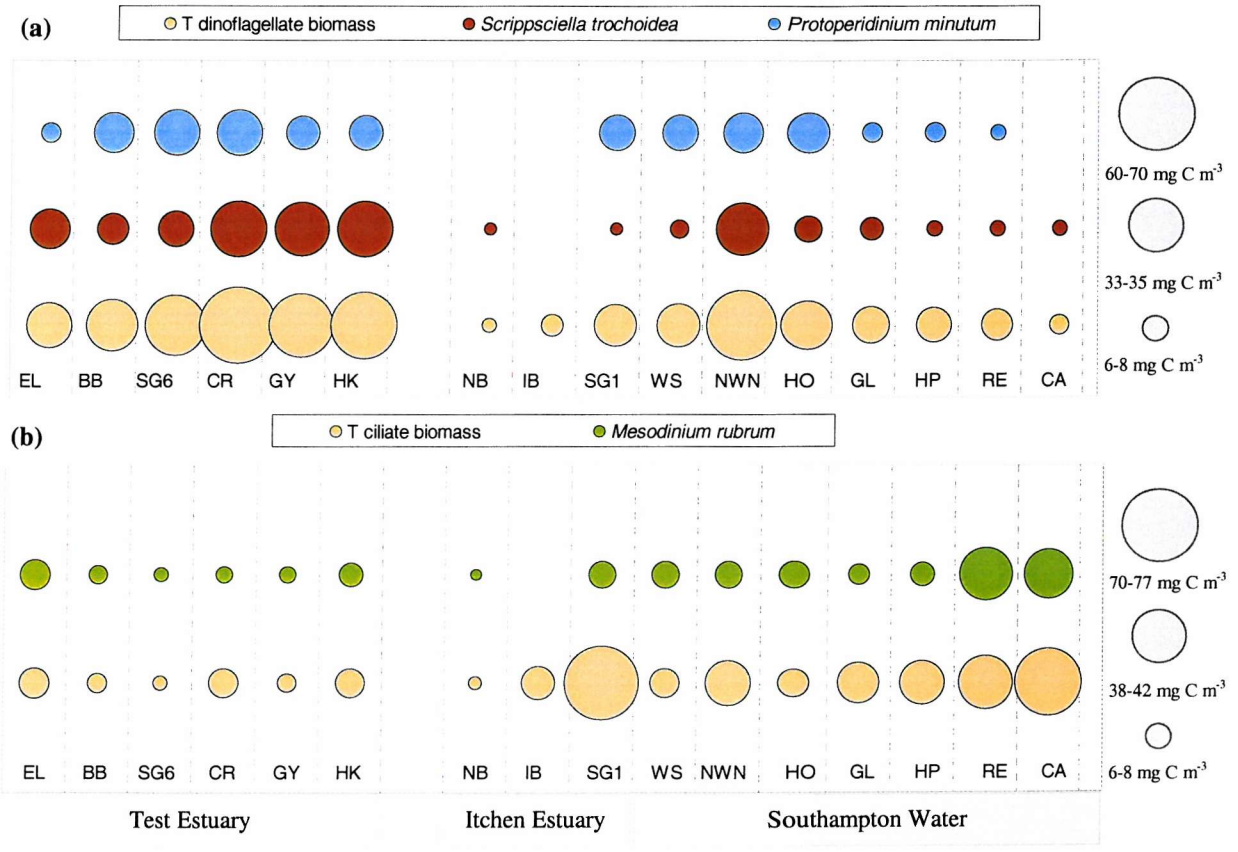


Figure 4.10. Horizontal distribution of (a) total dinoflagellates biomass & dominant dinoflagellates species (mg C m^{-3}) and (b) total ciliate biomass & dominant ciliate species (mg C m^{-3}) throughout the estuary on 10th June 1999.

Despite their lesser contribution to total phytoplankton biomass, flagellates were numerically the most abundant group (see figure 4.7a) at most sites along the Test estuary and Southampton Water comprising up to 74% of the total cell number at some sites. Meanwhile, no flagellate species were recorded along the Itchen estuary (figure 4.7a) with few recorded at the lower site (SG1) contributing approximately 1% of the total cell number at this site with diatoms being numerically most abundant (74% total cell number at some sites). The relatively small-sized flagellate, *Cryptomonas* sp., was the most dominant flagellate with maximum biomass 33.6 mg C m^{-3} and 43.6 mg C m^{-3} at CR and GY, respectively (figure 4.11). The flagellate *Eutreptiella*

marina was also recorded (2.5-7.5 mg C m^{-3}) at some sites particularly in the Test estuary (figure 4.11) with less contribution compared to *Cryptomonas* sp.

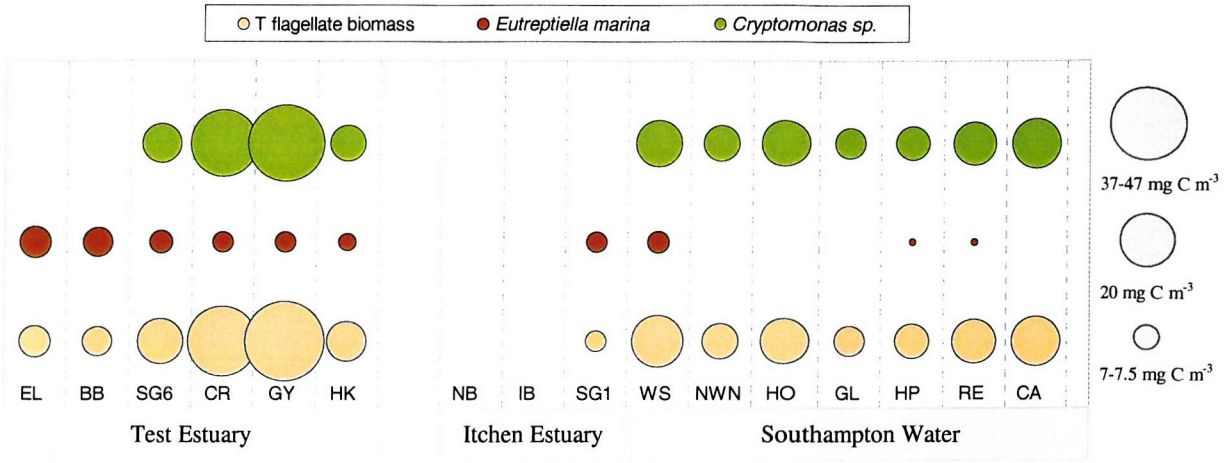


Figure 4.11. . Horizontal distribution of total flagellates biomass (mg C m^{-3}) and dominant flagellate species (mg C m^{-3}) throughout the estuary on 10th June 1999.

The spatial distribution of chlorophyll *a* (mg m^{-3}) and phytoplankton cell biomass (mg C m^{-3}) was strongly correlated ($r = 0.72$, $p < 0.01$) (figure 4.12). A discrepancy in the relationship between Chl *a* and total biomass was recorded, for example, when the large-celled diatoms *G. flaccida* was dominant (see figure 4.12) with a noticeable overestimate of total carbon biomass

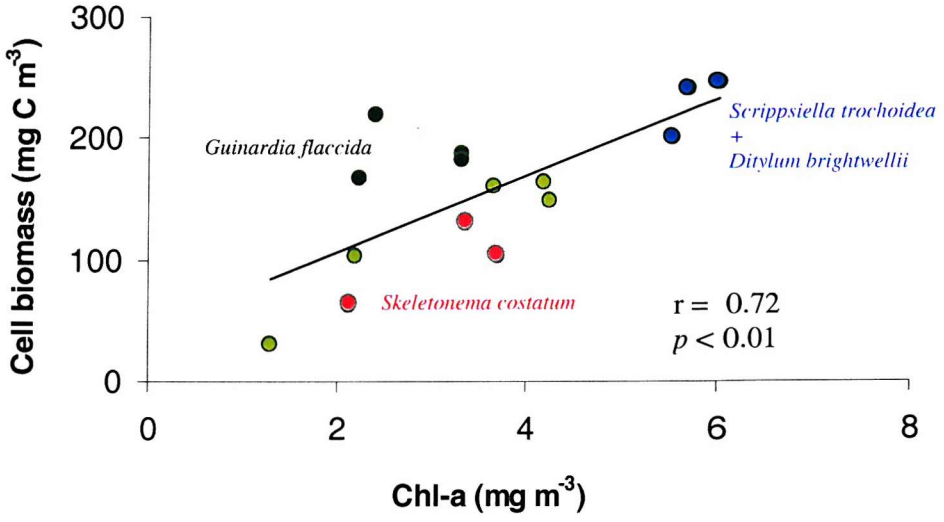


Figure 4.12. Relationship of total phytoplankton cell biomass (mg C m^{-3}) versus total Chl *a* (mg m^{-3}) measured on 10th June 1999.

4.4 22nd JULY 1999

A similar spatial sampling program was carried out on 22/7/99 as applied on 10/6/99, however four stations, NB, OS, SG1 and GY were not sampled on this date.

4.4.1 CTD PROFILES

Spatial changes of temperature (figure 4.13a), salinity (figure 4.13b), density (figure 4.13c), attenuance and chlorophyll (figure 4.14) on 22nd July 1999 were determined throughout the Test estuary, Itchen estuary and Southampton Water using the CTD data. Water temperature was slightly higher than that during June; ranging from 18.6 – 19.7 °C (figure 4.13a). Lower water temperature values were recorded at HP and RE (18.6, 18.8°C) with minimum vertical temperature gradient of 0.2 and 0.1, respectively.

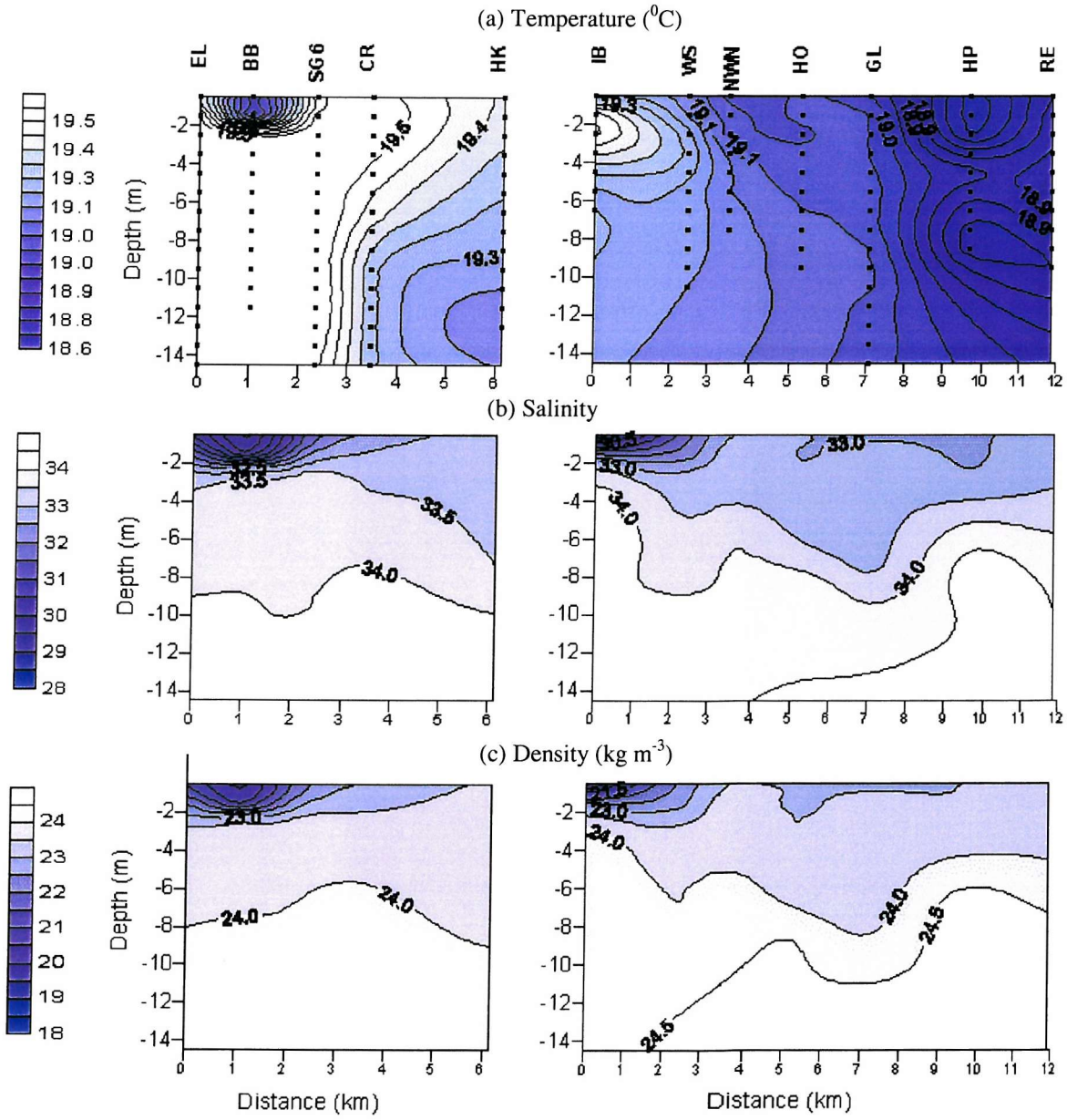


Figure 4.13. Vertical profiles of: (a) temperature, (b) salinity and (c) density throughout Southampton Water on 22nd July 1999. See figure 1.3 for station identification.

Maximum water temperatures (19.5-19.7 °C) were recorded towards the head of the Test estuary with a degree of thermal stratification restricted at BB station with a maximum vertical temperature gradient of 0.6 °C (figure 4.13a).

Salinity values in July varied from 28.2 to 34.7, with lower salinity values of 28.2 and 28.8 measured in surface water at IB and BB stations (at the head of both Itchen and Test estuaries), due to the input of the less-saline fresh water. Vertical salinity gradient reduced from 6.1 and 5.3 (at IB and BB) to 1.7 and 1.1 at CR and HK (figure 4.13b). Similar observations were recorded at the same stations during June, and may be attributed to the increased turbulence towards the mouth of the estuary.

A degree of salinity stratification existed throughout the sampling area and was more recognised at stations in the upper part of the estuary (see figure 4.13b). Horizontal variations in density for the sampling sites presented in the contour plot (figure 4.13c) show that water of lower density was restricted to the surface layers with minimum values upstream.

Horizontal variations of both transmission and chlorophyll *a* values at the sampling sites are presented in figure 4.14a & b. This shows turbidity throughout the water column to be maximal in deeper waters. Higher transmission values were recorded in surface water layers at HO, GL and RE with a highly transparent patch of water near the bottom at RE.

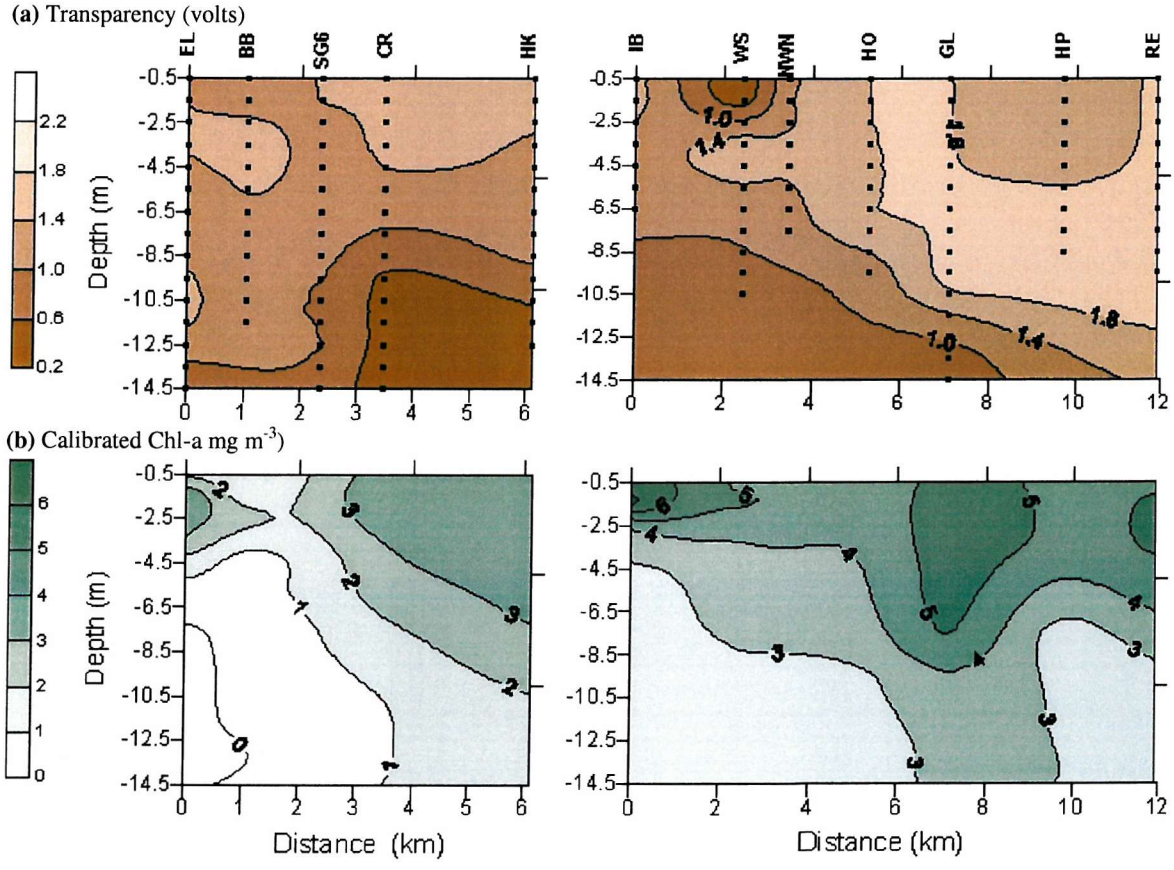


Figure 4.14. Vertical distribution of: (a) transparency and (b) calibrated Chl *a* on 22nd July1999. See figure 1.3 for station identification.

Higher surface chlorophyll concentrations were recorded at most of the sampling sites (figure 4.14b). Chl *a* seemed to be generally increased downstream towards the mouth of the estuary. In the upper estuary low chlorophyll deep water was recorded with a patch of high Chl *a* concentration (~5 mg m^{-3}) in near-surface water at IB (figure 4.14b). Maximum chlorophyll values (4-5 mg m^{-3}) were recorded in upper layers of the water column, particularly at GL and RE.

4.4.2 ATTENUATION COEFFICIENTS

Spatial distribution of attenuation coefficient (*k*) throughout the sampling sites on 22/7/99 is illustrated in figure 4.15. Maximum *k* values were measured at EL & SG6 (0.88 m^{-1} & 0.89 m^{-1}) compared to low values recorded at coastal sites, particularly at RE & CA with a value of 0.5 m^{-1}. % Irradiance profile at the sampling sites on 22/7/99 are presented in a surfer contour plot (figure 4.15) showing the depth of the 1 % light. The photic zone (1% irradiance) ranged from 5.2 m to 9.3 m throughout the whole estuary. Data indicated that water throughout the sampling sites (Test estuary and Southampton water) was horizontally homogenous with respect to light data. (irradiance data for Itchen estuary is not included).

Table 4.2. Values of attenuation coefficients (*k*) and 1% irradiance depth (m) throughout the estuary on 22nd July 1999.

	Test Estuary						Itchen Estuary				Southampton Water						
Site	EL	BB	SG6	CR	GY	HK	NB	IB	OS	SG1	WS	NWN	HO	GL	HP	RE	CA
k (m^{-1})	0.88	0.7	0.89	0.71	nd	0.7	nd	nd	nd	nd	0.78	0.85	0.56	0.55	0.74	0.5	0.5
photic depth (m)	5.2	6.4	5.2	6.5	nd	6.6	nd	nd	nd	nd	5.9	5.4	8.2	8.3	6.2	9.2	9.3

* nd = no data

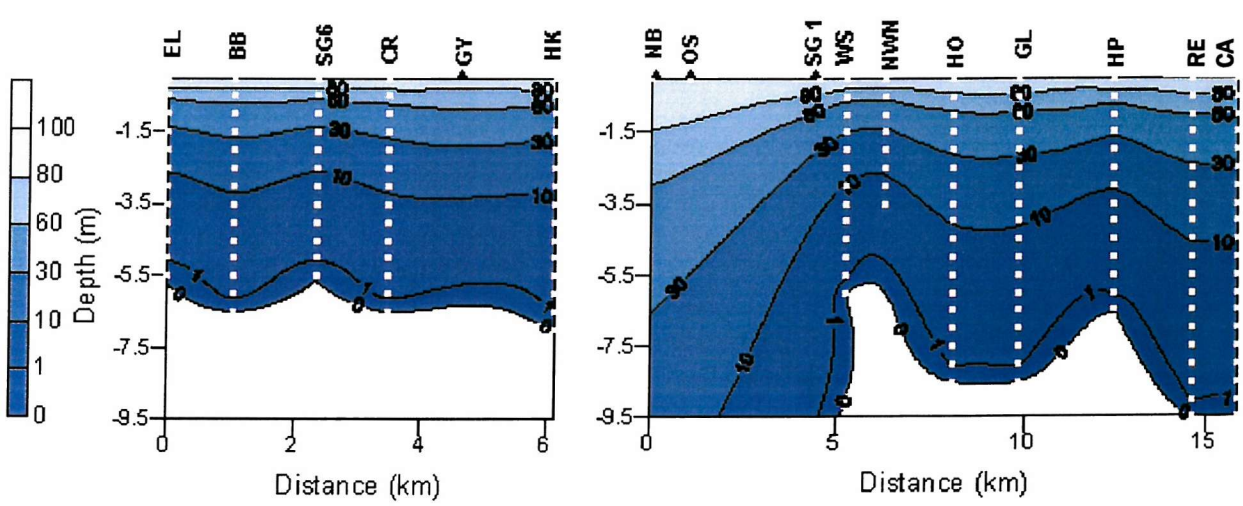


Figure 4.15. Spatial variations in % irradiation throughout Southampton Water on 22nd July1999. See figure 1.3 for station identification.

4.4.3 NUTRIENT CONCENTRATIONS

Figure 4.16 shows the spatial variability in nutrients for each depth (surface, middle, and bottom) at each of the sampling sites. Phosphate ranged from 0.8-4.5 μM, nitrate ranged from 0.5-40 μM, and silicate ranged from 3-35 μM (figure 4.16). Maximum nutrient concentrations were generally measured in surface layers throughout all the sampling sites however; higher phosphate concentrations were measured in deep water at EL (middle), BB (bottom) and GL (bottom).

4.4.4 CHLOROPHYLL A

Chl *a* had a relatively small range of concentration (1.3-5.4 mg m^{-3}) throughout the estuary. The highest values (figure 4.16) were recorded at estuarine sites particularly at IB, WS (Itchen estuary), EL, CR and HK (Test estuary) due to nutrient availability at these sites. High surface Chl *a* was measured throughout the whole estuary during the sampling day (22nd July) with a degree of homogeneity with respect to Chl *a* towards the mouth of the estuary.

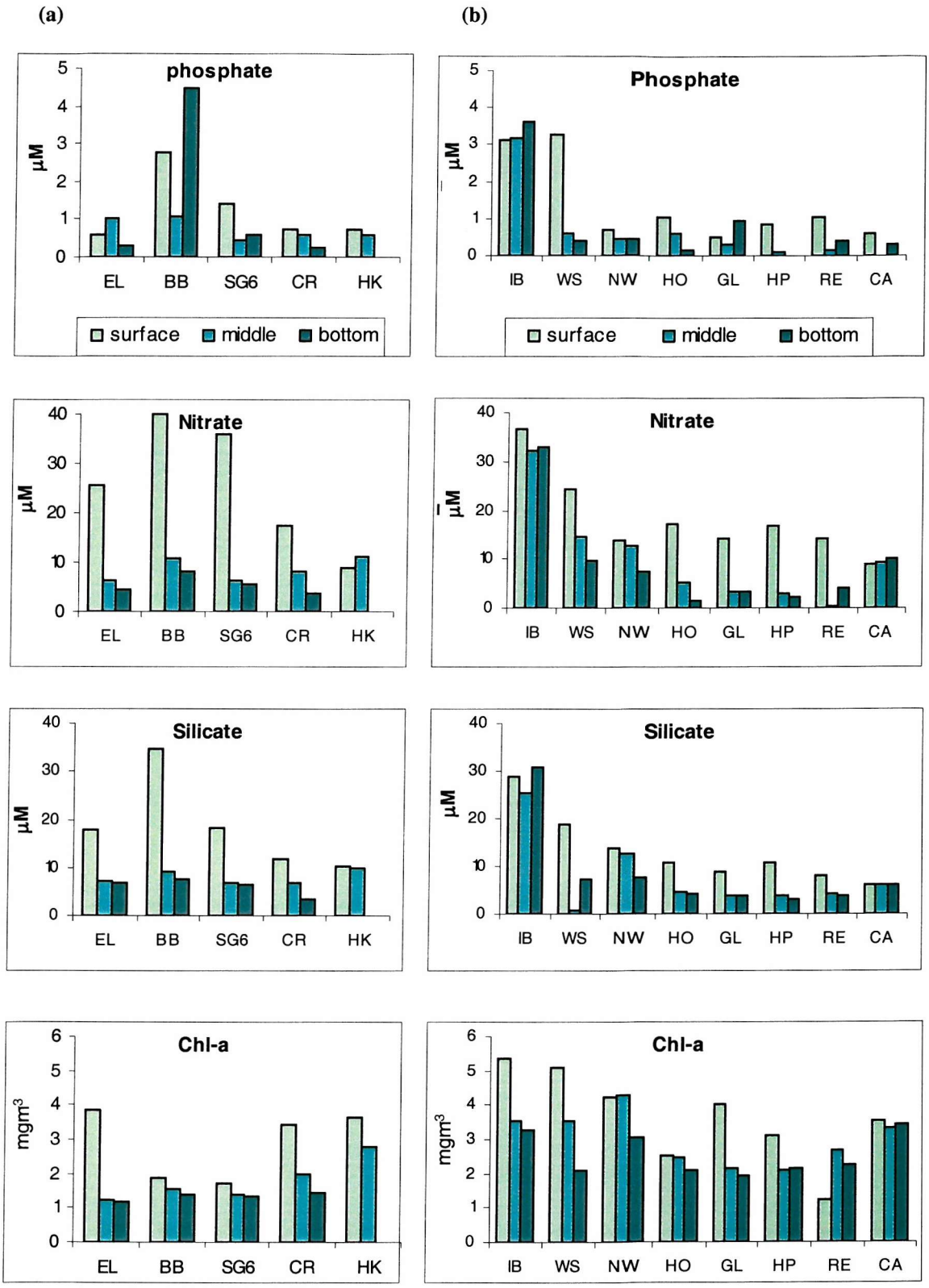


Figure 4.16. Spatial distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* through (a) Test estuary, (b) Itchen estuary and Southampton Water on 22nd July 1999. No bottom data for HK.

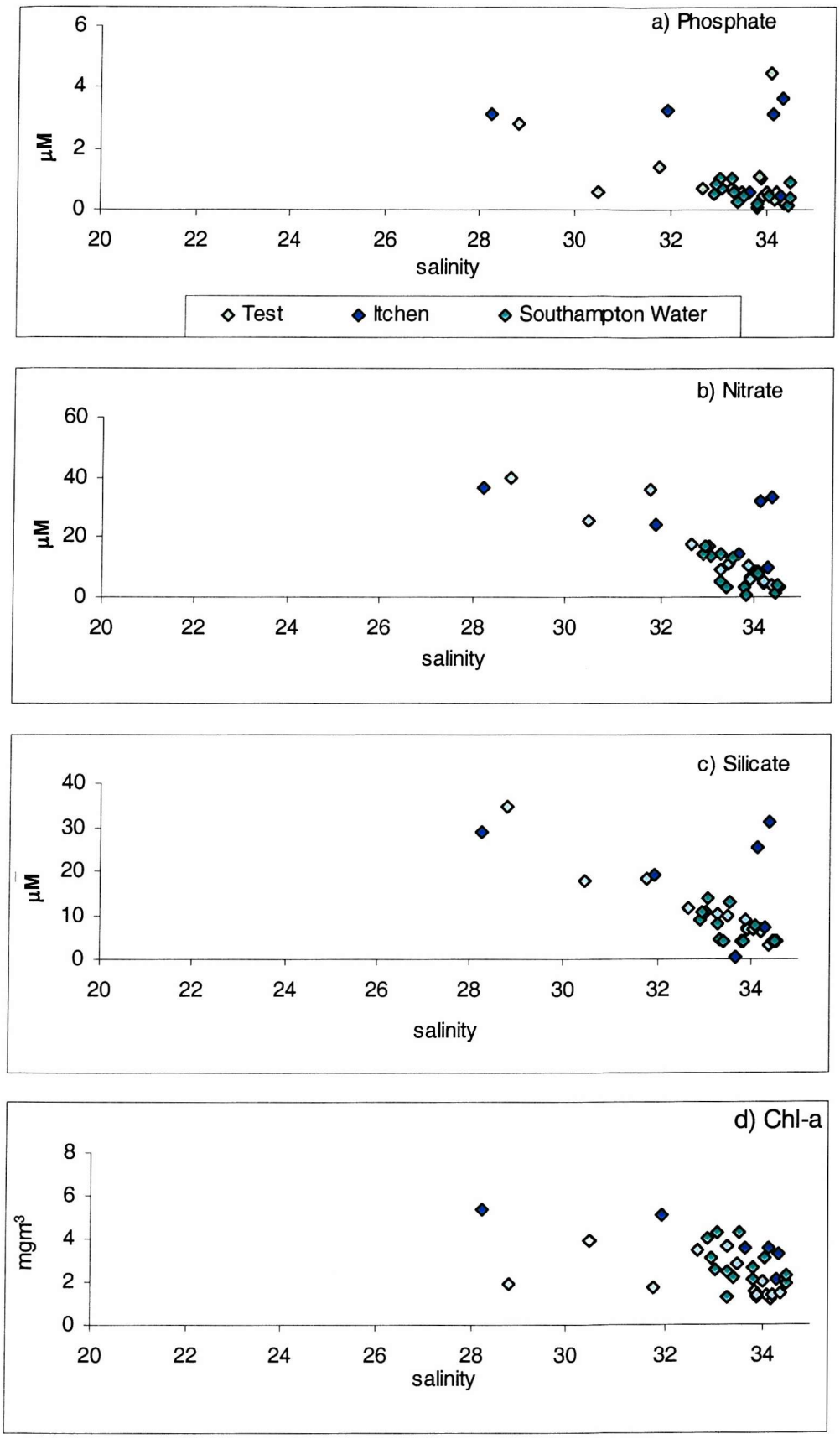


Figure 4.17. Distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* concentration against salinity throughout the estuary on 22nd July 1999.

4.4.5 PHYTOPLANKTON DISTRIBUTION ALONG THE ESTUARY ON 22nd JULY 1999

A diverse community of phytoplankton species was recorded in surface water samples collected throughout the estuary on 22/7/99. Phytoplankton population and species distribution along the estuary are represented in figure 4.18a (cells ml^{-1}) and 4.18b (mg C mg^{-3}). The population was relatively similar to that recorded on 10/6/99; however dinoflagellates were much more abundant in July comprising 7%-74% of total phytoplankton biomass along the estuary on 22nd July (figure 4.18b).

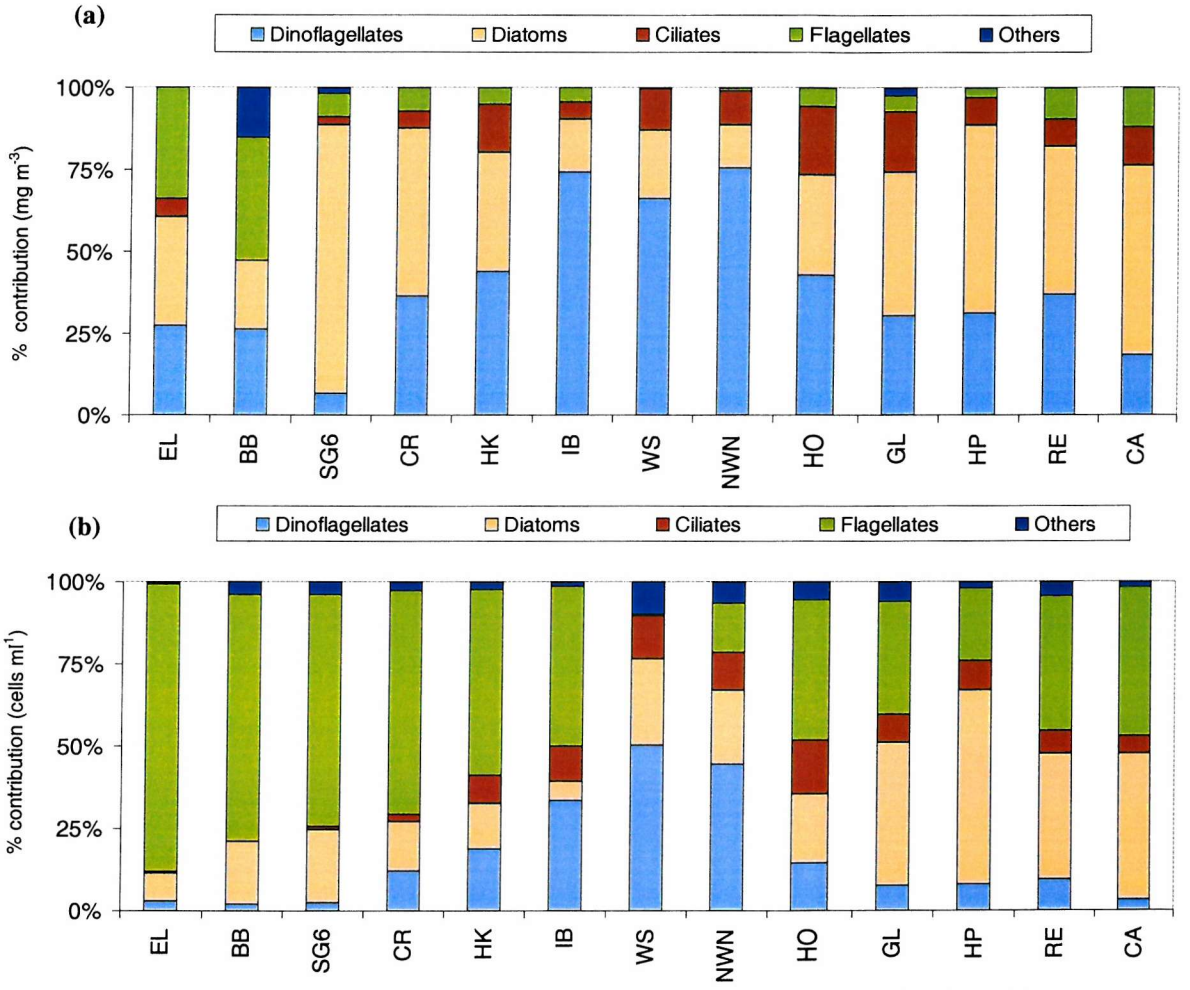


Figure 4.18. Horizontal distribution of dominant phytoplankton groups expressed as (a) cell biomass (mg C m^{-3}) and (b) cell density (cells ml^{-1}) on 22nd July 1999.

Total dinoflagellate biomass varied from 5.2 to 185.6 mg m^{-3} with highest biomass of 185.6, 117 and 172.2 mg C m^{-3} at IB (lower site in the Itchen estuary), WS and NWN (upper and mid sites in Southampton Water), respectively. *Scrippsiella trochoidea* (0.3-160 mg C m^{-3}), was the most dominant dinoflagellate species at most sites along the estuary with maximum biomass of 160 mg C m^{-3} in the middle of the estuary, particularly at NWN (figure 4.19) contributing 93% of total dinoflagellate biomass at this site. *Protoperidinium minutum* (0-35 mg C m^{-3}) and *Prorocentrum micans* (0-16.3 mg C m^{-3}) were also abundant along the estuary on the sampling day with less contribution to the dinoflagellate community compared to *Scrippsiella trochoidea.*

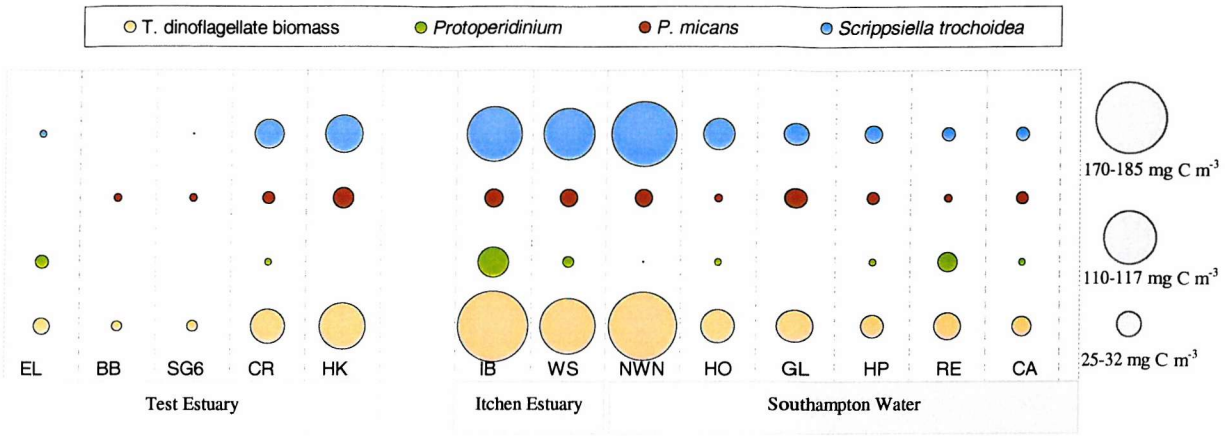


Figure 4.19. Horizontal distribution of (a) total dinoflagellates biomass and dominant dinoflagellates species (mg C m^{-3}) throughout the estuary on 22nd July 1999.

Diatoms were the second most numerous phytoplankton group recorded throughout the estuary comprising 13% to 84% of total phytoplankton biomass (see figure 4.18b). Diatoms were represented by many species along the estuary among which, *Odontella sinensis* (0-60.5 mg C m^{-3}), *Rhizosolenia stylformis* (0-32.2 mg C m^{-3}) and *Ditylum brightwellii* (0-10.2 mg C m^{-3}) were the most abundant species. The relatively large-celled diatom *Odontella sinensis* was much more abundant in estuarine waters with maximum biomass along the Test estuary (figure 4.20), while *Rhizosolenia stylformis* was more abundant in coastal waters. Other diatom species of relatively small-sized cells were numerically abundant, for example *Skeletonema costatum* (0-32.5 cells ml^{-1}) and *Chaetoceros* spp. (0-30.3 cells ml^{-1}) contributing up to 38% and 48% of total diatom cell number, respectively at some sites, (figure 4.21).

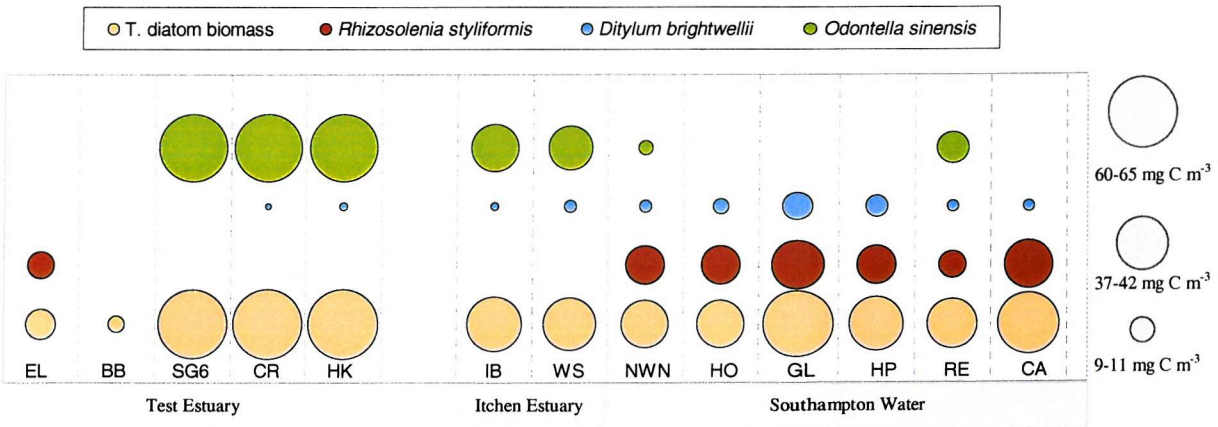


Figure 4.20. Horizontal distribution of total diatom biomass (mg C m^{-3}) and dominant diatom species (mg C m^{-3}) throughout the estuary on 22nd July 1999.

Both diatoms, S. *costatum* and Chaetoceros were more abundant at coastal sites with maximum cell numbers of *S. costatum* at RE and CA and of *Chaetoceros* spp. at GL and HP (figure 4.21). Moreover, a diverse mixture of small pennate diatoms (e.g. *Nitzschia* spp., 0-14.9 cells ml^{-1}) was numerically abundant at most sites comprising 73% of total diatom cell number at some sites (see figure 4.21).

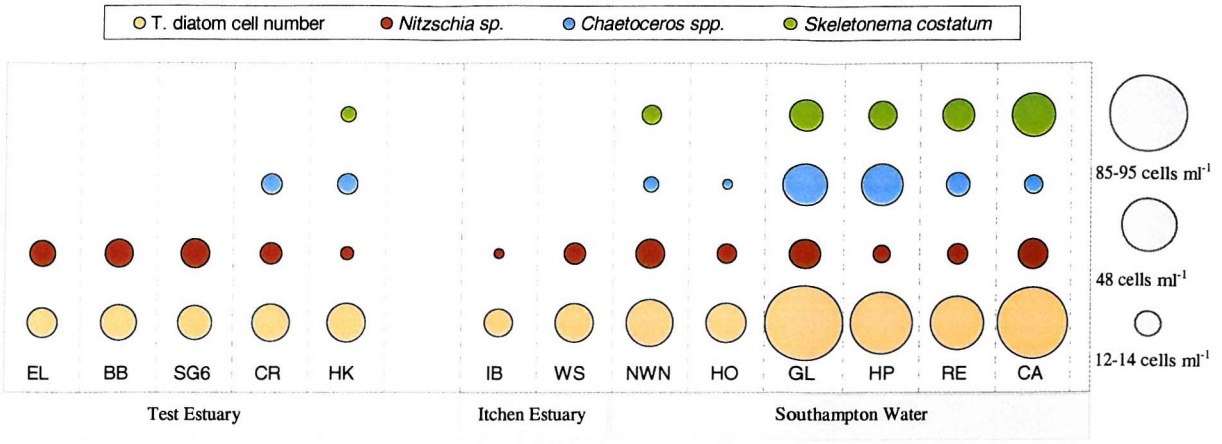


Figure 4.21. Horizontal distribution of total diatom density (cells ml^{-1}) and the numerically dominant diatom species (cells ml^{-1}) throughout the estuary on 22nd July 1999.

Despite their lower carbon biomass, flagellates were numerically the most abundant group at most of the sampling sites on 22nd July contributing up to 87% of total phytoplankton cell number (figure 4.18b). The flagellate *Cryptomonas* (23.8-160 cell ml^{-1} & 2-13 mg C m^{-3}) was seen to be the most abundant flagellate (figure 4.22) with maximum cell biomass (5.4-13 mg m^{-3}) recorded along the Test estuary, particularly at EL (13 mg m^{-3}) and CR (~9 mg C m^{-3}) and Itchen estuary, particularly at IB (9.5 mg C m^{-3}). The flagellate *Eutreptiella marina* (maximum contribution was 11% of total flagellate cell number) was also recorded throughout the estuary during the sampling day in low numbers (<10 cell ml^{-1}) at some sites with maximum biomass of 3.4 and 3.8 mg C m^{-3} at RE and CA, respectively (figure 4.22).

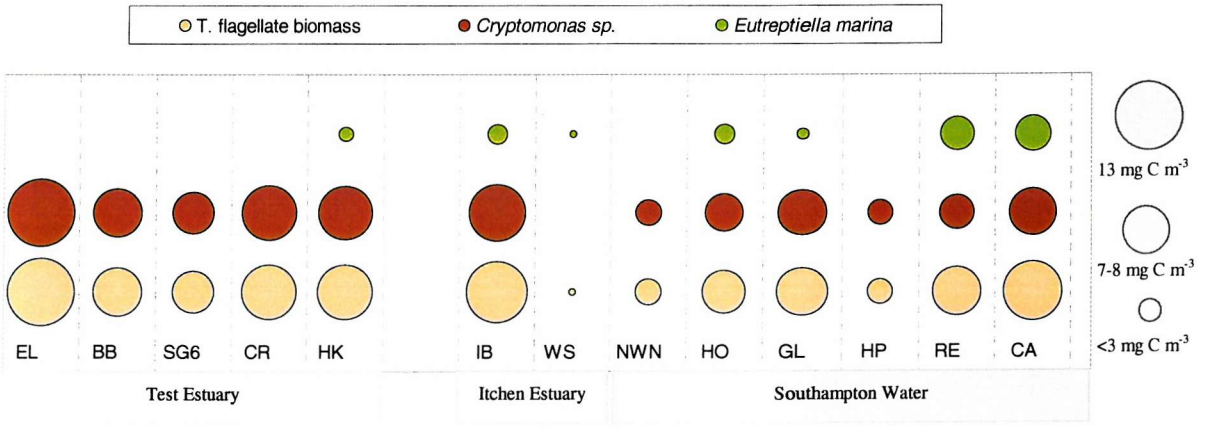


Figure 4.22. Horizontal distribution of total flagellates biomass (mg C m^{-3}) and dominant flagellates species (mg C m^{-3}) throughout the estuary on 22nd July 1999.

Ciliates (0-26.6 mg C m^{-3}) were also recorded throughout the estuary on 22nd July comprising <15% of total phytoplankton biomass at some sites (see figure 4.18b). Ciliates were mainly represented by the autotrophic ciliate *Mesodinium rubrum* (0-21.6 mg C m^{-3}) together with some heterotrophic ciliates (e.g. *Tintinnid* spp., 0-16.7 mg $m^{-3}$and *Strombidium* spp., 0-4.1 mg m^{-3}). *M. rubrum* was more abundant at intermediate sites with maximum biomass of similar value (21.6 mg C m^{-3}) at WS, NWN and GL (see figure 4.23).

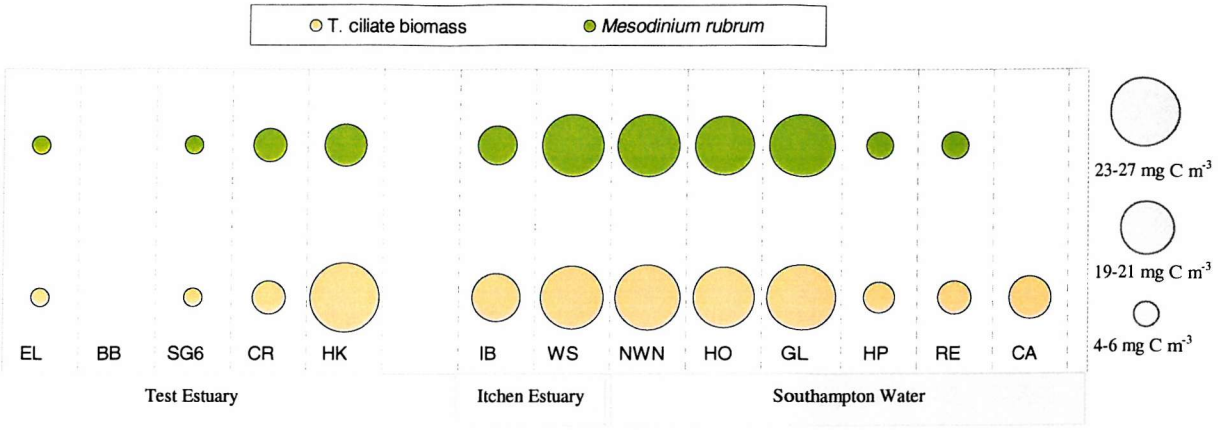


Figure 4.23. Horizontal distribution of total ciliates biomass (mg C m^{-3}) and dominant ciliate species (mg C m^{-3}) throughout the estuary on 22nd July 1999.

Phytoplankton biomass (expressed as mg C m^{-3}) was strongly correlated with that of chlorophyll *a* concentration ($r = 0.76$, $p < 0.01$) (figure 4.24), however the relationship showed some scatter . This was mainly attributed to the differences in cellular chlorophyll content among species of the same group (e.g. different cell size among diatom species) and/or different groups (e.g. flagellates and dinoflagellates). Maximum concentration of Chl *a* of 5.4, 5.1 and 4.3 mg m^{-3} were measured at IB, WS and NWN, respectively and were coincident with noticeable peaks in cell biomass of *S. trochoidea* (at the 3 sites) and *O. sinensis* (at IB and WS). Secondary peaks of 3.7 mg Chl *a* m^{-3} were measured at EL and CA but did not coincide with a relative increase in the total cell biomass (<40 mg C m^{-3}), this peak was mainly coincident with the numerical increase of the flagellate *Cryptomonas* sp. In contrast, relatively higher peaks in phytoplankton biomass (109.4, 74.3 and 66.9 mg C m^{-3}) were estimated at some sites when large-sized diatoms (e.g. *Rhizosolenia styliformis* and *Odontella sinensis* were present (figure 4.24) and probably represent an overestimate of carbon biomass.

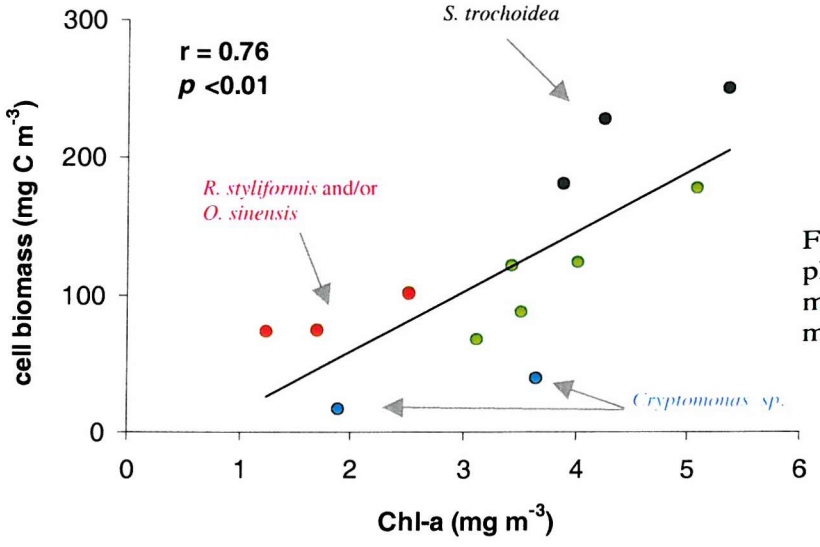


Figure 4.24. Relationship of total phytoplankton cell biomass (mg C m^{-3}) versus total Chl *a* (mg m^{-3}) measured on 22nd July 1999.

4.5 15th AUGUST 2000

14 sites along the estuary were sampled on 15th August 2000 using the same sampling program and procedures for field and laboratory measurements carried out in 1999. Six sampling sites were sampled in the Test estuary (from EL down to HK), four sites were sampled in Itchen estuary (from NB down to SG1) and four sites in Southampton Water (from WS down to GL). See figure 1.3 chapter 1 for detailed description of sampling locations. No attenuence data was included in the following analysis as the transmissometer sensor was not working properly on this sampling day. Moreover, the light profile data (% depth irradiance) was accidentally deleted from the light logger.

4.5.1 CTD PROFILES

Spatial changes in temperature, salinity, density and chlorophyll on 15th August 2000 are represented in figure 4.25 and 4.26. Water temperature through the water column ranged between 19.0-21 °C on 15th August with lower values (19.5-19.8 °C) recorded in the upper Itchen (NB & OS). Higher water temperatures were, however, recorded in surface water along Test estuary; at EL, BB and CR and Southampton Water; at GL (figure 4.25).

Surface water was generally warmer than deep water in all sampling sites, except at NB, OS and WS (along Itchen estuary), at which slightly warmer water masses occurred in deep waters with a minimum vertical gradients ranged between 0.18 °C and 0.22 °C (figure 4.25a) indicating a degree of water mixing and no obvious thermal stratification at these sites. In the Test estuary; the water column was clearly stratified with a higher range of (up to 0.7 °C), particularly at EL (0.64 °C), BB (0.66 °C) and SG6 (0.43 °C). Similarly, a greater gradient of vertical temperature was also recorded at sites towards the mouth of the estuary (see figure 4.25), particularly at HO (0.54 °C) and GL (0.59 °C).

Salinity values in surface waters varied from 22.5 to 33.9 on 15th August (figure 4.25b) with lower salinity values along the Itchen estuary (22.52-31.4) and Test estuary (27.8- of 30.8) due to the effect of the more fresh water input decreasing towards the mouth of the estuary due to mixing with high salinity sea water. The water column was more stratified, with respect to salinity, at estuarine sites with vertical salinity gradient ranged from 2.24 to 4.75 along the Test estuary and from 1.56 to 8.26 in the Itchen estuary. Values of vertical salinity gradient reduced to 1.84-2.4 at the coastal sites and this is mainly attributed to increased water mixing and reduced stratification towards the mouth of the estuary. Similar findings were also obtained from the contour plot of the vertical and horizontal variations in density along the estuary (figure 4.25c) with stratified water column at the head of the estuary (upper sites of Test and Itchen) and well mixed water column at the mouth of the estuary.

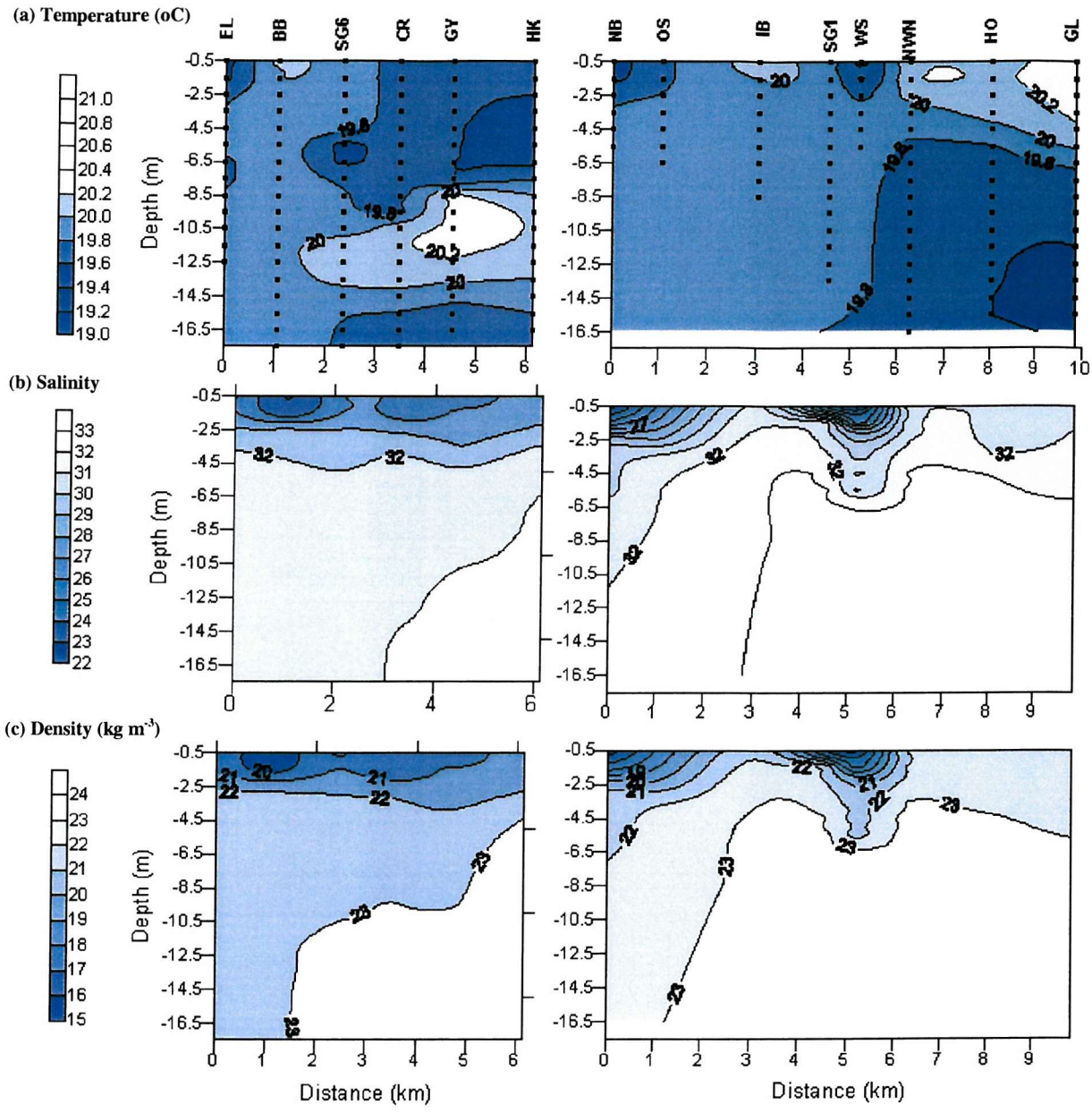


Figure 4.25. Vertical profiles of: (a) temperature, (b) salinity and (c) density throughout Southampton Water on 15th August 2000.

Values of calibrated Chl *a*, ranged between 3.6 and 20.2 along the estuary on 15th August (see figure 4.26). Horizontal and vertical distribution of Chl *a* along the estuary indicated that higher Chl *a* levels were recorded in the well mixed water column in the lower Test estuary (GY & HK) as well as at the intermediate sites in Southampton Water (WS, HO & NWN). This could be due to the sinking tendency of diatom species that were abundant at these sites. Low Chl *a* deep water was, however recorded at El, BB and SG6 with a patch of relatively higher concentration (8.4 & 12.2 mg m^{-3}) recorded in near surface waters at El & SG6 decreasing to < 0.3 mg m^{-3} at the bottom. A layer of low Chl *a* concentration (>2 mg m^{-3}) was recorded in surface water at BB followed by a Chl *a* maximum at 2.5-4.5 m depth (figure 4.26).

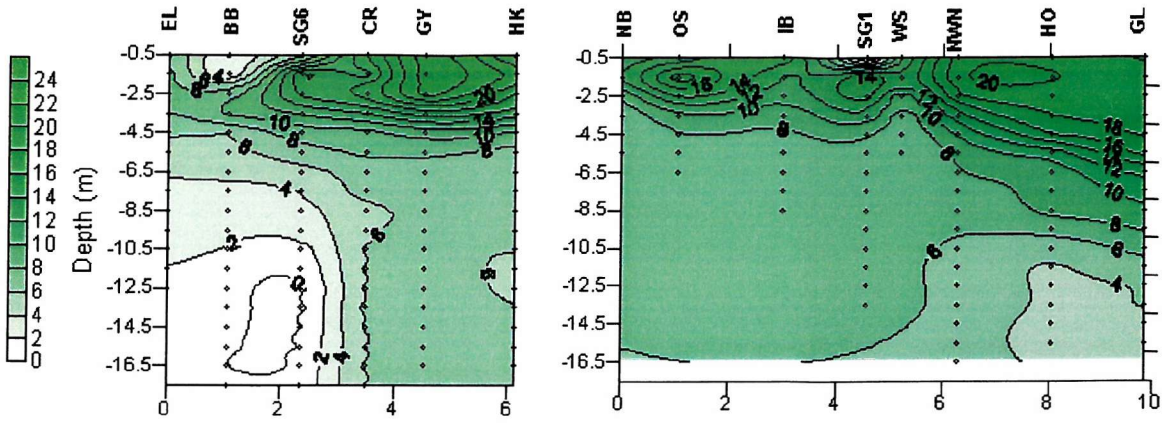


Figure 4.26. Spatial distribution of calibrated Chl *a* (mg m^{-3}) throughout the estuary on 15th August 2000.

4.5.2 CHLOROPHYLL A

Concentration of fluorometrically measured Chl *a* varied from 6.3 to 25.7 mg m^{-3} in surface waters throughout the sampling sites on 15th August (figure 4.27) with maximum values at GY and HK down the Test estuary (23-25 mg m^{-3}) and at intermediate sites along Southampton Water (13.8-18.8 mg m^{-3}). Relatively lower concentrations of Chl *a* were determined in middle (4.2–11.6 mg m^{-3}) and bottom (3.8–8.9 mg m^{-3}) waters with a more homogeneous water column throughout the estuarine sites (along the Test and the Itchen estuaries). A greater vertical Chl *a* gradient (difference between surface and bottom Chl *a* = 6.1-12.7 mg m^{-3}) was recorded at sites along Southampton Water and the lower Test estuary (figure 4.27). The relationship between Chl *a* concentration (at the 3 depths) and salinity (of the same depths) on the 15th August (figure 4.28) showed a noticeable increase in phytoplankton production at high salinity sites with a degree of conservative behaviour at salinity vales between 30-34, particularly for water samples collected from the Test estuary and Southampton Water. Despite the high range of salinity differences (22-29.5) in surface waters in the upper Itchen estuary, no obvious variations in Chl *a* concentration (ranged between 8.1-9.6 mg Chl *a* m^{-3}) were observed (figure 4.28).

4.5.3 NUTRIENT CONCENTRATIONS

Figure 4.27 shows the spatial variability in phosphate, nitrate and silicate measured in water samples collected from each depth (surface, middle, and bottom) at each of the sampling sites. Phosphate ranged from 0.51–6.6 μM, nitrate ranged from 6.9-131.4 μM, and silicate ranged from 4.4-71.2 μM with maximum nutrients levels along the upper Itchen estuary.

With respect to nutrient concentrations, a relatively stratified water column was recorded with a high-nutrient surface water layer followed by a relatively homogenous water column with nutrient concentrations in middle and bottom water layers of almost similar values (see figure 4.27).

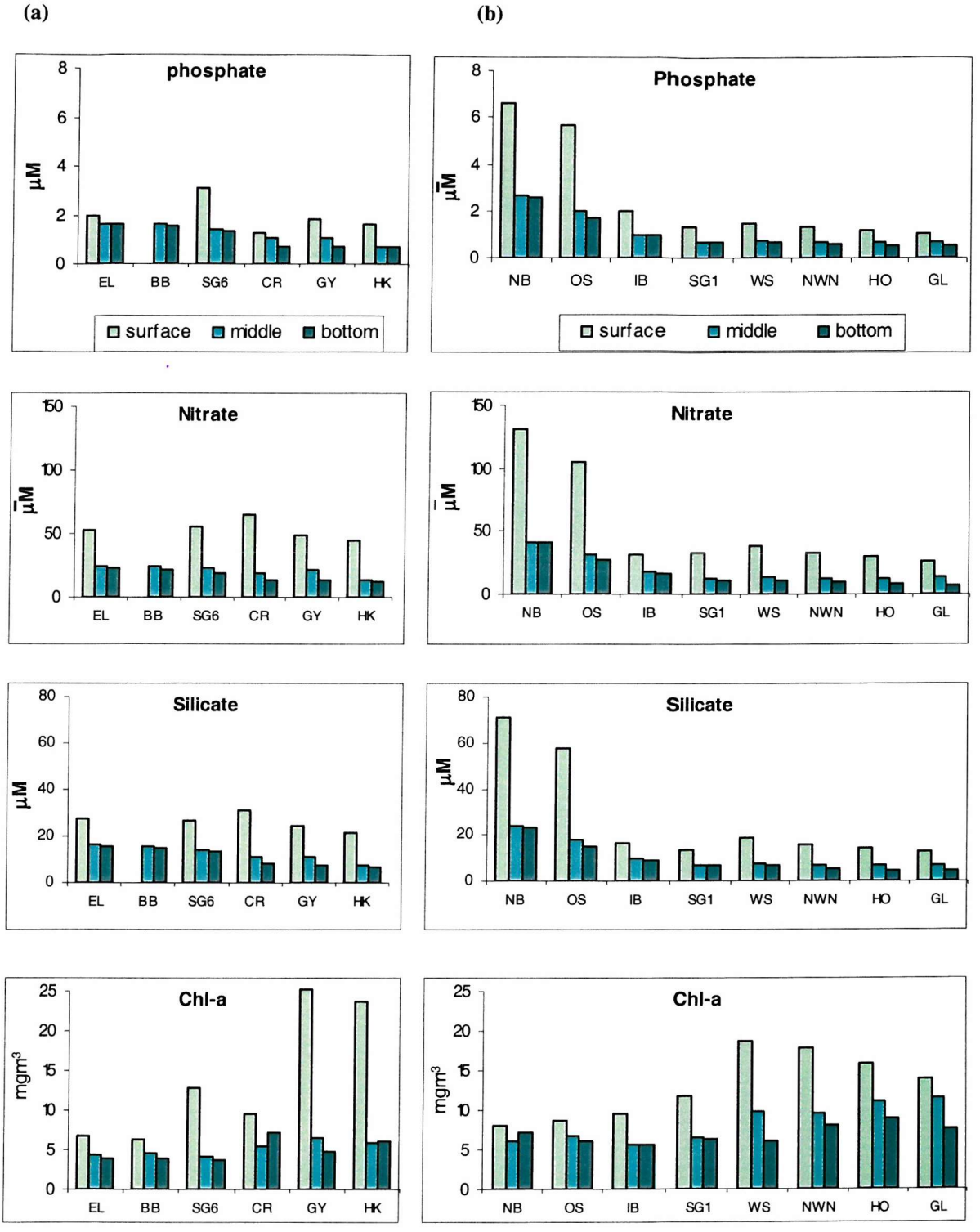


Figure 4.27 Spatial distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* through (a) Test estuary, (b) Itchen estuary and Southampton Water on 15th August 2000.

Figure 4.28 shows a comparison between the 3 nutrients measured at all sampling sites and salinity on 15th August 2000. It shows a noticeable decrease in nutrient concentrations at higher salinity sites suggesting some dilution of these nutrients against the salinity gradient along the

estuary. In addition, a degree of nutrient removal is indicated at some sites with high phytoplankton biomass (expressed as chlorophyll biomass, mg m^{-3}) (figure 4.28).

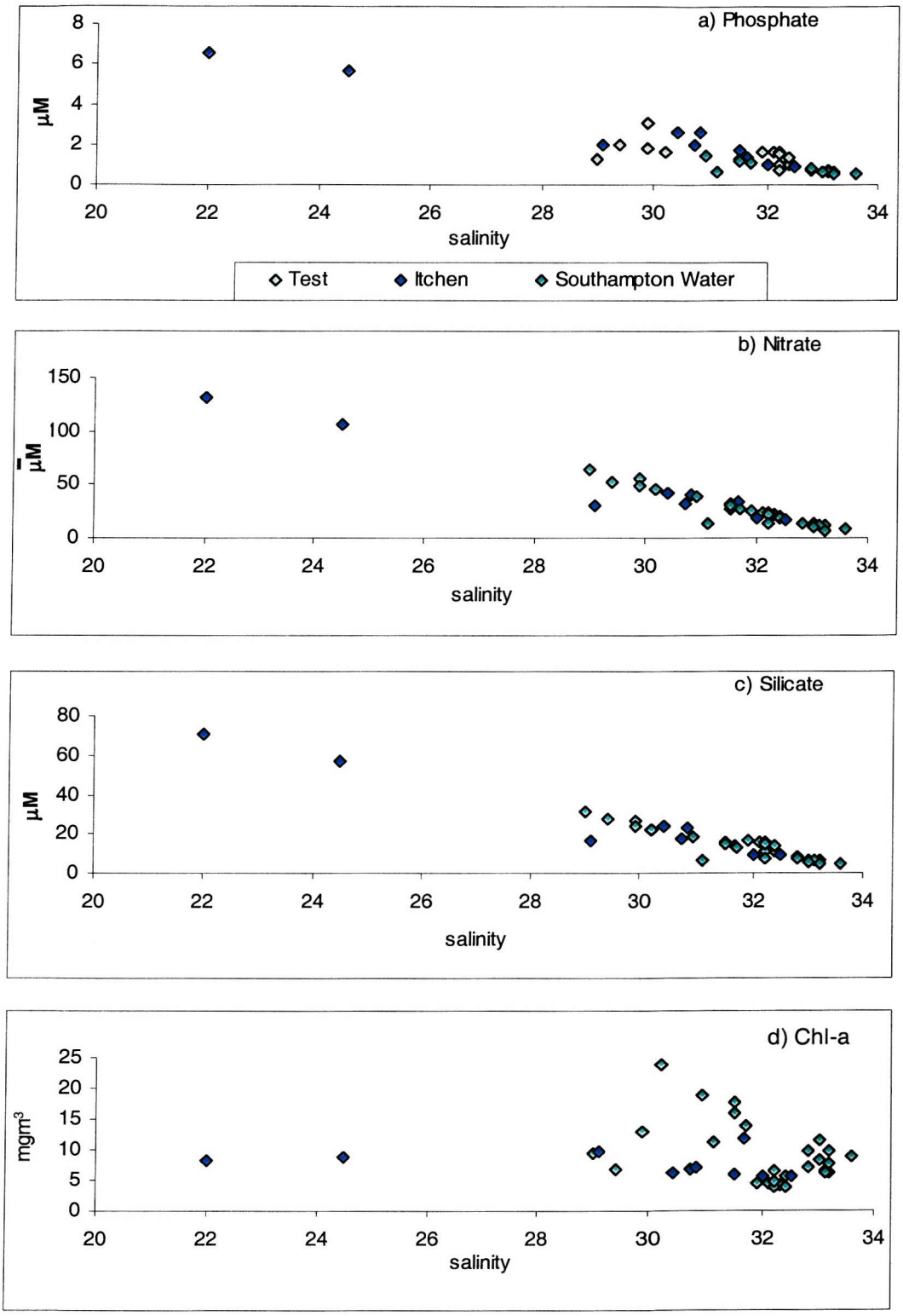


Figure 4.28. Distribution of nutrients (phosphate, nitrate and silicate) and Chl *a* concentration against salinity throughout the estuary on 15th August 2000.

4.5.4 PHYTOPLANKTON DISTRIBUTION ALONG THE ESTUARY ON 15th AUGUST

A community of different phytoplankton groups (figure 4.29) was recorded in surface water samples collected throughout the estuary on 15/8/00. Phytoplankton abundance, species composition (as cell numbers and cell biomass) and their distribution along the estuary are represented in figure 4.29 & 4.31. Similar to both surveys undertaken in 1999, phytoplankton population was numerically dominated by flagellates (see figure 4.29). The flagellate community (727-1984 cells ml^{-1}) during this survey (15th August, 2000) was however, dominated by a small (2-3 µm) flagellate (up to 1596 cells ml^{-1}) comprising up to 76% of total phytoplankton biomass and up to 93% of total flagellate cell number followed by *Cryptomonas* sp. (up to 844 cells ml^{-1}) as the second important species.

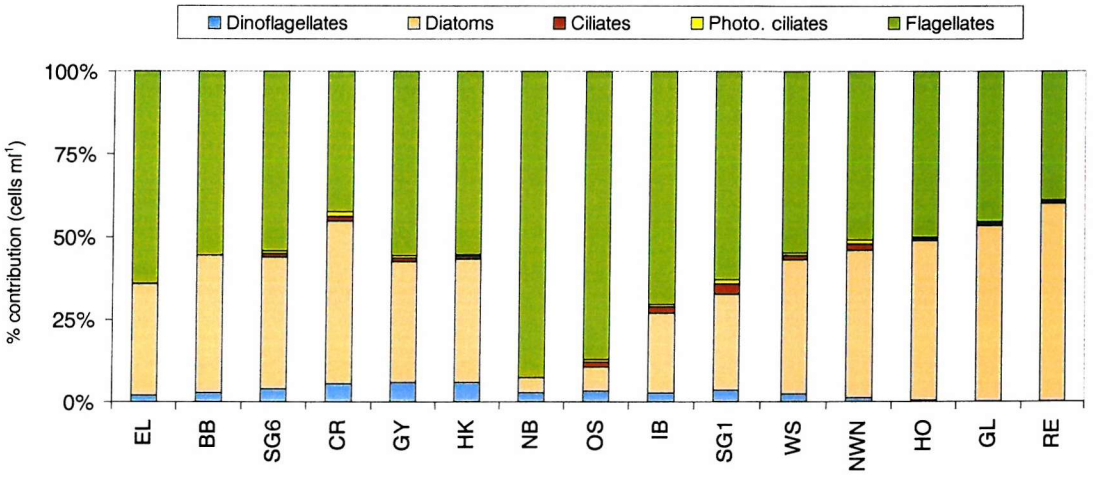


Figure 4.29. Horizontal distribution of dominant phytoplankton groups expressed as (a) cell density (cells ml^{-1}) and (b) cell biomass (mg C m^{-3}) on 15th August 2000.

Both species, *Cryptomonas* and the small flagellates, were abundant throughout the whole estuary with relatively similar contribution to total cell biomass. Maximum biomass peak of *Cryptomonas* sp. (~75 mg C m^{-3}) was measured at HK comprising >80% of total flagellate biomass. However, the other small flagellate was seen to be similarly distributed along the estuary with a slight increase in biomass in coastal waters, comprising up to 68% of total flagellate biomass at some coastal sites (figure 4.30).

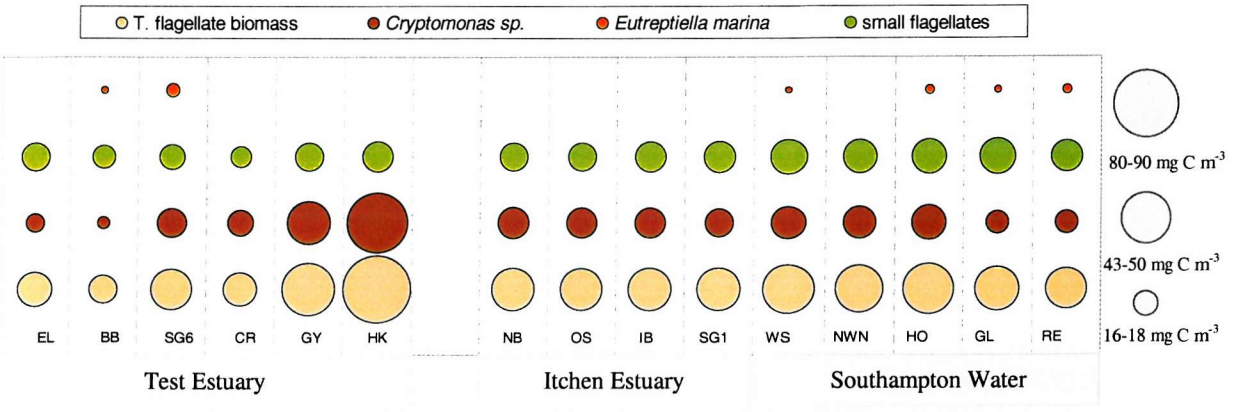


Figure 4.30. Horizontal distribution of total flagellates biomass (mg C m^{-3}) and dominant flagellate species (mg C m^{-3}) throughout the estuary on 15th August 2000.

This could be partially attributed to confusion during microscopic counting which might led to include other small flagellates (e.g. *Phaeocystis* sp.). *Eutreptiella marina* was also recorded at some sites with smaller numbers (< 12 cells ml^{-1}) and contributing also less to total flagellate biomass (see figure 4.30).

Ciliates (16-350 mg C m^{-3}) seemed to be the most important group contributing >55% of total phytoplankton biomass (see figure 4.31) at some sites, however autotrophic ciliates (maximum biomass 95.3 mg C m^{-3}), mainly *Mesodinium rubrum* contributed only 4-18% of total phytoplankton biomass (figure 4.31) and 16-43% of total ciliate biomass (figure 4.32) with maximum peaks at HK (96 mg C m^{-3}) and WS (110 mg C m^{-3}).

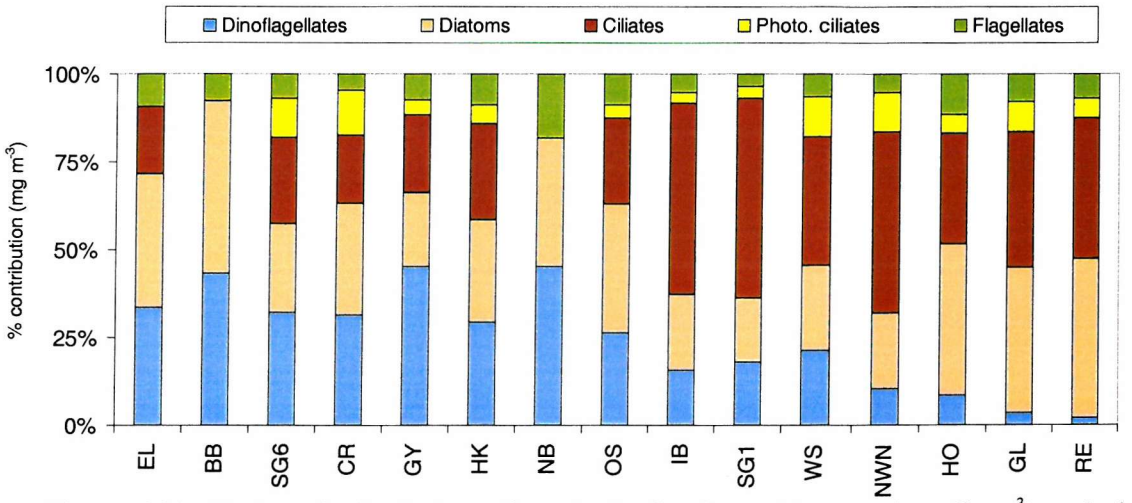


Figure 4.31. Horizontal distribution of total dinoflagellates biomass (mg C m^{-3}) and dominant dinoflagellates species (mg C m^{-3}) throughout the estuary on 15th August 2000.

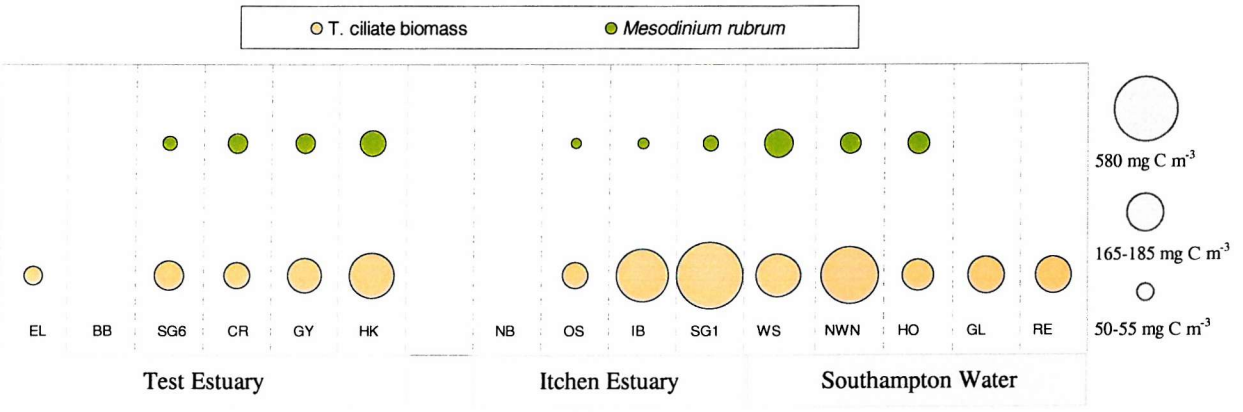


Figure 4.32. Horizontal distribution of total diatoms biomass (mg C m^{-3}) and dominant diatoms species (mg C m^{-3}) throughout the estuary on 15th August 2000.

Dinoflagellates (2%-45% of total phytoplankton biomass) and diatoms (19%-48% of total phytoplankton biomass) were very abundant along the estuary on 15th August (figure 4.31) with dinoflagellates being much more abundant in estuarine sites (along the Test and Itchen estuaries) and the intermediate sites in Southampton Water, while diatoms were abundant at all sites (see figure 4.31). Among the dinoflagellate community, *Prorocentrum micans* (3.2-131 mg C m^{-3}) and

Scrippsiella trochoidea (0-119.2 mg C m^{-3}) were the most dominant species at most sites contributing up to 71% and 39% of total dinoflagellate biomass, respectively at some sites (figure 4.33). A maximum biomass peak of both dinoflagellate species were measured at GY, HK (in the lower Test estuary) and SG1 (in the lower Itchen estuary) and WS (at the head of Southampton Water) (figure 4.33). *Protoperidinium minutum* was recorded at some sites with less contribution to total dinoflagellate biomass (maximum < 25%).

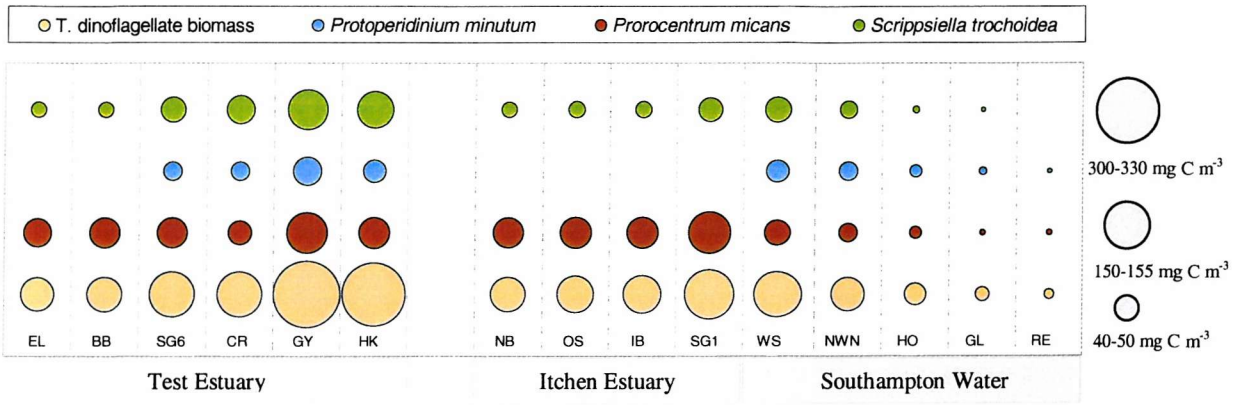


Figure 4.33. Horizontal distribution of total dinoflagellates biomass (mg C m^{-3}) and dominant dinoflagellate species (mg C m^{-3}) throughout the estuary on 15th August 2000.

Diatoms, the second most important photoautotrophic group, were represented by various species with *Odontella* spp. (*O. sinensis*; 0-52 mg C m^{-3} & *O. aurita*; 0-97 mg C m^{-3}) *Thalassiosira rotula* (0-60 mg m^{-3}) and *Rhizosolenia shrubsolei* (0-31 mg m^{-3}) being the most dominant species (figure 4.34a) contributing up to 71%, 28% and 13% of total diatom biomass, respectively (figure 4.34a). The relatively large diatom *Odontella* spp. were abundant in estuarine waters (figure 4.34a) with maximum biomass at HK (Test estuary), OS and IB (Itchen estuary), while the chain-forming diatom *R. shrubsolei* was much more abundant in Southampton water; from WS down to RE as well as at the lower sites in the Test estuary; from CR down to HK (figure 4.34a). The centric diatom *T. rotula* was, however, abundant at most sites along the estuary with noticeable increase in biomass towards the coastal sites (near to the mouth of the estuary).

Other less important, with respect to cell biomass, diatom species were numerically very abundant (see figure 4.34b). For example, a small (6-10 μm) centric diatom (*cf. Thalassiosira* sp.; 342-1151 cells ml^{-1}), a chain-forming narrow-celled diatom (*cf. Rhizosolenia* sp.; 18.2-262 cells ml^{-1}) and *Skeletonema costatum* (6.8-212 cells ml^{-1}) were the most numerically dominant diatoms contributing up to 55%, 21% and 19% of total diatom cell number at some sites (mainly in coastal waters), respectively. The small *Thalassiosira* species was recorded at all sampling sites throughout the whole estuary; however, *Rhizosolenia* as well as *S. costatum* were much more abundant at coastal sites (figure 4.34b).

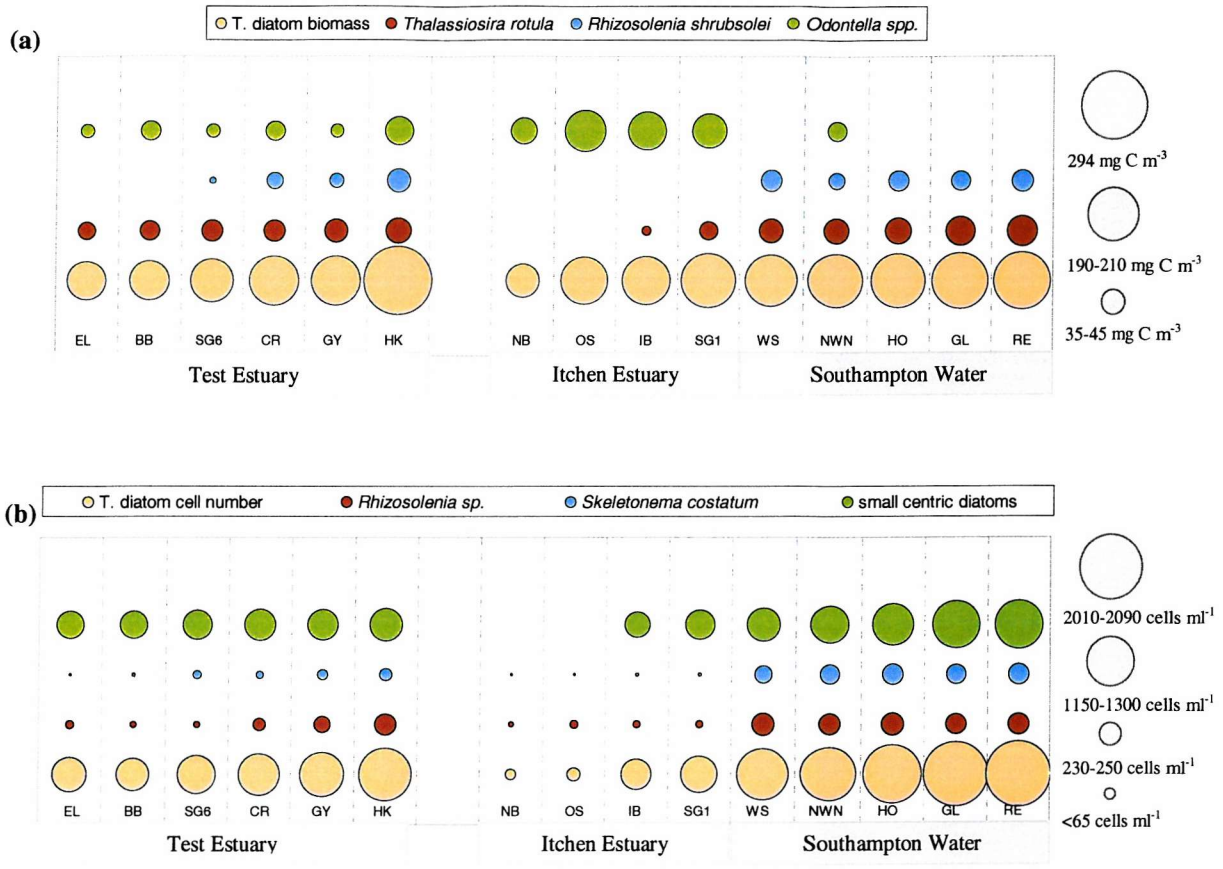


Figure 4.34. Horizontal distribution of: (a) total diatom biomass with dominant species (mg C m^{-3}) and (b) total diatom density with numerically dominant diatoms species (cells ml^{-1}) throughout the estuary on 15th August 2000.

Total phytoplankton biomass (mg C m^{-3}) and Chl *a* (mg m^{-3}) of water samples collected from the estuary on 15th August 2000 were correlated ($r = 0.65$, $p < 0.01$) although showed some scatter (see figure 4.35). This is partially due to the different community structure at each site, i.e. cell carbon was probably overestimated when large celled diatom species (e.g. *O. sinensis*) and/or carbon-rich species (e.g. dinoflagellates) were dominant (see figure 4.35).

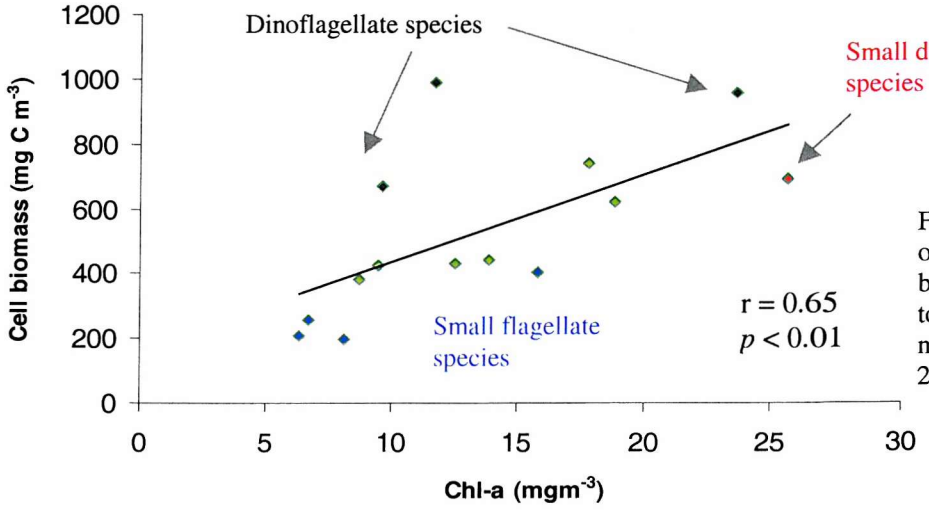


Figure 4.35. Relationship of total phytoplankton cell biomass (mg C m^{-3}) versus total Chl *a* (mg m^{-3}) measured on 15th August 2000.

4.6 DISCUSSION

Most of the previous phytoplankton studies in Southampton Water were only confined to a part of the estuary and involved only a few sampling sites e.g. not more than 5 sites (Iriarte, 1991; Kifle, 1992; Proenca, 1994; Lauria, 1998). The main objective of this part of the research was to investigate the variability of phytoplankton biomass and species abundance in relation to different environmental conditions (i.e. salinity, turbidity and nutrients) throughout the estuary.

4.6.1 CHANGES OF ENVIRONMENTAL PARAMETERS ALONG THE ESTUARY

Values of water temperature were very similar throughout the whole estuary, during each survey with a surface temperature difference of <1 ^{0}C (see table 4.3). Water temperature was warmer during the August (15/8/00) (19 – 21 ^{0}C) than during June (15.8 – 16.3 ^{0}C) and July (22/7/99) (18.6 – 19.7 ^{0}C) surveys. Higher water temperature values were measured in deeper waters on 22/7/99 (particularly, in the Test estuary), however surface waters were always warmer on 10/6/99 and 15/8/00 (see table 4.3).

Salinity structure along Southampton Water generally depends on the seasonal cycle of fresh water flow as well as on the tidal state (Phillips, 1980). Patterns of salinity as well as density structure were similar during all three surveys with higher salinity values recorded in July (maximum = 34.7) and August (maximum = 33.9) due to the reduced rainfall and lower freshwater input towards summer months. Higher transmission values (i.e. clearer water) were generally measured in surface waters during the June and July surveys (no data for August survey). Despite the higher concentrations of Chl *a* recorded in more transparent waters on 10/6/99, higher turbidity in deeper waters was mainly due to the effect of non-living particulate matter stirred up from bottom sediments as previously recorded in Southampton Water (Kifle, 1992; Lauria, 1998). However low Chl *a* concentrations were recorded in less transparent waters at the head of the estuary indicating the effect of increased turbidity on the growth of phytoplankton.

Relatively high values of attenuation coefficient (k) were recorded during both surveys in 1999 (no data for August 2000 survey) however light conditions were suitable for the onset of net phytoplankton growth as the euphotic layer was almost > 15% of the water depth as detected by Allen et al. (1998). Lower k values were measured along the Test estuary on 10th June 99, compared to other sites throughout the estuary with deeper photic depths (> 10 m) due to less turbidity and more-transparent water column. In contrast, sites throughout Southampton Water (coastal waters) were much clearer on 22nd July with maximum 1% irradiance depth (up to 9.3 m) compared to only 5.2 – 6.5 m along the Test estuary (see table 4.3). The increase in k values and the reduced photic depth along the Test estuary could be related to the mixing process of nutrient-

rich fresh waters containing more sediment, which often reduce the light penetration (Lauria, 1998).

Table 4.3: Difference in range of magnitude measured (for selected parameters) during the one-day spatial surveys undertaken in June, July, 1999 and August 2000.

Category	**10/6/99**	**22/7/99**	**15/8/00**
Salinity	25.9 - 33.7	28.2 - 34.7	22.5 – 33.9
Temperature (^{0}C)	15.8 - 16.3	18.6 - 19.7	19.0 – 21.0
Attenuation coefficient (m^{-1})	0.46 – 0.77	0.8 – 0.88	nd
1% Irradiation depth (m)	0.8 - 10	5.2 – 9.3	nd
Chl *a* (mg m^{-3})	2.24 - 6.2	1.18 - 5.1	6.3 – 25.7
Total diatoms (mg m^{-3})	24 - 160	4 - 65	72 – 294
Total dinoflagellates (mg m^{-3})	3 - 70	5 - 186	9 – 297
Total photo. ciliates (mg m^{-3})	3 – 77	0 - 22	0 – 95
Total flagellates (mg m^{-3})	0 - 47	0.2 -13	16 - 54
Phosphate (μM)	0 - 9.5	0 - 8	0.51 – 6.6
Nitrate (μM)	0 - 226	0.5 - 40	6.9 – 131.4
Silicate (μM)	0 - 105	3 - 35	4.4 - 71.2
Dominant phytoplankton species	*R. shrubsolei* *D. brightwellii* *Guinardia* spp. *Cryptomonas* sp.	*S. trochoidea* *R. styliformis* *O. sinensis* *Cryptomonas* sp.	*P. micans* *S. trochoidea* *T. rotula* *Cryptomonas* sp.

Nutrient distributions throughout the estuary showed conservative behaviour with dilution of freshwater nutrient-rich inputs with high salinity nutrient-depleted waters (Wright & Hydes, 1999). The more scattered data points for a phosphate versus salinity are due to the different inputs of phosphate along the estuary. Maximum nutrient (phosphates, nitrates and silicates) concentrations were measured at low salinity sites in both estuaries (Test and Itchen) during all surveys with maximum concentrations along the Itchen estuary. However, the Test estuary is known to have much higher mean annual discharge of (8.81 m^{-3} s^{-1}) compared to that (3.26 m^{-3} s^{-1}) of the Itchen estuary (Howard et al., 1995; Sylaios & Boxall, 1998).

As expected in a typical estuary (Pennock & Sharp, 1994), the euryhaline part of the estuary (from NWN down to CA) had a relatively low N:P ratio compared to that of the oligohaline part

(considering the Test estuary). This was not obtained in the Itchen estuary although a smaller salinity range was occurred. During June N:P ratio increased from SG1 to RE indicating P-limitation (see figure 4.36). A similar finding was also recorded in the hypernutrified highly-turbid Colne Estuary (Kocum et al., 2002b). Phosphate limitation is sometimes recorded in Southampton Waters particularly after extensive blooms (e.g. after the diatom spring bloom in May 1999).

P-limitation is also sometimes evident at coastal sites due to the removal of P which resulted from P adsorption to particulates in estuaries with high SPM such as the Colne estuary (Kocum et al., 2002b). Southampton Water estuary does not have high SPM and P-limitation in coastal waters is more likely to be due to phytoplankton removal through growth in the estuary.

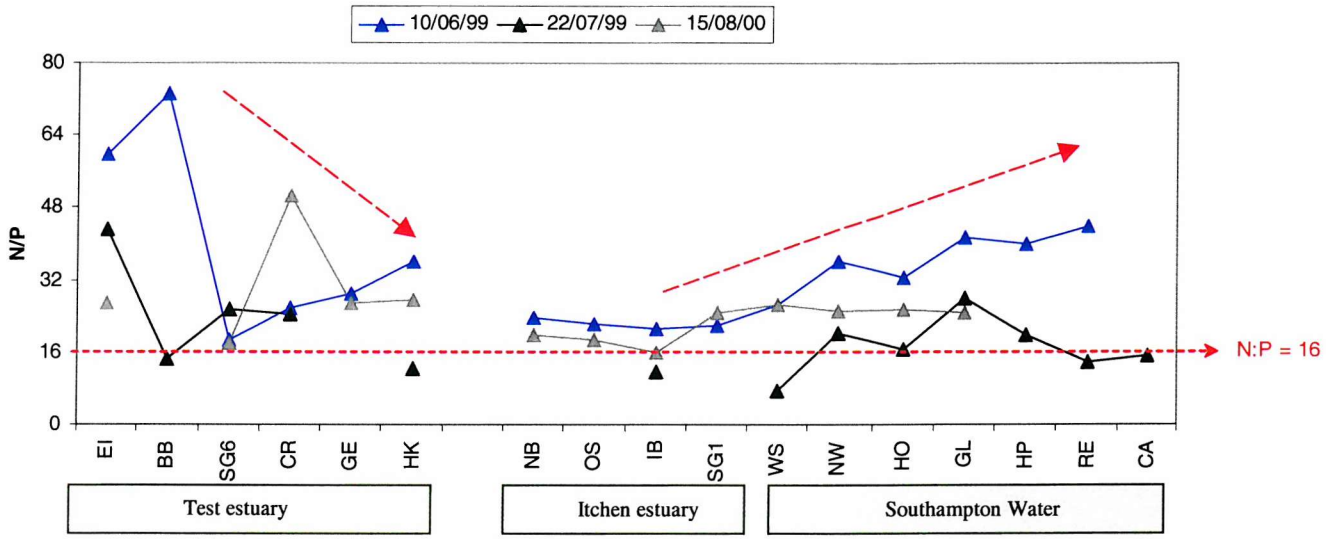


Figure 4.36. Changes in N:P ratio throughout the estuary during the 3 one-day surveys conducted in 1999 (10^{th} June & 22^{nd} July) and 2000 (15^{th} August).

4.6.2 SEASONAL DISTRIBUTION AND ABUNDANCE OF PHYTOPLANKTON SPECIES ALONG THE ESTUARY

Relatively similar patterns of phytoplankton distribution in surface waters were recorded at high and low salinity points during all three surveys suggesting that salinity variations along Southampton Water are not a major barrier to growth of most phytoplankton species in the estuary (Kifle, 1992).

The phytoplankton community was mainly dominated by diatoms in June and then changed to dinoflagellate-dominated in summer surveys (22^{nd} July 1999 and 15^{th} August 2000). Similar phytoplankton species succession was previously recorded in the Southampton Water Estuary (Kifle, 1992; Kifle & Purdie, 1993; Howard et al., 1995) and in similar estuaries, e.g. Peconic Bay (Bruno et al., 1980), in lower Westerschlde (Tripos, 1991) and inner Oosterscheld (Bakker et

al., 1994). Flagellates were exclusively numerically dominant in all surveys with the small-flagellate *Cryptomonas* sp. the most numerically dominant species during June and July surveys, while it was co-dominant with other much smaller flagellates during August survey.

A large diatom community of relatively large-celled species (e.g. *Guinardia flaccida* and *Ditylum brightwellii*,) was identified (figure 4.8) in surface water samples on 10/6/99 which was mainly the reason for the chlorophyll maximum recorded in deeper waters (figure 4.3b), particularly in the lower estuary because of the diatoms tendency to sink (Newell & Bulleid, 1975; Hallegraef, 1981) and their re-suspension into surface water in response to tidal mixing particularly during spring tide (see figure 4.1a) (Lauria, 1998).

During the July survey, an increase in dinoflagellates (*Scrippsiella trochoidea*) and flagellates (*Eutreptiella marina*) was recorded in surface waters (figure 4.19 & 4.22) causing a noticeable increase in surface Chl *a* throughout the whole estuary. Both species, *E. marina* and *S. trochoidea* are known to aggregate at surface waters and show well-marked stratification with near-surface Chl *a* maximum (Lauria, 1998), particularly at NWN (mid estuary, see map figure 1.3) during May-June (Kifle, 1992). A relatively similar finding was recorded during the August survey with flagellates (mainly small flagellate species) and dinoflagellates (mainly *P. micans*) dominanting the high surface Chl *a* on 15th August.

Phytoplankton abundance and species composition showed some variations throughout the estuary. For example, *Guinardia* species (*G. flaccida* and *G. delicatula*) were mostly dominant at high-salinity sites (i.e. coastal waters) (see figure 4.8). *Guinardia*, particularly *G. delicatula* is a stenohaline organism that does not grow at salinities below 14.5 and has been recorded in high cell numbers at high salinities (Rijstenbil, 1987). This species is known to preferably grow in saline water with optimal salinity value of 34-35 (Grall, 1972, cited in Kifle, 1992). *Guinardia*, particularly *G. delicatula* was previously noticed forming blooms during spring in Southampton Water (Kifle, 1992; Anning, 1995; Lauria, 1998) and also in other estuarine and coastal waters (Sournia et al., 1987; Peperzak et al., 1993). *Rhizosolenia styliformis* had a similar distribution to that of the *Guinardia* species.

In contrast, the relatively large-sized diatom, *O. sinensis* was only recorded in estuarine waters (see figure 4.20 & 4.34a). Similarly, the small-celled diatoms *Nitzschia* (with many species) was exclusively dominant in less-saline waters in the Test and the Itchen estuaries (see figure 4.9).

Other diatom species, e.g. *Thalassiosira rotula*, *Skeletonema costatum*, *Rhizosolenia shrubsolei* and *Chaetoceros* spp. were distributed throughout the whole estuary, indicating a wide-range of conditions of these species for growth. For examble, *S. costatum* has a broad range of salinity

tolerance (5-40) (Brand, 1984) with an optimum range of 15-18 (Brand, 1984; Kifle, 1992). *S. costatum* can also survive in freshwater for a few days (Qasim et al., 1972). *T. rotula* is described as a moderately euryhaline organism and grows in a salinity range of 12-38 (Schön, 1972) or 10-40 (Krawiec, 1982) with optimum growth of 25-30.

Among the dinoflagellate species identified in this part of the research, *S. trochoidea*, *P. micans* and *P. minutum* were recorded at upper and mid sites (figure 4.10 & 4.19 &4.33) decreasing towards the coastal sites, probably due to the increased turbulence at the mouth of the estuary. It is well known (e.g. White, 1976; Pollinger & Zemel, 1981; Thomas & Gibson, 1990; Berdalet, 1992; Berdalet & Estrada, 1993; Thomas et al., 1995) that most dinoflagellate species prefer to grow and form blooms in calmer water conditions. Brand (1984) and Kifle (1992) noticed that *P. micans* was dominant at a salinity of 32 with optimum range of 25-33 as observed *in situ* (Brand, 1984) and 18 in laboratory experiments (Kifle, 1992). *S. trochoidea* was previously recorded in higher cell densities in the mid estuary (NWN) than that recorded at the coastal site (CA) (Kifle, 1992).

The flagellate *Eutreptiella* was much more abundant at the coastal sites during July (figure 4.22) and August (figure 4.30); however it was recorded mostly at estuarine sites during the June survey (figure 4.11). *E. marina*, which is an oligohaline species of freshwater origin (Rijstenbil, 1987) can grow to a high cell density in waters with salinity <12.5.

Ciliates were less abundant during most surveys and generally contributed less to total phytoplankton biomass. The increased number of ciliates during the August survey was mainly due to heterotrophic species. *Mesodinium rubrum* was the most abundant photosynthetic ciliate along the estuary in 1999 and 2000. *M. rubrum* was very abundant in the mid estuary during July (figure 4.23) and August (figure 4.32). This could be attributable to the intermediate estuarine conditions (e.g. nutrient concentration and water mixing) at these sites. A noticeable increase in the biomass of *M. rubrum* was, however recorded at coastal waters (at RE and CA) during June survey (figure 4.10b). This is likely attributed to the increased flow rate of the River Test reflected by the higher nutrient concentrations measured in June and the state of the tide (i.e. spring tide). Therefore, the residence time of the upper part of the estuary is reduced and increase advection causes flushing of phytoplankton cells out of the estuary. A similar finding was previously recorded in Southampton Water by Kifle (1992).

4.6.3 RELATIONSHIP BETWEEN PHYTOPLANKTON BIOVOLUME AND CHL A

The temporal and spatial relationship between phytoplankton carbon biomass (mg C m^{-3}) and Chl *a* (mg m^{-3}) showed a good correspondence (see figure 4.12, 4.24, 4.35) and both variables showed a similar seasonal temporal and spatial pattern, but some spatial and temporal differences were observed. Some evidence of decoupling was recorded between both variables. Carbon biomass was in some water samples, overestimated. This may have occur when relatively large-celled diatoms (e.g. *O. sinensis, R. styliformis*) or/and dinoflagellates (e.g. *S. trochoidea*) were exclusively abundant. The relationship between these two biomass estimators could be considered as a phenomenon related to species succession induced by environmental changes (Felip & Catalan, 2000). For example, the C : Chl *a* ratio is influencing by stress conditions such as, nutrient limitation, light stress and seasonal variations in the phytoplankton community (Falkowski & LaRoche, 1991; Leeuwe & Stefels, 1998; Breton et al., 2000). These variable conditions can greatly affect the pigment content of phytoplankton cells. In addition, fixation can alter phytoplankton cell volume and this damage varies according to the fixative used and/or the species fixed (Montagnes et al., 1994).

CHAPTER FIVE

CHAPTER FIVE

5- CONTRASTING NUTRIENTS AND IRRADIANCE AS GROWTH PROMOTING CONDITIONS FOR PHYTOPLANKTON IN SOUTHAMPTON WATER

5.1 INTRODUCTION

In estuarine and coastal waters, phytoplankton are exposed to rapidly changing conditions that may have pronounced effects on their dynamics and community structure. Phytoplankton blooms in Southampton Water are known to be generally short lived, although concentrations of nutrients are high enough to support phytoplankton growth throughout the whole year (Wright et al., 1997, see section 5.4.2 in this chapter). Consequently, it is hypothesised that the growth of phytoplankton in Southampton Water is light limited rather than nutrient-limited. To test this hypothesis, two parallel sets of measurements were carried out during spring – summer 2000:

I- Field Sampling: A frequent sampling programme (approximately biweekly) was conducted at 3 sites representing a range of different environments in the Southampton Water Estuary during the productive period of the year in 2000.

II- Laboratory Incubations: A series of incubation experiments were undertaken in the laboratory using the surface water collected on 4 occasions from the same 3 sites. The collected samples were incubated under non-limiting irradiance levels for up to 14 days.

The data obtained from the field and experimental work were used to:

1- Quantify the temporal and spatial phytoplankton distribution and species composition along the estuary in relation to the changing environmental conditions.

2- Compare phytoplankton growth and species succession under essentially non-limiting light conditions in the laboratory with changes that occurred in the estuary over a similar period of time (i.e. 14 days)
3- Determine the degree to which a phytoplankton bloom can develop if supplied with sufficient irradiance.
4- Determine the extent to which nutrients and/or light are limiting factors for phytoplankton growth in different regions of Southampton Water.

5.2 SAMPLING STRATEGY

Surface water samples (1-meter depth) were collected approximately fortnightly from 3 different sites (figure 1.3) within Southampton Water, at SG6 (upper estuary), NW Netley (middle estuary) and Calshot or Reach (coastal waters) from mid May to end of August 2000. On return to the laboratory, sub-samples were taken for later measurements of Chl *a* (using the fluorometric method and HPLC pigment analysis) and nutrients (nitrate, phosphate and silicate). Water samples for phytoplankton cell counts were also taken and preserved with Lugol's iodine solution (see chapter 2 for detailed methods). On four occasions at monthly intervals (16th May, 19th June, 17th July and 14th August) unfiltered water samples were collected from these 3 sites and used for the incubation experiments.

5.3 EXPERIMENTAL METHODOLOGY

Water samples were collected from 1-meter and pre-filtered through a 100-μm net to remove larger zooplankton. Initial sub-samples were taken for Chl *a*, nutrients (nitrate, phosphate, silicate) and phytoplankton cell counts. Water samples were then placed in duplicate clean 2-L polycarbonate bottles (see figure 5.1) and incubated for a period of 14 days at 15 –16 ^{0}C and mean irradiance level of about 120 μmol. m^{-2} s^{-1} on a 16L: 8D cycle. Culture bottles were mixed and their positions in the incubator changed daily to randomise the incubation conditions over the period of the experiment.

During the incubation period, water sub-samples, for the experiments conducted in June, July and August, were taken every two days (for a period of 14 days) from each bottle for later analysis of Chl *a*, nutrients (nitrate, phosphate, silicate) and phytoplankton cell counts. For the first experiment (May), water sub-samples were collected daily for a period of 8 days. Cell count and carbon data is presented only for the initial day (day 0) and for the day with the maximum Chl *a* (peak day). Details of procedures for the conversion of phytoplankton cell number to cell biomass (carbon) was described in section 2.3.2.4 of chapter 2.

Figure 5.1. Picture of the culture bottles (in duplicates) during the incubation experiments collected from the three sampling sites; SG6 (UE; A1 + A2), NW Netley (ME; B1+ B2) and Calshot (LE; C1 + C2)

5.4 FIELD RESULTS

5.4.1 SALINITY AND TEMPERATURE

Spatio-temporal variations in salinity and temperature of the surface water collected from the three chosen sites (upper & mid estuary and coastal waters) along Southampton Water are represented in figure 5.2 a & b. Horizontal variations in temperature were similar along the estuary during each sampling date, with a mean temperature difference of about 0.3-0.5 ^{0}C between the upper and lower part of the estuary. Lower temperature values, 14.1,14.3 and 14.0 ^{0}C were measured in May at SG6, NWN and Calshot/Reach, respectively (figure 5.2b), while temperatures increased during summer reaching maximum values (24.9, 24.7 and 24.4 ^{0}C) during July.

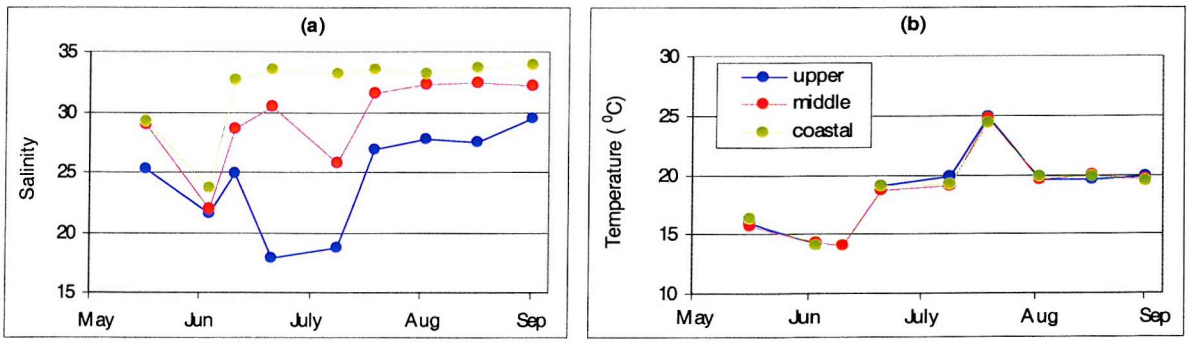


Figure 5.2. Temporal variation in surface salinity (a) and temperature (b) at the 3 sampling sites (upper, middle and coastal) along Southampton Water Estuary from May to August 2000.

Surface water salinity varied from 17.8 to 33.9 along the estuary during the sampling period (16/5–29/8/00), with lower salinity values (figure 5.2a) at the head (SG6) of the estuary due to the freshwater input of the river Test (see map figure 1.3).

5.4.2 TEMPORAL CHANGES OF NUTRIENT AND CHL A CONCENTRATION

The fortnightly changes (from May to August 2000) in Chl *a* of the water collected from the 3 sampling sites in relation to variations in nutrient (silicate, nitrate, and phosphate) concentrations are presented in figure 5.3.

The maximum concentrations of these nutrients were measured in the lower-salinity waters (figure 5.3) due to the influence of nutrient-rich fresh water input from the River Test (see map, figure 1.3). In the upper part of the estuary (SG6) nutrient concentrations ranged between 45-190 μM, 30-100 μM, and 1.1-3.8 μM for nitrate, silicate and phosphate, respectively with maximum nitrate (190 μM) and silicate (100 μM) concentrations measured in mid June (figure 5.3). The highest phosphate value (3.8 μM) recorded at the head of the estuary was measured in mid May. However, nutrient concentrations at the mouth of the estuary ranged between 0.9-19.9 μM (silicate), 3.0-76.3 μM (nitrate) and 0.1-0.6 μM (phosphate) with higher concentrations of nitrate and silicate recorded on 16th May (figure 5.3).

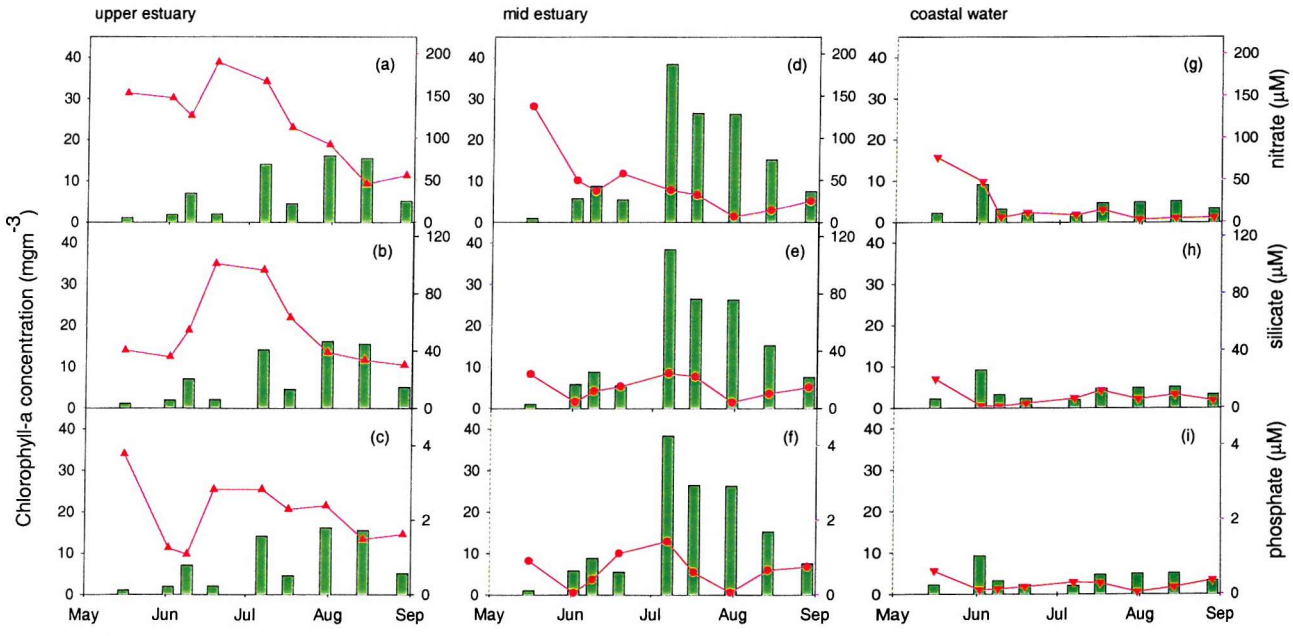


Figure 5.3. Temporal changes in nitrate, silicate, phosphate (red lines) and Chl *a* (green bars) in surface waters of the upper estuary (a, b, c), middle estuary (d, e, f) and coastal waters (g, h, i) along Southampton Water during spring-summer 2000.

Figure 5.3 shows the variations in phytoplankton biomass (expressed as Chl *a*) at the 3 sampling sites during the period of study. Chl *a* concentration varied from ~1.0 to 16.0 mg m^{-3} (at SG6) and ~1.0 to 38.0 mg m^{-3} (at NW Netley) in the estuarine waters, while it varied from ~2.0 to 9.0 mg m^{-3} in the coastal waters. In the upper part of the estuary, discrete Chl *a* concentration (figure5.3) showed lower values (<2 mg m^{-3}) in May and early June. A smaller peak of Chl *a* with a value of ~7 mg m^{-3} (mainly diatoms) was measured after the first week of June (9th June) followed by 3 larger peaks (~15 mg m^{-3}) in July (7th & 31st July) and mid August (14th August). Lower concentrations of Chl *a* (2.0 – 5.0 mg m^{-3}) were mostly measured in the outer part of the estuary (coastal waters), however, a noticeable increase in phytoplankton biomass (~9 mg m^{-3} Chl *a*) occurred in early June during the time of minimum nutrient concentrations. This increase followed a higher nutrient levels in mid May, particularly silicate. The chain forming diatom *Guinardia delicatula* mainly dominated this peak. Other smaller peaks (~5 mg m^{-3}) were recorded later in summer (figure 5.3) and were mostly dominated by other smaller diatoms (see section 5.4.3).

Maximum concentrations of nutrients were recorded at the upper estuarine site (SG6), however the maximum phytoplankton biomass (expressed as Chl *a*) was measured at the mid-estuarine site (NW Netley) where nutrient concentrations were intermediate between the upper and lower sites. The highest Chl *a* values (38.3, 26.4, 26.2 and 15.2 mg m^{-3}) were recorded in July (7th July, 17th July, 31st July) and August (14th August) at NW Netley during the phototrophic ciliate (*Mesodinium rubrum*) bloom, see figures 5.3 & 5.10.

The scatter plot (figure 5.4) presents the horizontal distribution of nutrients measured along the salinity gradient within the estuary. It shows the dilution effect of the low- salinity nutrient rich water at the mouth of the estuary, in late June and early July. Apparent removal of nutrients (i.e. dramatic decrease in concentration) is seen, particularly of silicate and phosphate during the first week of June.

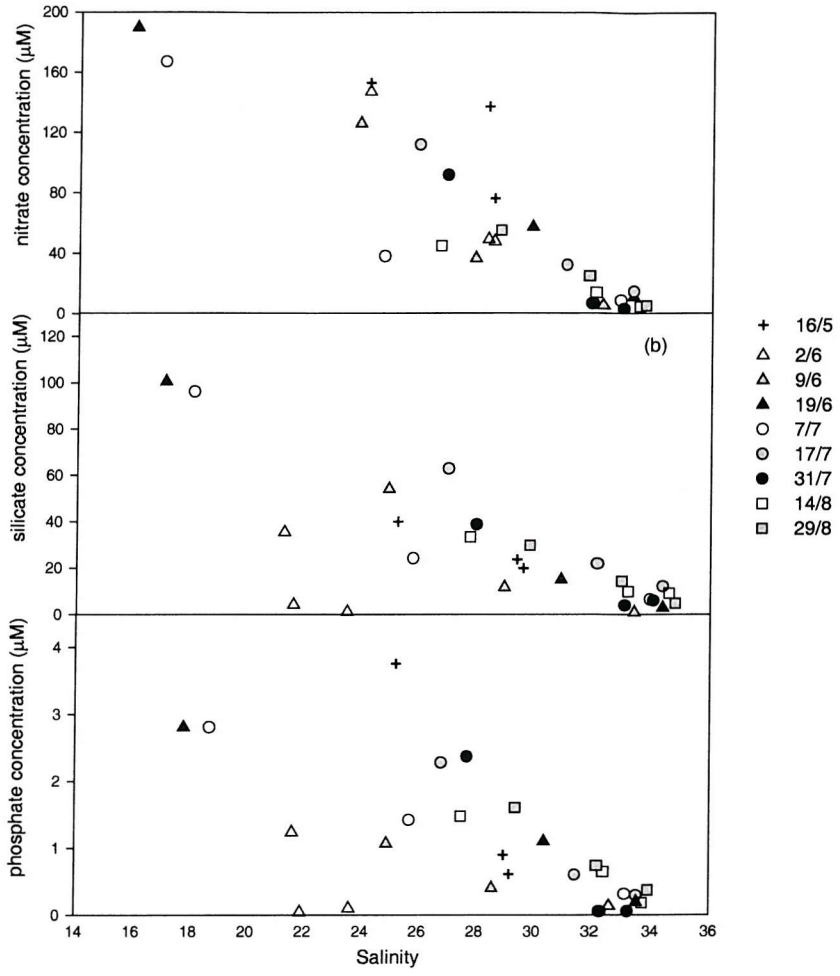


Figure 5.4. Horizontal distribution of nitrate, silicate and phosphate along salinity gradient of Southampton Water during spring-summer 2000.

5.4.3. TEMPORAL CHANGES IN PHYTOPLANKTON BIOMASS AND COMMUNITY STRUCTURE

Temporal and spatial distribution of total phytoplankton cell numbers (cells ml^{-1}) and total biomass (mg C m^{-3}) are presented in figure 5.5 & 5.6. A mixture of different phytoplankton groups/species was recorded (figure 5.6, 5.7 & 5.10) from the microscopic analysis of water samples collected from the 3 sampling sites. It is noticeable that phytoplankton biomass was maximal in the estuarine waters (upper and middle) compared to the coastal waters (figure 5.5), being maximum in the mid estuarine environment particularly in July during the period of the *M. rubrum* bloom.

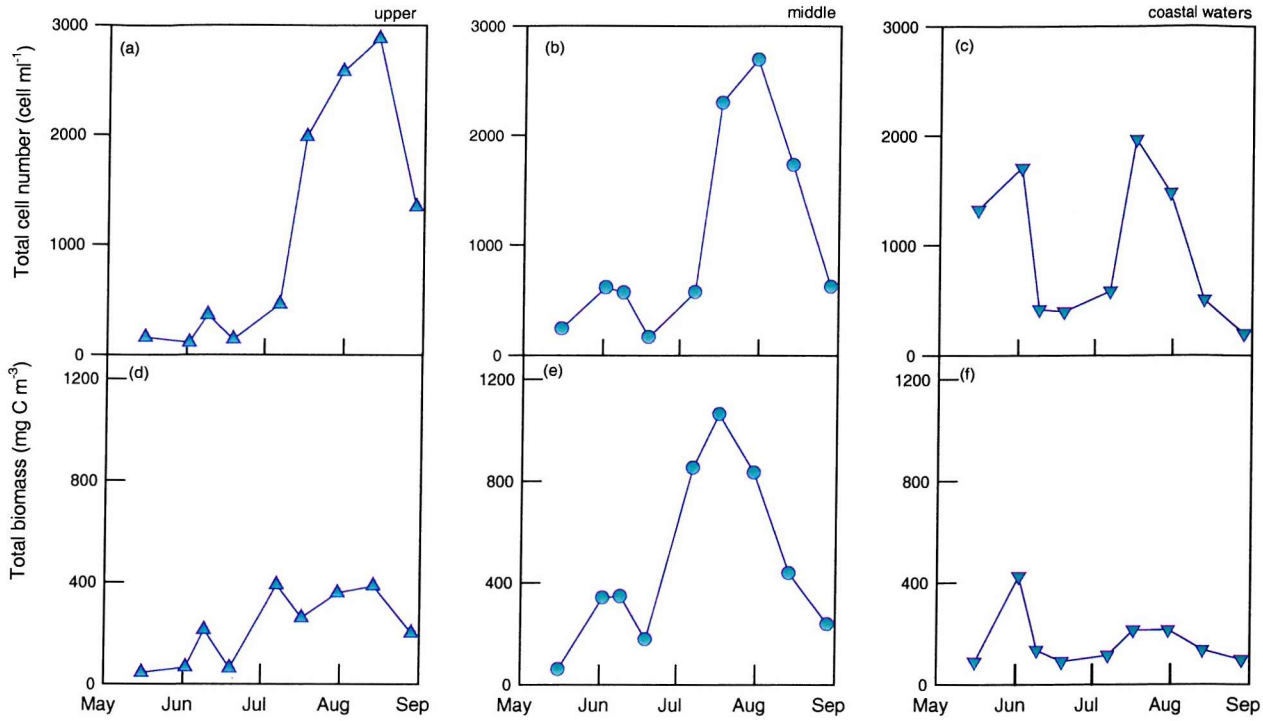


Figure 5.5. Temporal variation in total phytoplankton cell number (a, b, c) and total phytoplankton biomass (d, e, f) in the upper estuary (a, d), middle estuary (b, e) and coastal waters (c, f) of Southampton Water during spring-summer 2000.

Figure (5.5 a, b, c) shows that the highest phytoplankton cell numbers of ~ 2900 cells ml^{-1} (upper), ~2700 cells ml^{-1} (middle), and ~2000 cells ml^{-1} (coastal waters) were recorded along the estuary in late summer. A small unidentified flagellate was numerically dominant (figure 5.12) during this period with a contribution of ~70% and 66% of the total cell number during summer (July-August) in the estuarine waters and the coastal waters, respectively. The peak in cell numbers (1100 cells ml^{-1}) in coastal waters (figure 5.5f & 5.6k) in mid May was mainly dominated by *Phaeocystis sp.*

The highest phytoplankton biomass (~1050 mg C m^{-3}) was recorded in July in the middle estuary (figure 5.5) during the bloom of the relatively large-celled and carbon-rich phototrophic ciliate *Mesodinium rubrum* (figure 5.10 & 5.11).

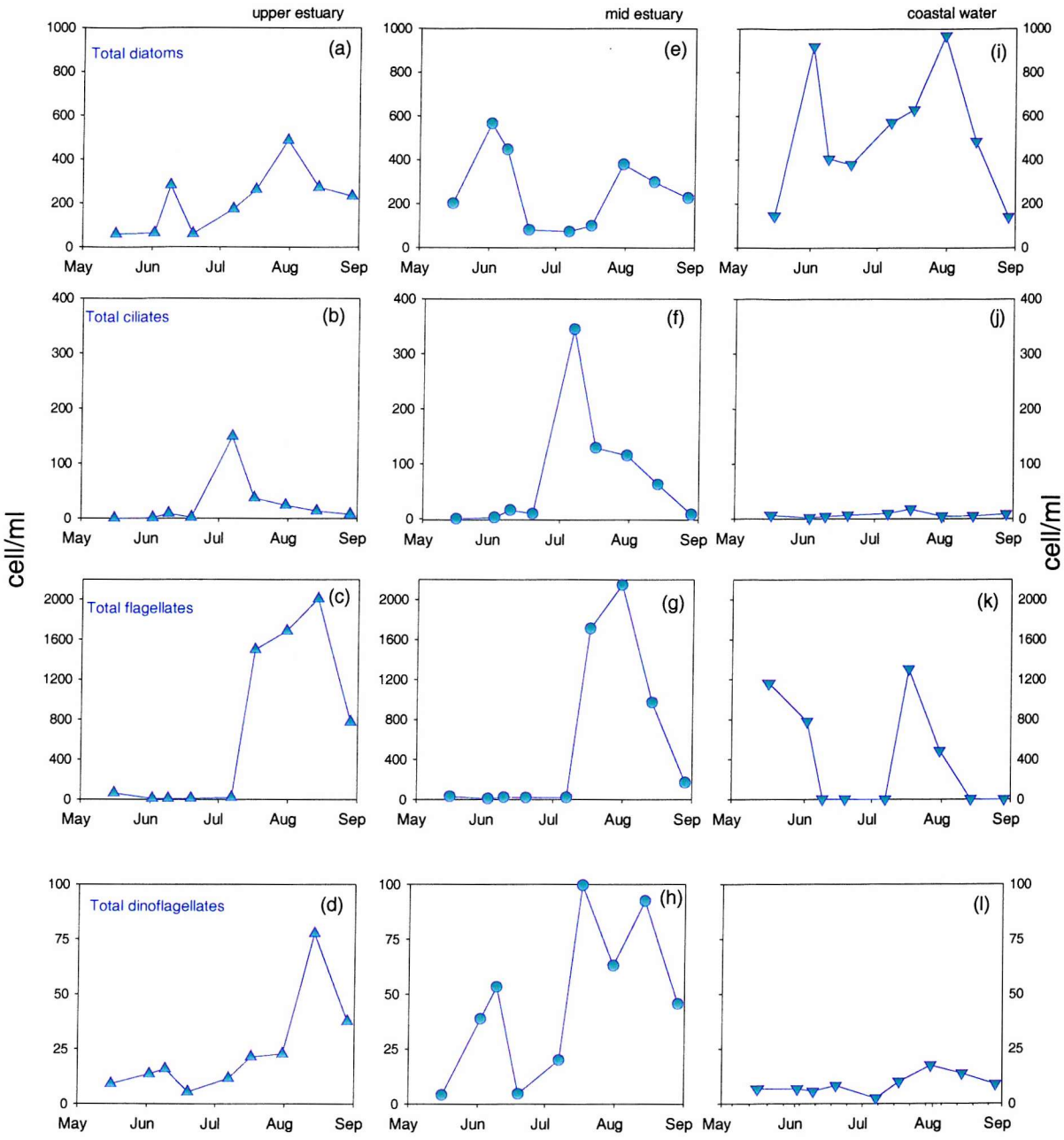


Figure 5.6. Temporal variation in cell numbers of total diatoms (a, e, i), total ciliates (b, f, j), total flagellates (c, g, k) and total dinoflagellates (d, h, l) in the upper estuary (a, b, c, d), middle estuary (e, f, g, h) and coastal waters (i, j, k, l) along Southampton Water during spring-summer 2000.

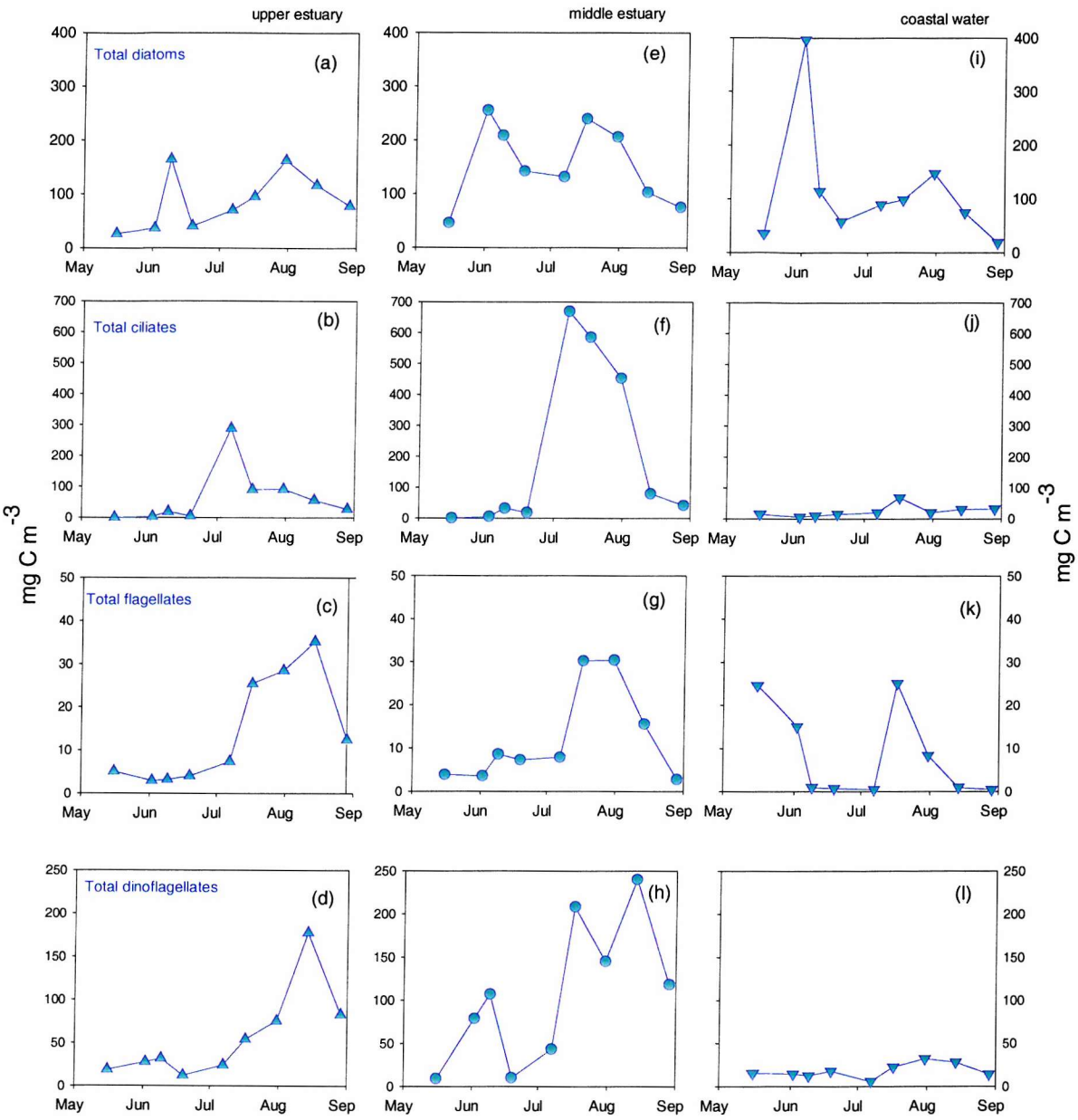


Figure 5.7. Temporal variation in phytoplankton biomass expressed as carbon content (mg C m^{-3}) of total diatoms (a, e, i), total ciliates (b, f, j), total flagellates (c, g, k) and total dinoflagellates (d, h, l) in the upper estuary (a, b, c, d), middle estuary (e, f, g, h) and coastal waters (i, j, k, l) along Southampton Water during spring-summer 2000.

Figure 5.8 shows that temporal changes in total phytoplankton biomass, expressed as carbon content (mg C m^{-3}) had a relatively similar pattern to that expressed as Chl *a* (mg m^{-3}) at the three sampling sites. The C:Chl *a* ratio of samples collected throughout the whole estuary ranged between 20 and 60 (figure 5.9), with higher C: Chl *a* ratios (~40–60) measured in May and June,

during the growth of the chain forming diatom *Guinardia delicatula*, and the second half of July during the summer bloom of dinoflagellates (figure 5.10 & 5.11). During the bloom of the phototrophic ciliate *Mesodinium rubrum* in July, the C: Chl *a* ratio had a lower range of 22 to 40 in the middle estuary, with minimum values in early July when *M. rubrum* was ~80% of the total phytoplankton biomass. Relatively higher C: Chl *a* ratios of 32 and 40 were measured, at the same site, at the end of July when the ciliate bloom contributed ~50% of the total biomass.

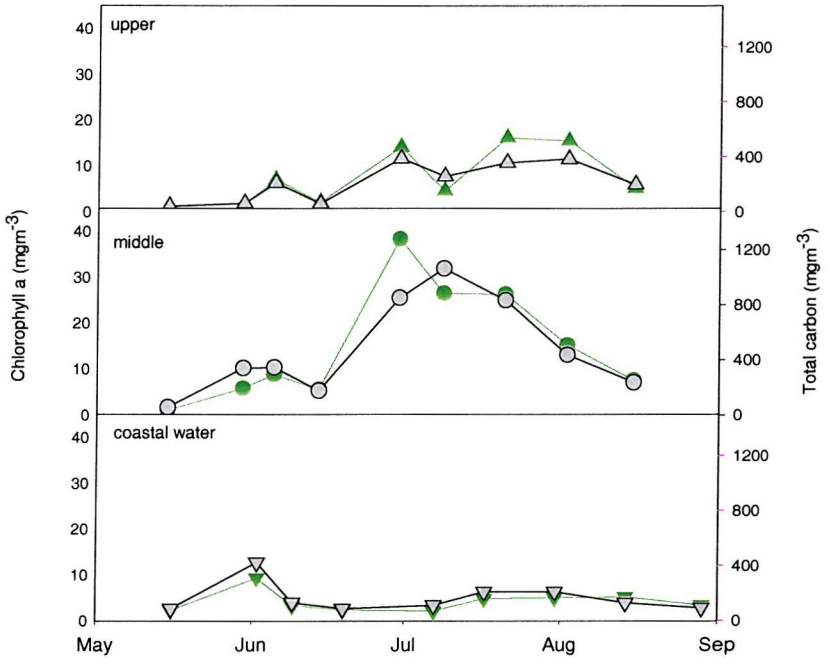


Figure 5.8. Temporal changes in phytoplankton biomass presented as Chl *a* in mg m^{-3} (green lines) and total carbon as mg C m^{-3} (grey lines) at each of the three sites.

Data presented in figure 5.6, 5.7 & 5.11 shows a seasonal pattern of phytoplankton succession, with diatoms being the dominant group of the phytoplankton community in spring; however the late summer bloom was mainly dominated by dinoflagellate species (figure 5.11). A large increase in total ciliates, expressed as cell numbers (figure 5.6) and cell carbon (figure 5.7) followed the spring diatom bloom. This increase was mainly due to the bloom of the autotrophic ciliate *Mesodinium rubrum* which contributed > 95% to the total biomass of ciliates (figure 5.10) and 32-83% of the total phytoplankton biomass (figure 5.11) during this time of the year. Total flagellates showed a distinctive increase in cell numbers (55–85% of the total cell number) before and during the dinoflagellate bloom in late summer (figure 5.6 & 5.12), although they showed little contribution to the total cell carbon (<12%; figure 5.11). The main flagellate blooms (2008 cells ml^{-1} & 35.2 mg C m^{-3} at SG6, 1875 cells ml^{-1} & 30 mg C m^{-3} at NWN and 1306 cells ml^{-1}&

25.1 mg C m^{-3} at Calshot) were recorded during the period 17th July – 14th August (figure 5.10 & 5.11). This increase was mainly due to the increase in cell numbers of small flagellates and *Cryptomonas* sp.

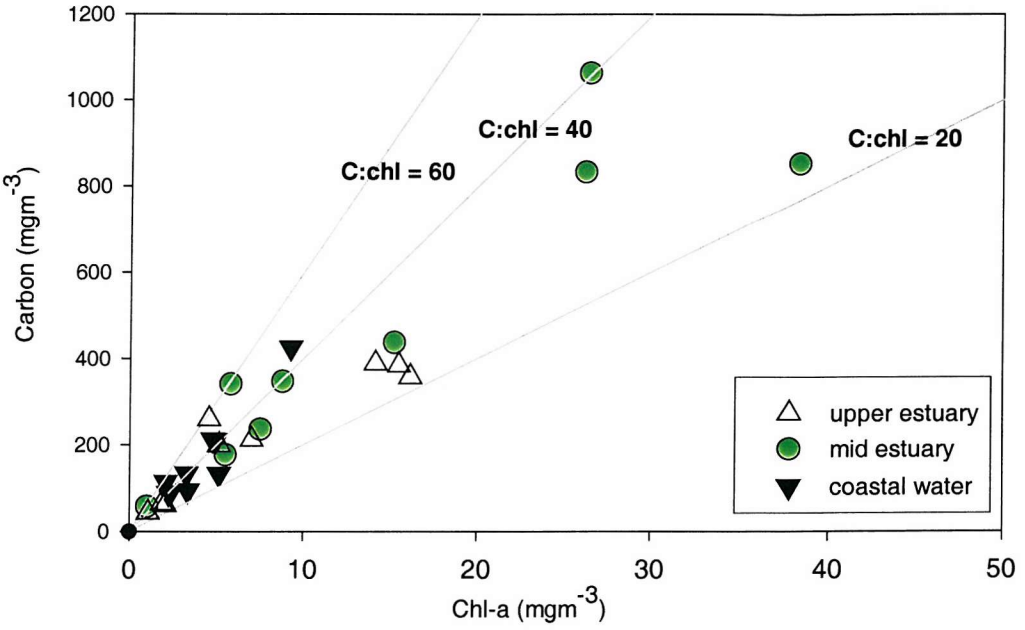


Figure 5.9. Temporal distribution of phytoplankton total biomass (mg C m^{-3}) versus Chl *a* (mg m^{-3}) for Southampton Water during the sampling period (16th May – 29th August 2000), compared to different theoretical C: Chl *a* ratios (C: Chl *a* = 20, 40, 60).

Phytoplankton succession of dominant species during the sampling period (mid May–end of August 2000) is shown in figure 5.10 at the 3 sampling sites. A mixture of several diatom species was recorded at all sampling sites during spring/summer period of this study, with four species (*Guinardia delicatula*, *Thalassiosira cf. rotula*, *Odontella sinensis*, and *Nitzschia closterium*) mainly dominating the total diatom biomass (figure 5.10). The chain-forming diatoms, *Guinardia delicatula* and *Thalassiosira cf. rotula* were the most dominant species in the early diatom bloom (9th June) followed by a pronounced increase in the biomass of the larger (> 60 μm) centric diatom *Odontella sinensis* (figure 5.11). However, a later diatom bloom recorded on 31st July, was mainly dominated by the small-sized pennate (<10 μm) diatom *Nitzschia closterium.*

Some diatom species formed a high proportion of the total diatom cell numbers but represented a small proportion of total diatom biomass. For example, some small centric diatoms were recorded during the period from mid June to the end of August (figure 5.12) contributing up to 90% of the total diatom cell number along the whole estuary, in general, and the coastal waters in particular (see figure 5.12). These small-sized species contributed less than 16% to the total diatom biomass (figure 5.11). Similarly, some small flagellates (including *Phaeocystis sp.*) contributed 35-75% of the total phytoplankton cell numbers identified during July - August throughout the estuary (figure 5.12), but contributed less than 10% to the total species biomass at the same time (figure 5.11).

A higher phytoplankton biomass recorded in July in the mid estuary (figure 5.10), was due to motile cells (e.g. *Mesodinium rubrum*) and dinoflagellates (figure 5.11), with some large-celled diatom species (e.g. *Odontella sinensis*). The lowest phytoplankton biomass was recorded at the mouth of the estuary (coastal waters), although *Phaeocystis sp.* and unknown small species grew well in this region. The chain forming diatom *Guinardia delicatula* was recorded with a relatively higher biomass peak in early June at the higher salinity station (Calshot/Reach).

The summer dinoflagellate bloom was well represented by three dominant species, among which *Scrippsiella trochoidea* and *Prorocentrum micans* were the most abundant species along the estuary (figure 5.10). The third abundant dinoflagellate, *Protoperidinium minutum*, contributed 5-17% to total dinoflagellate biomass (mg C m^{-3}) at the three sites compared to the contribution of 40 – 80% for *Scrippsiella trochoidea* and 30 – 70% for *Prorocentrum micans.* The later dinoflagellate species apparently grew better in the middle part of the estuary (figure 5.10).

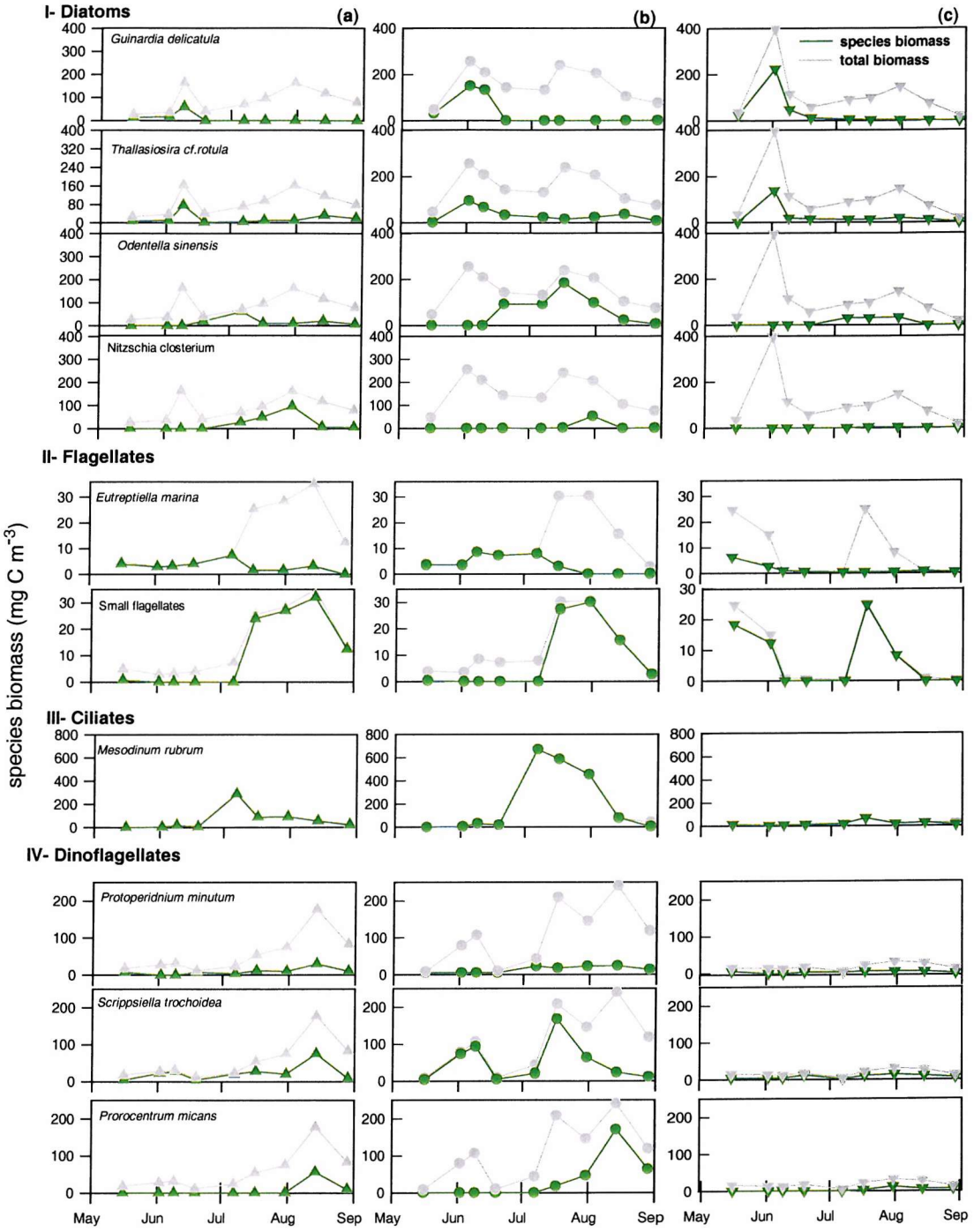


Figure 5.10. Temporal changes in dominant species biomass (green line) in relation to the total biomass (grey line) of the relevant group,(I) diatoms, (II) flagellates, (III) ciliates and (IV) dinoflagellates in the upper estuary (a), middle estuary (b) and the coastal water (c) during spring-summer 2000.

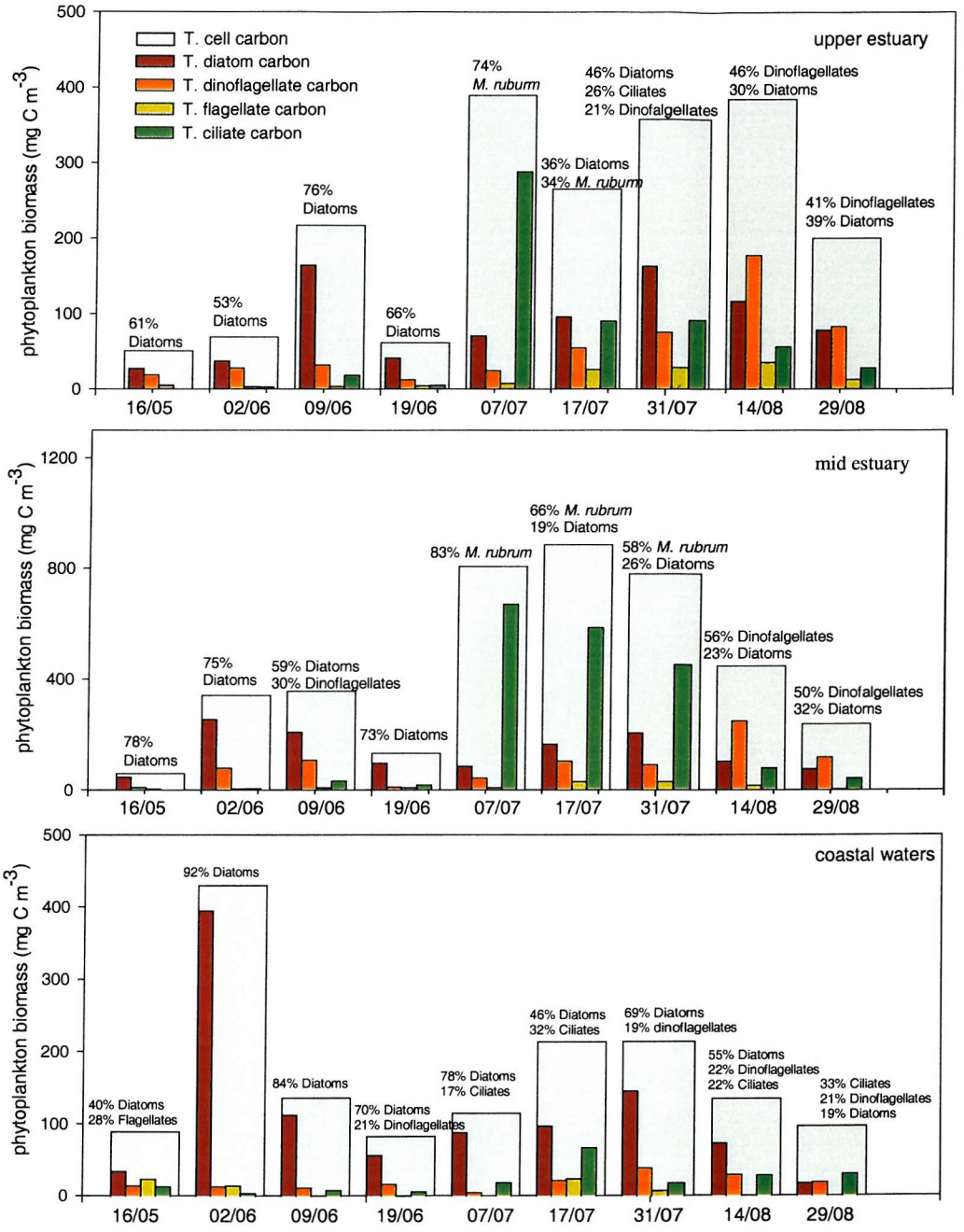


Figure 5.11. Temporal changes in total phytoplankton biomass (mg C m^{-3}) and biomass contribution of the dominant phytoplankton group and/or species. Numbers indicates the % contribution of the most dominant phytoplankton group and/or species to the total species biomass.

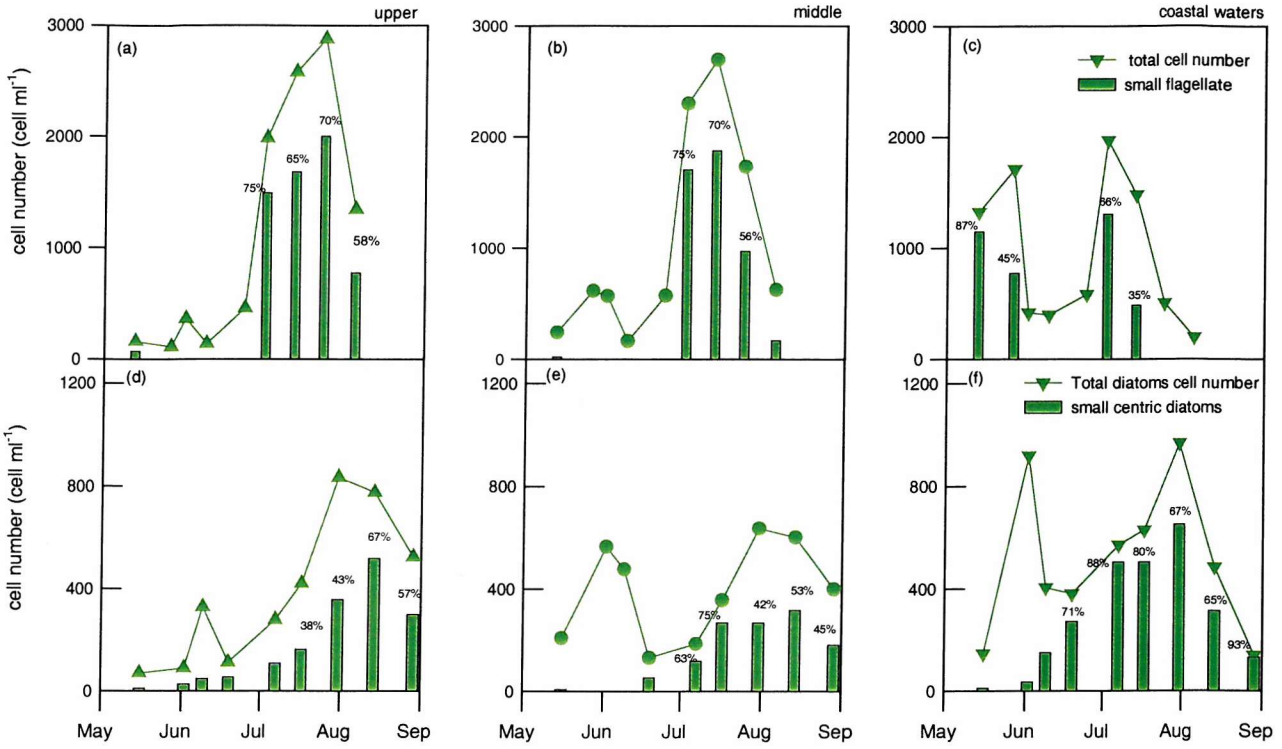


Figure 5.12. Temporal variations in numerically (cell ml^{-1}) dominant small flagellate species (a, b, c) in relation to the total phytoplankton cell numbers and small centric diatoms (d, e, f) in relation to total diatom cell number in the upper estuary (a, d), middle estuary (b, e) and coastal waters (c, f) of Southampton Water during spring-summer 2000.

5.5 EXPERIMENTAL RESULTS

Duplicate 2 litre surface water samples (1meter depth) collected from upper, middle estuary and coastal waters were incubated under good light conditions (figure 5.1) for a period of 2 weeks (1 week for the first experiment). Analyses of Chl *a*, nutrients (nitrate, phosphate, and silicate) as well as phytoplankton cell number/carbon were conducted for sub-samples taken every second day from each culture (daily for the first experiment). Incubation experiments were conducted at monthly intervals for four months (15th May, 19th June, 17th July and 15th August). Figure 5.13 showed the flow rate of the River Test during the period of this study (from May to August 2000).

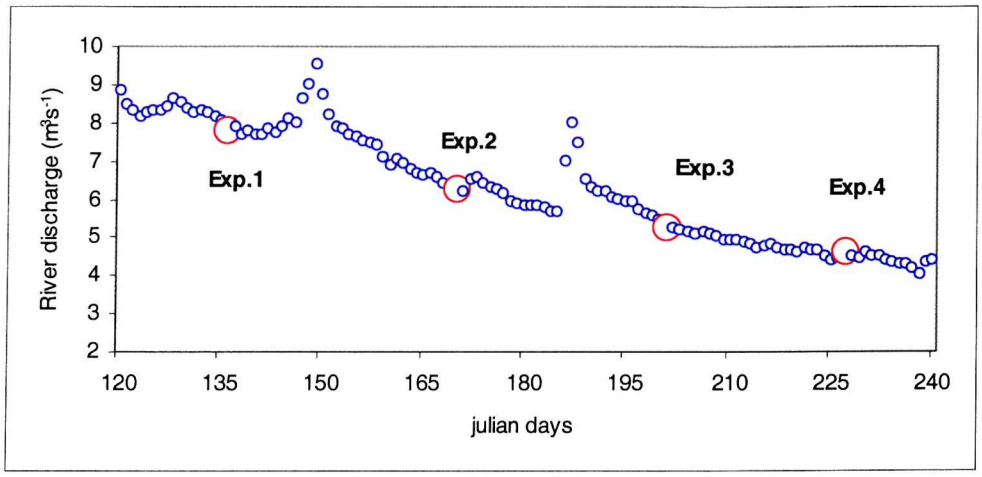


Figure 5.13. Flow rate of the Test River from May to August 2000. Red symbol indicates the flow rate on the sampling day for each incubation experiment.

5.5.1 CHANGES IN CHL A CONCENTRATION, NUTRIENT REMOVAL AND PHYTOPLANKTON COMMUNITY STRUCTURE

NB: **The following abbreviations are used to refer to cultures from each site:**

- Culture of the upper estuary: **UE** culture
- Culture of the mid estuary: **ME** culture → Estuarine water
- Culture of the lower estuary: **LE** culture → Coastal waters

5.5.1.1 Spatial Study

- Experiment 1 (May, 2000):

Daily variations in Chl *a* concentration and nutrient removal during the May experiment are presented in figure 5.14 for UE, ME and LE cultures. Phytoplankton biomass (as Chl *a*) increased in all culture bottles, with a maximum mean value of 184.4 mg m^{-3} measured in UE culture (5.14 a, b, c) on day 6. However, lower biomass peaks, 26 mg Chl *a* m^{-3} (figure 5.14 d, e, f) and 34 mg Chl *a* m^{-3} (figure 5.14 g, h, i), were measured in the ME culture (day 4-5) and LE culture (day 4), respectively. Highest nutrient concentrations were initially measured in the UE

cultures in the beginning of the experiment (day 0) with minimum values recorded in the LE culture. Initial nitrate and silicate concentration (day 0) in the UE culture decreased from 153 μM and 40 μM, respectively, to minimum values of 94 μM and ~1 μM (peak day), and changed from 76 μM and 20 μM (day 0) to 44μM and ~5 μM (peak day); in LE culture. Phosphate concentrations became depleted after the Chl *a* peak in ME and LE cultures and declined from 3.8 μM (day 0) to 0.1 μM (peak day) in the UE culture (figure 5.14). Residual nitrate of > 40 μM was measured in all cultures after 7 days.

Microscopic analysis of phytoplankton community structure showed that the initial (day 0) community carbon in UE and ME cultures was mainly dominated by diatoms, contributing 53% and 78% of the initial total biomass of both cultures, respectively (figure 5.18), however diatoms comprised 40% of the total initial carbon in the LE culture (figure 5.18). Flagellates, mainly *Phaeocystis* sp., comprised 28% of the initial total carbon in LE culture (figure 5.18). Dinoflagellates (mainly, *P. minutum* and *S. trochoidea*) contributed ~30% of UE culture, but contributed only 14% and 16% total initial biomass of ME and LE cultures, respectively.

During the incubation experiment, diatoms were the most exclusively abundant group on the peak biomass day in UE and ME cultures (figure 5.18) contributing 100% and 92% of the total phytoplankton biomass, respectively (figure 5.18).

Table 5.1. Summary of the percentage (%) contribution of the dominant phytoplankton species to relative group total carbon (Diatoms, Dinoflagellates, Flagellates and Ciliates) on day of collection (day 0) and on the peak day for all cultures (UE, ME and LE) in Exp.1 (May).

	Dominant Species					
	Exp. 1 (May)					
	UE	ME	LE	UE	ME	LE
	Initial day			**Peak day**		
Diatoms	*Guinardia delicatula* 50% *Thallassiosira rotula* 33%	*Guinardia delicatula* 67% *Thallassionema sp.* 20%	*Guinardia delicatula* 66% *Thallassionema sp.* 7%	*Thallassiosira rotula* 94%	*Thallassiosira rotula* 86%	*Guinardia delicatula* 50% pennate diatoms 40%
Dinoflagellates	*Protoperidinum minutum* 40% *Scrippsiella trochoidea* 25% *Gyrodinium sp.* 35%	*Protoperidinum minutum* 60% *Scrippsiella trochoidea* 40%	*Protoperidinum minutum* 35% *Scrippsiella trochoidea* 30% unknown species 35%	unidentified species 100%	*Protoperidinum minutum* 100%	*Protoperidinum minutum* 66%
Ciliates	*Mesodinum rubrum* 100%		small ciliates (20m) 100%			
Flagellates	*Eutreptiella marina* 78%	*Eutreptiella marina* 91%	*Phaeocystis* sp. 71%			small flagellates (2-3 μm) 99%
				6	5	4
				Peak day		

The small centric diatom *Thalassiosira rotula* was the most dominant species among the peak diatom community encountered in UE culture, ME culture and LE culture comprising 94%, 86% and 50% of the total diatom carbon, respectively (see table 5.1). The biomass peak in the LE cultures were mainly dominated by flagellates (75% of the total peak carbon), with small unidentified flagellates (2-4 μm) being the most dominant comprising 99% of the total flagellate carbon on the peak day (figure 5.18).

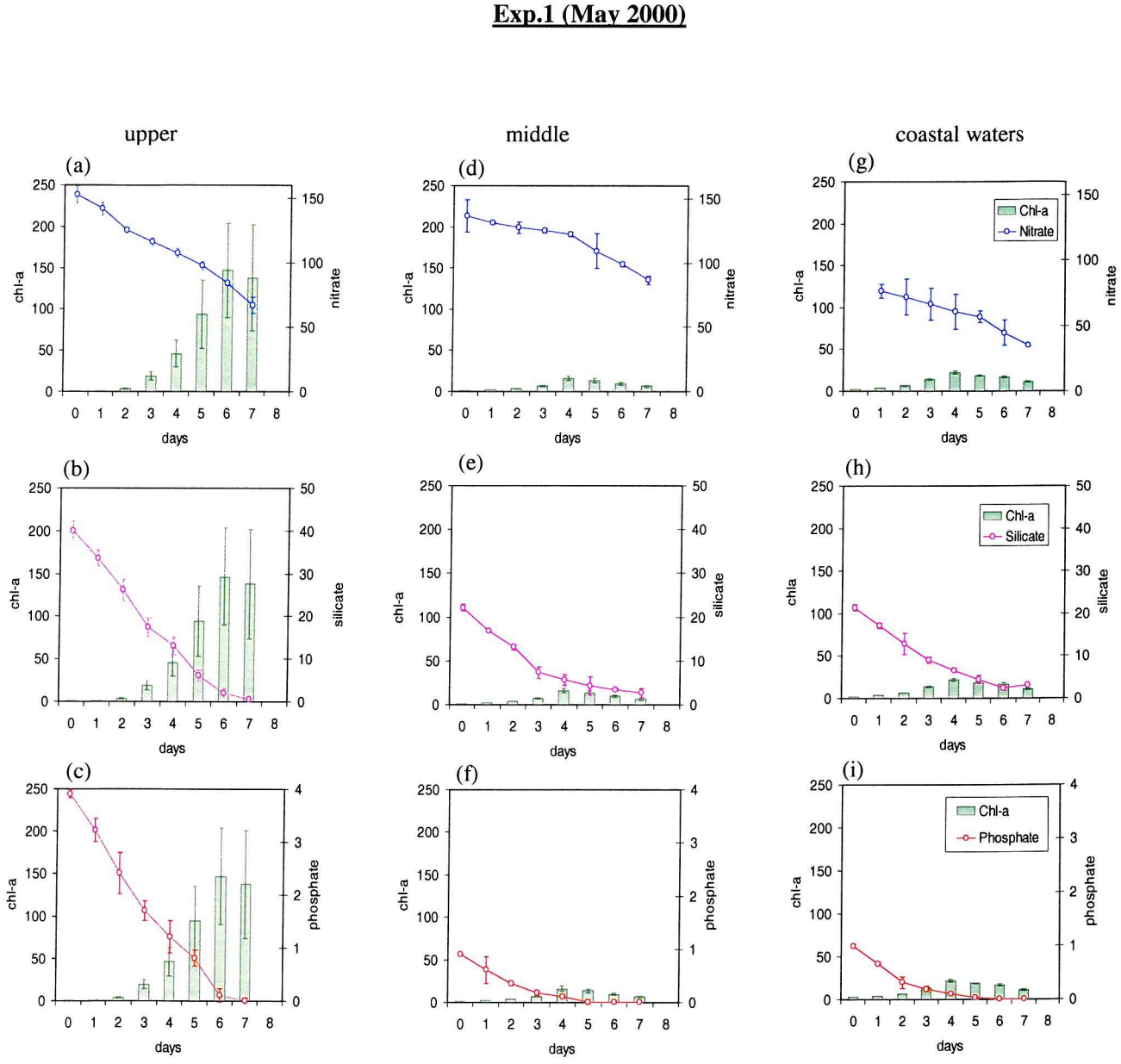


Figure 5.14. Changes in phytoplankton biomass (as mg Chl *a* m^{-3}) and nutrient concentrations (nitrate, silicate, and phosphate as μM) in the upper estuary (a, b, c), middle estuary (d, e, f) and coastal water (g, h, i) incubations in May 2000. Green bars are the average variations in Chl *a* concentrations of two replicate cultures for each site with standard difference. Lines are the average variation in nutrient concentration of both replicates with standard difference.

- Experiment 2 (June, 2000):

Variations in Chl *a* and nutrient concentrations in UE, ME and LE cultures during the incubation period of the June experiment are presented in figure 5.15. In this experiment Chl *a* concentration increased from initial values of 2.1, 5.5 and 2.4 mgm^{-3} in UE, ME and LE respectively to 146 mg m^{-3} (day 8), 35 mg m^{-3} (day6) and 5 mg m^{-3} (day 2) (figure 5.15). Silicate concentrations were dramatically reduced after the peak day of UE and ME cultures at the same time as diatom growth increased (96.5% and 63% of the total peak carbon, respectively). In the UE culture, *Chaetoceros* spp. comprised 60% of total diatom carbon, however, a mixture of different diatom species (*Chaetoceros* spp., *Nitzschia closterium and Lithodesmium undulatum*) were the dominant diatoms during the peak in the ME culture (table 5.2). Ciliates accounted for 27% of the total peak carbon in the ME culture (figure 5.18) with the autotrophic ciliate *Mesodinium rubrum* being the most dominant (34% of total ciliate peak carbon) with other unidentified large ciliates (66% of total ciliate peak carbon, table 5.2). Maximum Chl *a* in the LE culture occurred at a lower concentration (5 mg m^{-3}) compared to the other two cultures (UE and ME) and was measured on the second incubation day and then decreased. Low nitrate and silicate concentrations were initially measured in the LE culture and dramatically decreased following the phytoplankton growth peak (day 2) to values of 1.3 µM for nitrate and 0.9 µM for silicate, with complete removal of phosphate.

Table 5.2. Summary of the percentage (%) contribution of the dominant phytoplankton species to relative group total carbon (Diatoms, Dinoflagellates, Flagellates and Ciliates) on day of collection (day 0) and on the peak day for all cultures (UE, ME and LE) in Exp.2 (June). Unidentified species are referred to a specific group "Others".

	Dominant Species					
	Exp. 2 (June)					
	UE	ME	LE	UE	ME	LE
	Initial day			**Peak day**		
Diatoms	*Biddulphia sinensis* 50% *Ditylum brightwellii* 30% *Thallassiosira rotula* 10%	*Biddulphia sinensis* 48% *Thallassiosira rotula* 37%	*Guinardia delicatula* 66% *Thallassionema sp.* 7%	*Chaetoceros sp.* 61% *Biddulphia sinensis* 17% *Ditylum brightwellii* 11%	*Chaetoceros sp.* 26% *Nitzschia closterium* 22% *Lithodesmium. undulatum* 17% *Skeletonema costatum* 5%	*Thallassiosira rotula* 78% *Chaetoceros sp.* 4%
Dinoflagellates	*Protoperidinum minutum* 62% *Scrippsiella trochoidea* 48%	*Protoperidinum minutum* 56% *Scrippsiella trochoidea* 42%	*Protoperidinum minutum* 35% *Scrippsiella trochoidea* 30% unknown species 35%		*Protoperidinum minutum* 70% *Scrippsiella trochoidea* 30%	*Protoperidinum minutum* 32% unidentified species 68%
Ciliates	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 50% small ciliates (20 µm)100%			*Mesodinium rubrum* 34% Big ciliate (100 µm) 66%	
Flagellates	*Eutreptiella marina* 100%	*Eutreptiella marina* 100%	*Phaeocystis* sp. 71%	*Eutreptiella marina* 100%		small flagellates (2-3µm) 100%
Others						Thin filamentous species
				8	6	2
				Peak day		

Diatoms accounted for 67% of the initial total carbon in the LE culture (figure 5.18) but contributed less to the total peak carbon (16%) with *Thalassiosira rotula* being the most dominant diatom species on peak day (78% of total diatom peak carbon, see table 5.2). Other unidentified thin filaments (may be cyanobacteria) were abundant among the phytoplankton peak community of this culture (figure 5.18). Among the dinoflagellate community that comprised 32% of the total peak carbon in LE culture (figure 5.18), a heterotrophic species was dominating this group, comprising 68% of the total dinoflagellate peak carbon (table 5.2).

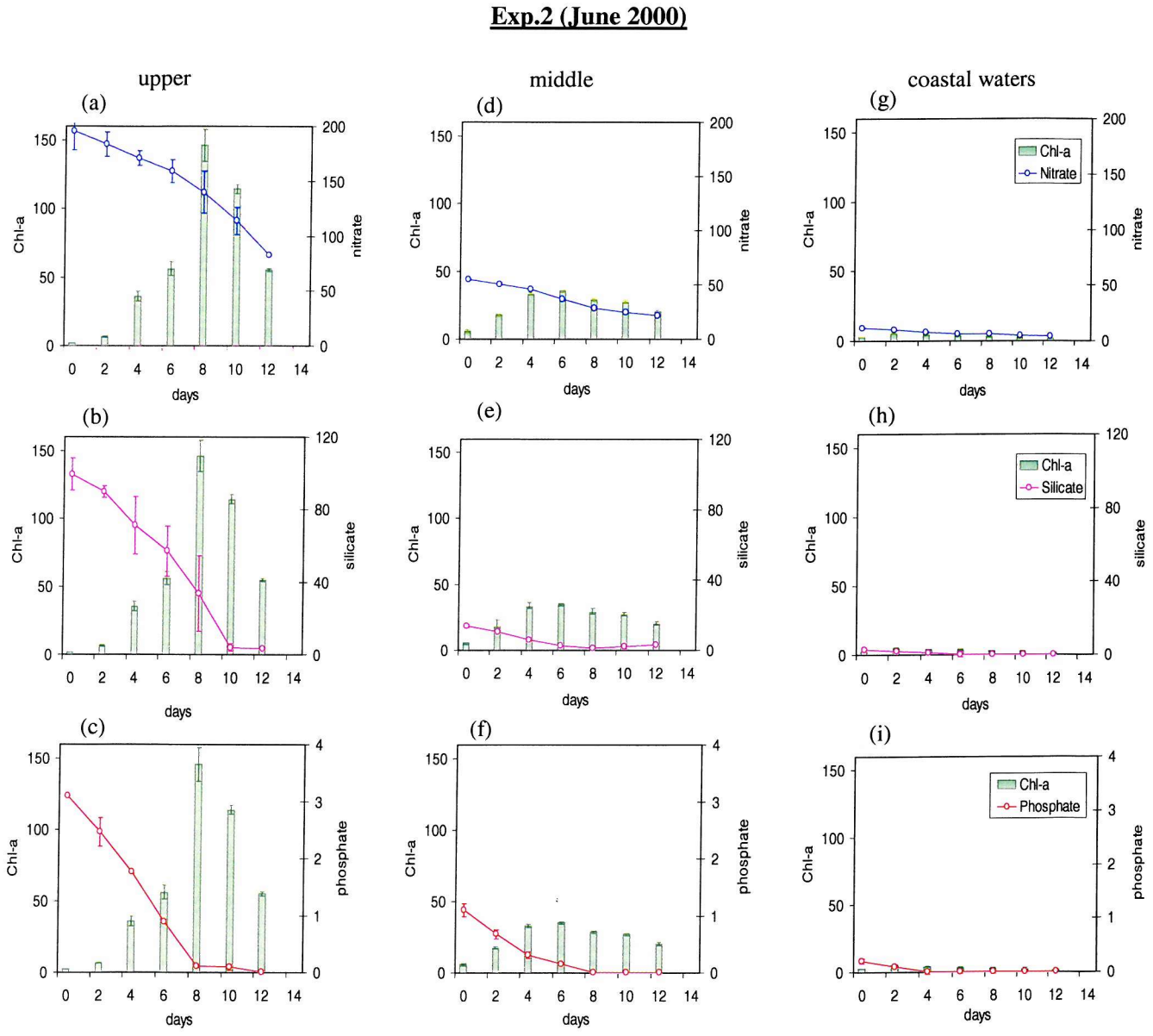


Figure 5.15. Changes in phytoplankton biomass (as mg Chl *a* m^{-3}) and nutrients concentrations (nitrate, silicate, and phosphate as μM) in the upper estuary (a, b, c), middle estuary (d, e, f) and coastal water (g, h, i) incubations in June, 2000. Green bars are the average variations in Chl *a* concentrations of two replicate cultures for each site with standard difference. Lines are the mean nutrient concentration of both replicates with standard difference.

Experiment 3 (July 2000)

Data presented in figure 5.16 show the variations in Chl *a* and nutrient concentrations during the incubation period of July experiment for UE, ME and LE cultures. Phytoplankton biomass in the UE culture increased from an initial concentration of 3.8 mg Chl *a* m^{-3} to a maximum value of 193 mg Chl *a* m^{-3} on day 6 (figure 5.16). In the UE culture, silicate concentrations noticeably decreased (figure 5.16) after the phytoplankton peak on day 6. Diatoms, mainly *Thalassiosira rotula* (78% of total diatom peak carbon, table 5.3) were the most abundant group on the peak day in UE culture (97% of total peak carbon; figure 5.18), however diatoms comprised 36% of total initial carbon (day 0). Although ciliates contributed 34% of the total initial carbon they comprised only 2.4% of the total peak carbon (figure 5.18).

Table 5.3. Summary table of the percentage (%) contribution of the dominant phytoplankton species to relative group total carbon, Diatoms, Dinoflagellates, Flagellates and Ciliates before the incubation (day 0) and on the peak day for all cultures (UE, ME and LE) in Exp.3 (July).

	Dominant Species					
	Exp. 3 (July)					
	UE	ME	LE	UE	ME	LE
	Initial day			Peak day		
Diatoms	*Nitzschia. closterium* 51%	*Biddulphia sinensis* 56%	*Biddulphia sinensis* 31%	*Thallassiosira rotula* 78%	*Thallassiosira rotula* 36%	*Thallassiosira rotula* 41%
	Thallassiosira rotula 15%	*Thallassiosira rotula* 15%	*Thallassiosira rotula* 32%	*Biddulphia sinensis* 11%	*Biddulphia sinensis* 23%	*Rhizosolenia shrubsolei* 33%
		Nitzschia. closterium 13%			*Nitzschia closterium* 15%	*Nitzschia closterium* 14%
					Rhizosolenis fragillissimus 3%	
Dinoflagellates	*Scrippsiella trochoidea* 52%	*Scrippsiella trochoidea* 62%	*Scrippsiella trochoidea* 48%		*Scrippsiella trochoidea* 12%	
		Prorocentrum micans 17%	*Prorocentrum micans* 14%		*Prorocentrum micans* 48%	
	Protoperidinium minutum 22%	*Protoperidinium minutum* 17%	*Protoperidinium minutum* 24%		unidentified species 40%	
Ciliates	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 99%	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 100%	
Flagellates	small flagellates (3-5 µm) 94%	small flagellates (2-3 µm) 90%	small flagellates (2-3 µm) 36%			
			Phaeocystis sp. 62%			
				6	6	4
				Peak day		

In ME and LE cultures maximum phytoplankton growth (as Chl *a*) was recorded after 4 incubation days and then began to decrease when phosphate became depleted in both cultures (figure 5.16). During the bloom of the autotrophic ciliate *M. rubrum* in Southampton Water, ciliates were mostly dominant in the initial phytoplankton community (68% of the total initial carbon) and contributed 30% of the total peak carbon (figure 5.18). Among the phytoplankton community identified on the peak day, diatoms (*T. rotula, O. sinensis, N. closterium and R. cf. fragilissimus*) comprised 60% of the total peak carbon in ME culture, although they comprised only 19% of the total initial carbon (figure 5.18). In the LE culture the phytoplankton peak community was mostly represented by diatoms (100%), with the chain forming diatoms *Thalassiosira rotula* (41%) and *Rhizosolenia shrubsolei* (33%) the most abundant among this group (table 5.3).

Exp.3 (July 2000)

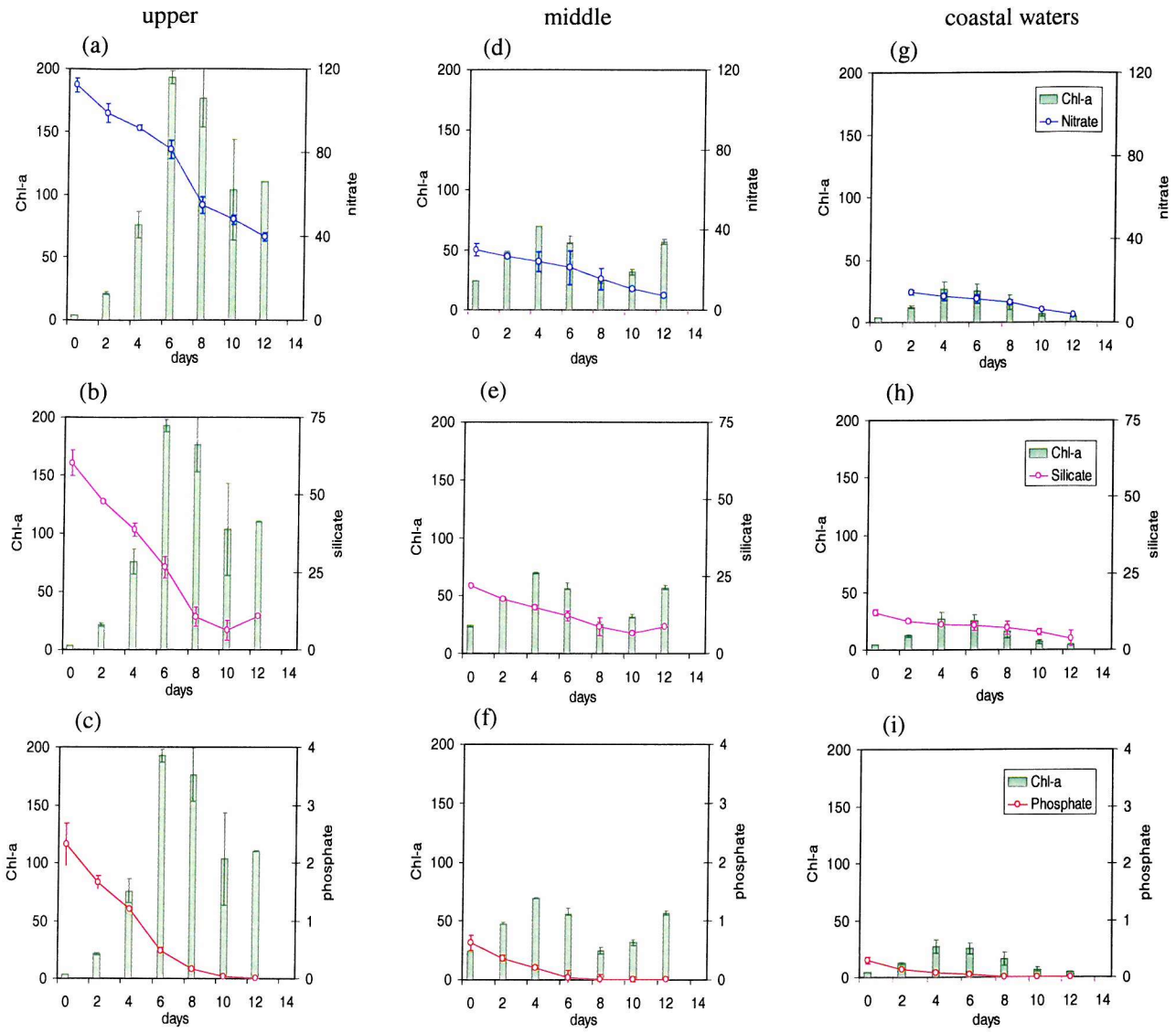


Figure 5.16. Changes in phytoplankton biomass (as mg m^{-3} Chl *a*) and nutrients concentration (nitrate, silicate, and phosphate as μM) in the upper estuary (a, b, c), middle estuary (d, e, f) and coastal waters (g, h, i) incubations in July 2000. Green bars are the average variations in chlorophyll-a concentrations of two replicate cultures for each site with standard difference. Lines are the mean nutrient concentration of both replicates with standard difference.

- Experiment 4 (August 2000)

Variations in Chl *a* and nutrient concentrations during the August experiment are presented in figure 5.17 for UE, ME and LE cultures. Maximum peaks in Chl *a* with mean values of 205 mgm^{-3}, 82 mgm^{-3} and 30 mgm^{-3} were recorded in UE (day 6), ME (day 4) and LE (day 4) respectively. In UE culture, nitrate concentration decreased from an initial value (day 0) of 45 μM to 18.5 μM after peak day, however it then decreased to undetectable levels in the ME and the LE cultures after the peak day. Phosphate was completely removed after the peak day in all incubated cultures (figure 5.17).

At the time of summer blooms in Southampton Water, the phytoplankton community was mainly dominated by dinoflagellates comprising 64%, 56% and 23% of the total initial carbon in UE, ME and LE incubations, respectively during the August experiment (figure 5.18). *Scrippsiella trochoidea* and *Prorocentrum micans* were the most abundant species among this dinoflagellate community (see table 5.4).

Table 5.4. Summary table of the percentage (%) contribution of the dominant phytoplankton species to relative group total carbon, Diatoms, Dinoflagellates, Flagellates and Ciliates before the incubation (day 0) and on the peak day for all cultures (UE, ME and LE) in Exp.4 (August).

	Dominant Species					
	Exp. 4 (August)					
	UE	ME	LE	UE	ME	LE
	Initial day			**Peak day**		
Diatoms	*Thallassiosira rotula* 40% *Biddulphia sinensis* 17%	*Thallassiosira rotula* 43% *Biddulphia sinensis* 23% *Skeletonema costatum* 4%	*Thallassiosira rotula* 40% *Biddulphia sinensis* 24% *Chaetoceros sp.* 11%	*Thallassiosira rotula* 95% *Skeletonema costatum* 3%	*Thallassiosira rotula* 71% *Skeletonema costatum* 3% *Biddulphia sinensis* 14%	*Thallassiosira rotula* 93%
Dinoflagellates	*Scrippsiella trochoidea* 43% *Prorocentrum micans* 32% *Protoperidinium minutum* 17%	*Scrippsiella trochoidea* 13% *Prorocentrum micans* 68% *Protoperidinium minutum* 10%	*Scrippsiella trochoidea* 38% *Prorocentrum micans* 21% unidentified species 18%	*Prorocentrum micans* 100%	*Prorocentrum micans* 97%	*Prorocentrum micans* 100%
Ciliates	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 100%	*Mesodinium rubrum* 100%			
Flagellates	small flagellates (2-3 μm) 91%	small flagellates (2-3 μm) 100%	*Eutreptiella marina* 100%			
				6	4	4
				Peak day		

On the peak day of UE, ME and LE cultures, diatoms were recorded as the most abundant group comprising 99.5%, 60% and 90% of the total peak carbon at these cultures, respectively (figure 5.18). A small (30–50 μm) centric diatom *Thalassiosira rotula* was recorded, in all cultures, as the most abundant species among the diatom community, comprising 95%, 71% and 93% of the total diatom

biomass peak in UE, ME and LE cultures, respectively (table 5.4). However, *Prorocentrum micans* was recorded as the most exclusively abundant dinoflagellate in the ME and LE cultures on the peak day. It comprised 97% and 100% of the total dinoflagellate carbon in LE and ME cultures, respectively. In all cultures no ciliates were identified on the peak day, although they contributed (mainly *Mesodinium rubrum*) 15%, 18% and 22% (figure 5.18) to the total initial carbon in UE, ME and LE cultures, respectively.

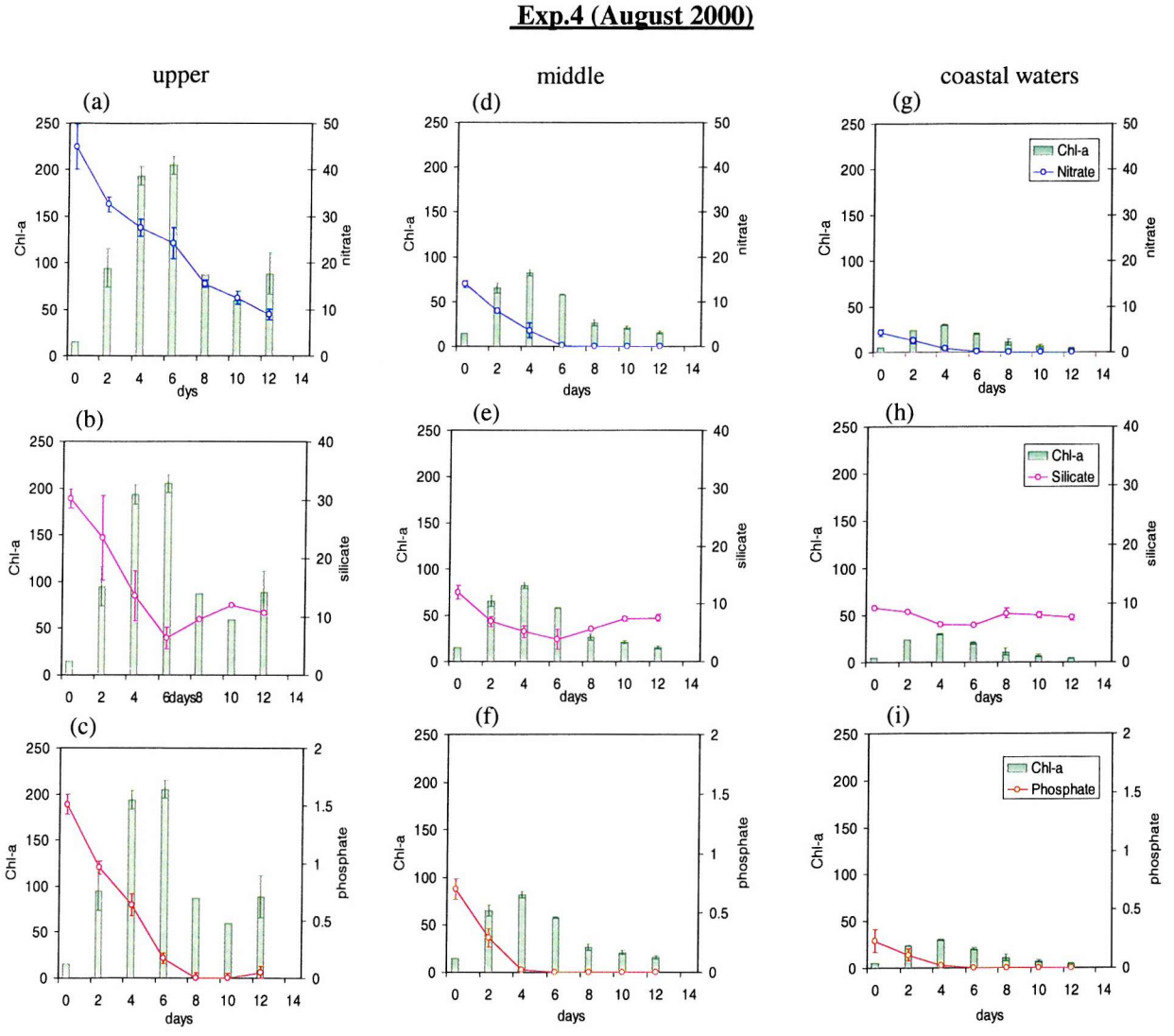


Figure 5.17. Changes in phytoplankton biomass (as mg Chl-a m^{-3}) and nutrients concentration (nitrate, silicate, and phosphate as μM) in the upper estuary (a, b, c), middle estuary (d, e, f) and coastal water (g, h, i) incubations in August 2000. Green bars are the average variations in Chl-a concentrations of two replicate cultures for each site with standard difference. Lines are mean nutrient concentration of both replicates with standard difference.

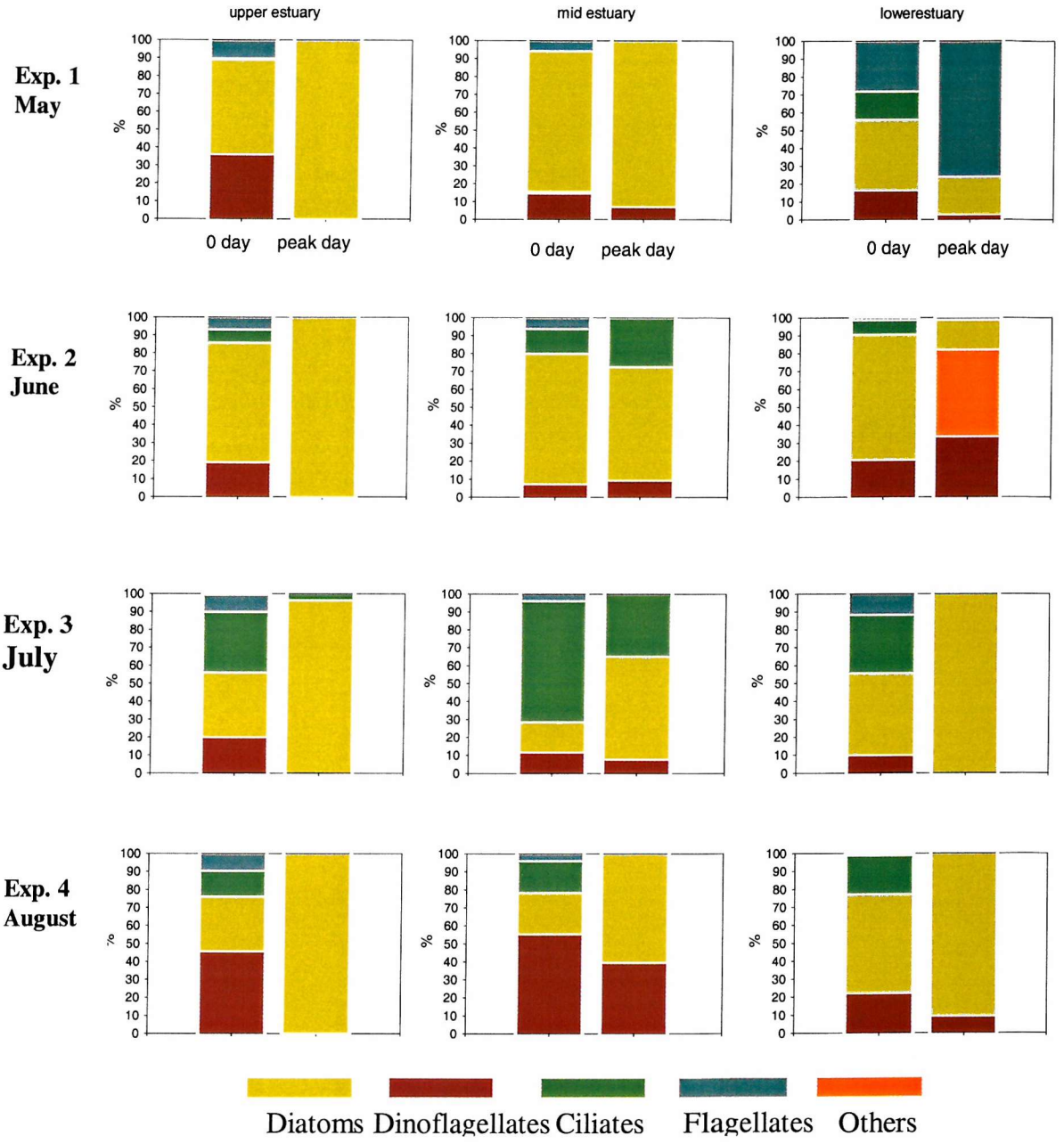


Figure 5.18. Summary of changes in phytoplankton assemblages (as % of carbon contribution of the dominant phytoplankton groups) before incubation (day 0) and during incubation (peak day) in all incubated samples (UE, ME and LE) during the 4 experiments (May, June, July and August).

5.5.1.2 TEMPORAL STUDY

Changes occurring in Chl *a* and nutrient concentrations during the incubation period (in each culture) in relation to the time of water sample collection (in each experiment) are presented in figures 5.21, 5.23 & 5.24. At all sampling sites nutrient concentrations were generally higher in spring/early summer and decreased over the summer to the lowest levels in August.

- Upper Estuary (SG6)

Higher nutrient concentrations were initially measured in the UE culture during May and June (figure 5.21) before incubation due to the nutrient-rich fresher water input of the River Test. In UE cultures, although, different nutrient levels were initially (day 0) measured, similar levels of Chl *a* were measured on the peak day during the four incubation experiments. Maximum Chl *a* in the four experiments ranged between 150 – 200 mg Chl *a* m^{-3} and phytoplankton biomass ranged between 3 x 10^3 and 5 x 10^3 mg C m^{-3} in the UE culture. Despite the species structure of the initial phytoplankton community diatoms were the most abundant group on the peak day (figure 5.19). Diatoms comprised 97-100% of the total peak carbon of all experiments and comprised 53%, 66%, 36% and 30% of the total initial carbon in Exp.1 (May), Exp.2 (June), Exp.3 (July) and Exp.4 (August), respectively (figure 5.19).

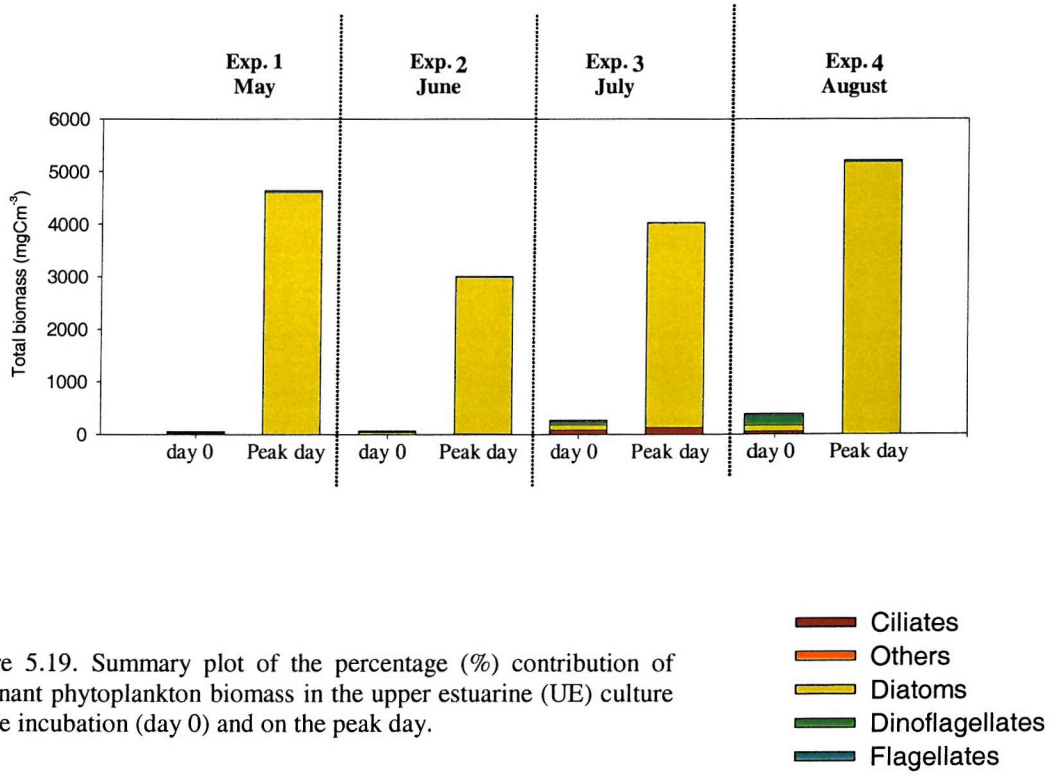


Figure 5.19. Summary plot of the percentage (%) contribution of dominant phytoplankton biomass in the upper estuarine (UE) culture before incubation (day 0) and on the peak day.

Dinoflagellates contributed less to the total peak carbon in all experiments, although they dominated the initial phytoplankton community, particularly in experiments conducted in July and August (figure 5.19). Similarly, ciliates that comprised 34% of the initial total carbon in Exp.3 (July) only accounted for 3% of the total peak carbon (figure 5.19).

- Middle Estuary (NWN)

Chl *a* concentrations increased in the ME culture from the initial values (1–27 mg m^{-3}) to peak values ranging between 30 and 70 mgm^{-3} in the four experiments (figure 5.23). The highest Chl *a* peak of 70 mg Chl *a* m^{-3} was measured in Exp.4 (August) and was mainly dominated by diatoms (57% of the total peak carbon) and dinoflagellates (43% of the total peak carbon). Diatoms dominated the peak community in May experiment (92%), with *Thalassiosira rotula* being the most dominant species, however ciliates, mainly *Mesodinium rubrum,* comprised 27% and 35% of the total peak carbon in Exp.2 (June) and Exp.3 (July) (figure 5.20).

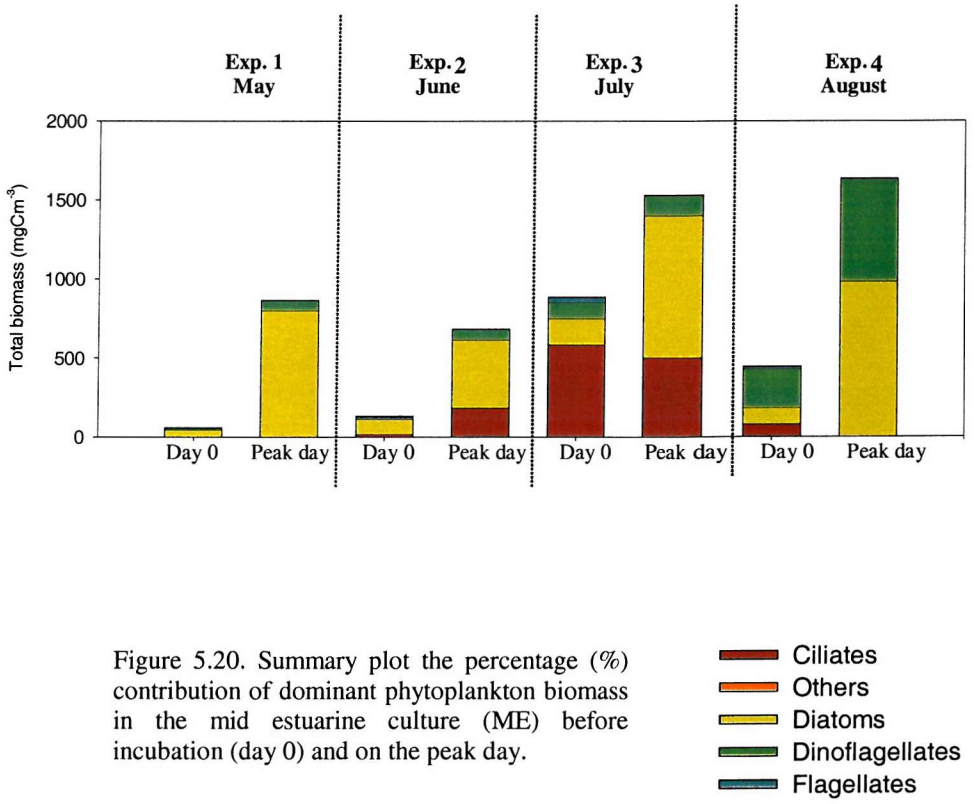


Figure 5.20. Summary plot the percentage (%) contribution of dominant phytoplankton biomass in the mid estuarine culture (ME) before incubation (day 0) and on the peak day.

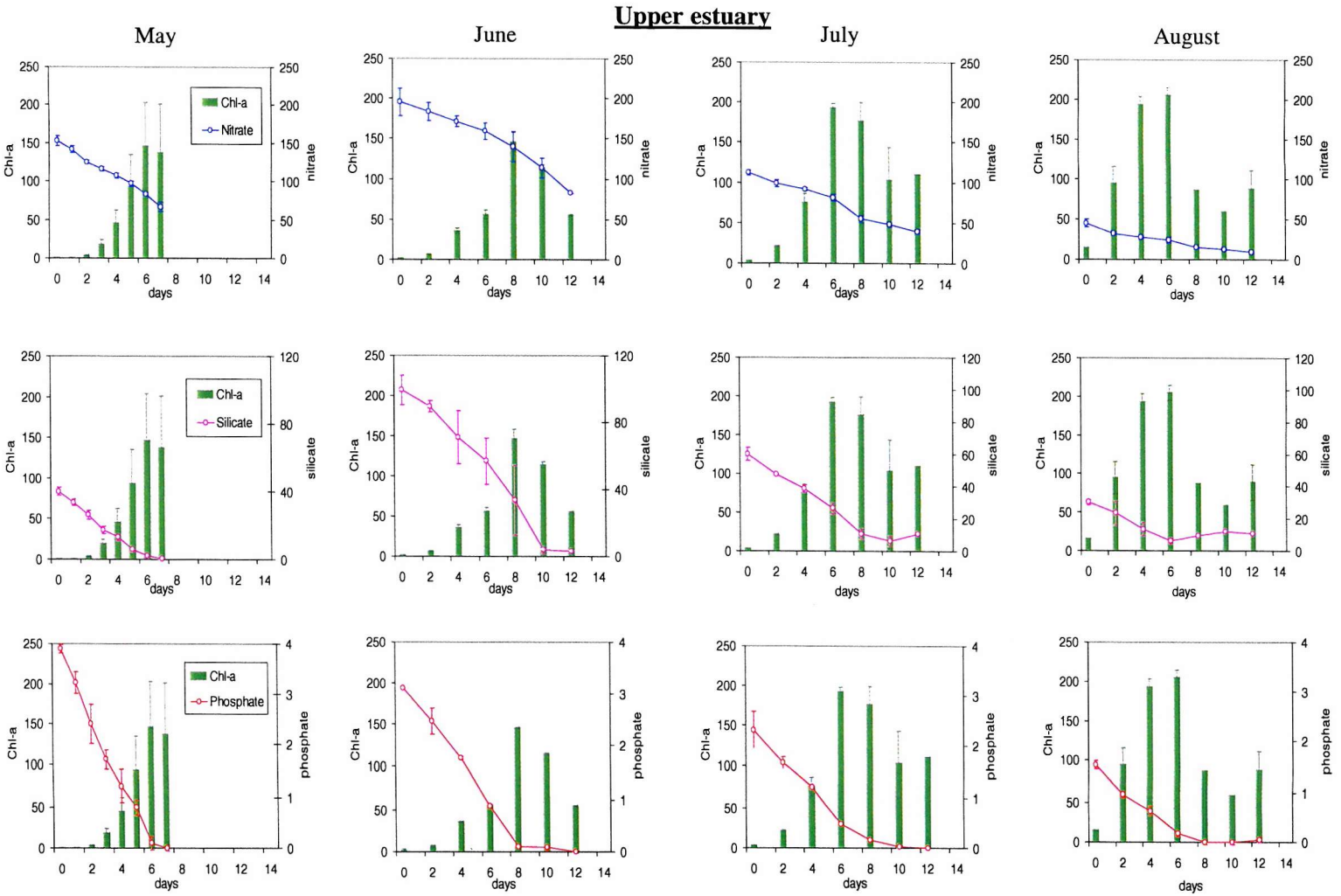


Figure 5.21. Variations in chlorophyll-a total (as mg m^{-3}) and nutrient concentrations (nitrate, silicate, and phosphate as μM) during incubation experiments, Exp. 1 (May), Exp. 2 (June), Exp. 3 (July) and Exp. 4 (August) of water collected from the upper estuary. Green bars are the average Chl *a* concentration of two duplicate cultures for each site with standard differences. Lines are the average nutrient concentration of both replicates with standard differences. Bars/Lines with no error bars have no replicate or the replicate sample was lost.

- Coastal Water (Calshot/Reach)

Lower nutrient levels were generally measured at the coastal site, with minimum concentrations (1.7 μM nitrate, 3.0 μM silicate and 0.2 μM phosphate) in mid June (figure 5.24). Lower peaks of Chl *a* (5-30 mg m^{-3}) were measured in the LE culture compared to the other two cultures, during the four experiments. A maximum peak was measured in Exp.1 (May) and the phytoplankton community was dominated by flagellates (small flagellates & *Phaeocystis*, figure 5.21). The lowest peak in Chl *a* (5 mg m^{-3}) was measured during the June experiment, and was dominated by thin unidentified filamentous algae (51% of the total peak carbon) and heterotrophic dinoflagellate species (30% of the total peak carbon). A mixture of small diatoms *Thalassiosira* sp., *Nitzschia closterium, Skeletonema costatum* and *Rhizosolenia cf. fragilissimus* dominated the carbon peak in Exp.3 (100% of the total peak carbon) and Exp.4 (90% of the total peak carbon) (figure 5.22)

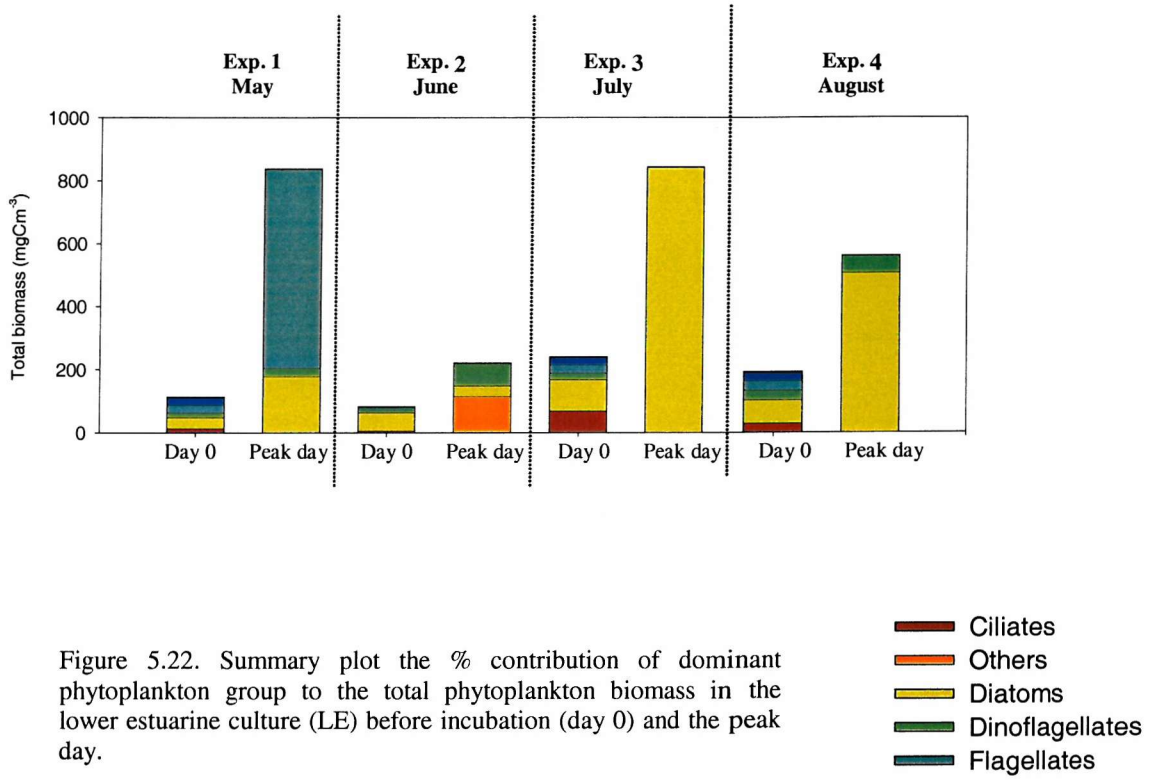


Figure 5.22. Summary plot the % contribution of dominant phytoplankton group to the total phytoplankton biomass in the lower estuarine culture (LE) before incubation (day 0) and the peak day.

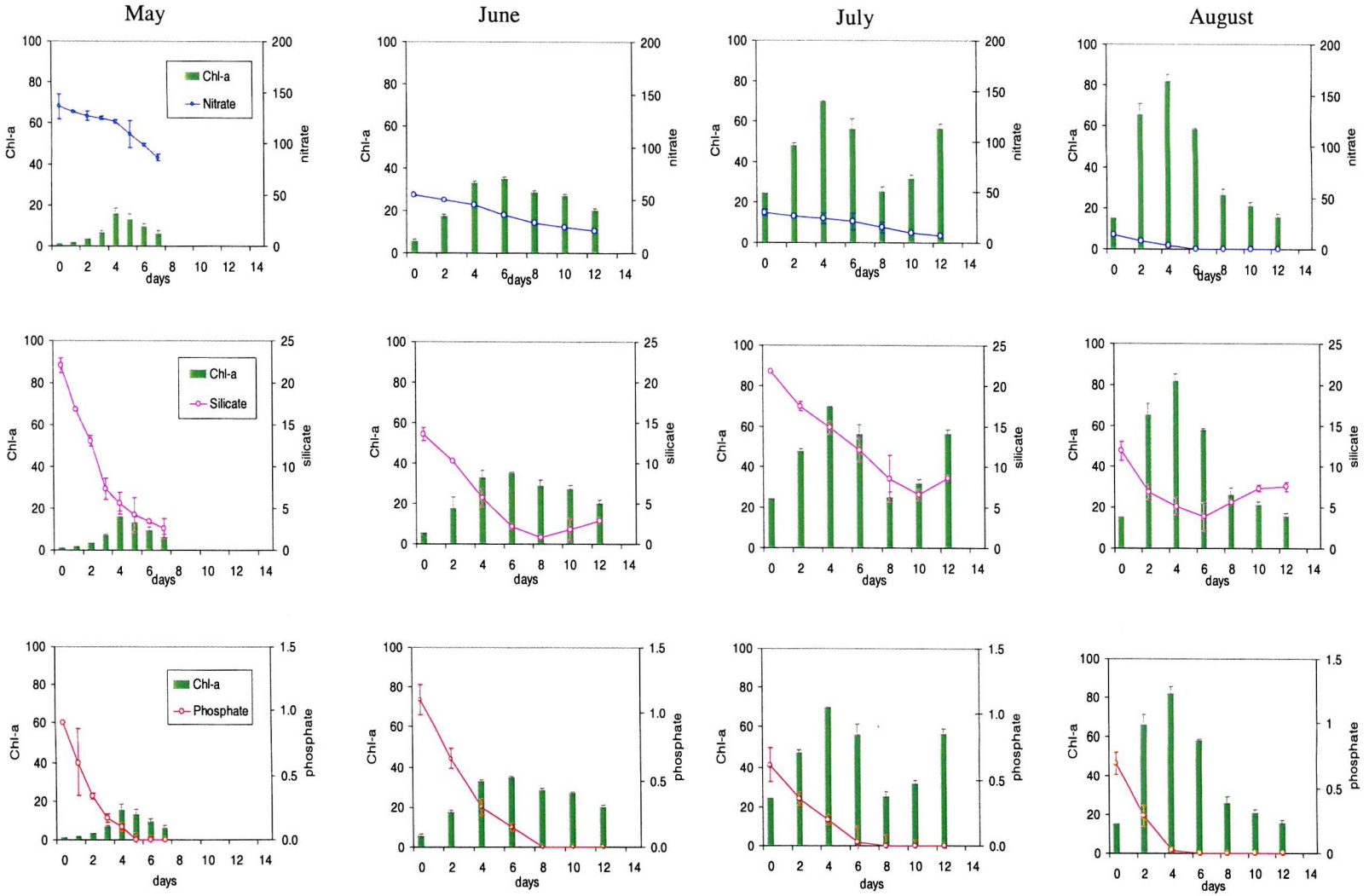


Figure 5.23. Variations in chlorophyll-a (as mg m-3) and nutrient concentrations (nitrate, silicate, and phosphate as μM) during incubation experiments, Exp. 1 (May), Exp. 2 (June), Exp. 3 (July) and Exp. 4 (August) of water collected from the middle estuary. Green bars are the average chlorophyll-a concentration of two duplicate cultures for each site with standard differences. Lines are the average nutrient concentration of both replicates with standard differences. Bars/Lines with no error bars have no replicate or the replicate sample was lost.

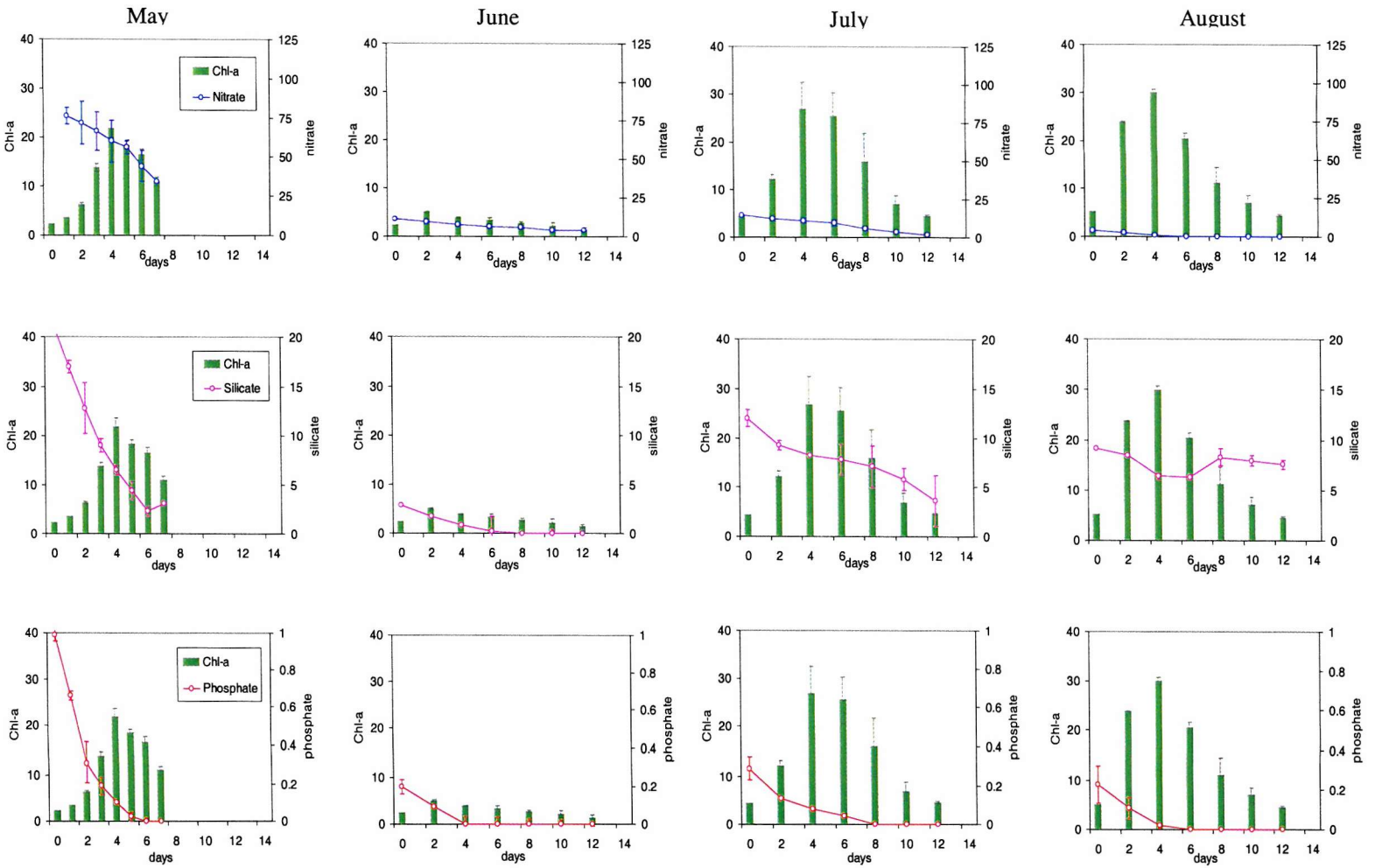


Figure 5.24. Variations in chlorophyll-a (as mg m^{-3}) and nutrient concentrations (nitrate, silicate, and phosphate as μM) during incubation experiments, Exp. 1 (May), Exp. 2 (June), Exp. 3 (July) and Exp. 4 (August) of water collected from the coastal waters. Green bars are the average Chl *a* concentration of two duplicate cultures for each site with standard differences. Lines are the average variation in nutrient concentration of both replicates with standard differences. Bars/Lines with no error bars have no replicate or the replicate sample was lost.

5.6 DISCUSSION

5.6.1 SPATIO-TEMPORAL VARIATIONS OF NUTRIENTS AND CHL A IN SOUTHAMPTON WATER

Southampton Water is a hypernutrified estuarine system (Hydes, 2000; Holley & Hydes, 2002), receiving high inputs of nutrients, mainly nitrate (>400 μM) from the river Test and Itchen (Xiong, 2000), and is classified as a 'Nitrate Vulnerable' Zone (Hornung, 1999). It also receives high inputs of phosphate (>20 μM) from sewage treatments works (Xiong, 2000, Nedwell et al., 2002). Silicate loads along the U.K. coast are of similar magnitude to that of total input of both nitrate and nitrite as concluded by Nedwell et al. (2002) and are relatively independent of anthropogenic influences (Hessen, 1999).

During the period of this study (May-August 2000), nutrient concentrations varied temporally ranging from highest levels in spring/early summer, particularly for nitrate (86-122 μM, mean value for the 3 sites) and silicate (14-42 μM mean value for the 3 sites) to minimum levels (20 μM nitrate and 16 μM silicate, mean value for the 3 sites) towards end of summer. Nutrient concentration also varied with respect to salinity distribution along the estuary with highest levels of up to 189.6 μM for nitrate, 3.76 μM for phosphate and 100.6 μM for silicate observed in the lower-salinity waters due to the influence of nutrient-rich freshwater input from the River Test and the River Itchen. Minimum values of nutrients (2.96 μM for nitrate, 5.9 μM for silicate and <0.1 μM for phosphate) were, however, measured at the mouth of the estuary (coastal waters). A similar trend of seasonal variations and conservative behaviour of nutrients was previously observed in Southampton Water in earlier studies (e.g. Antai, 1989; Kifle & Purdie, 1993; Wright & Hydes 1997). Chl *a* concentration was generally high in spring periods of higher nutrient input and decreasing towards the summer season when nutrients inputs were minimum due to reduced rainfall and lower river flow rates (see figure 5.13).

Chl *a* concentration varied, regionally and seasonally, with highest Chl *a* concentration (~ 16-38 mg m^{-3}) recorded in less-saline (salinity range between 21.6 & 31.5) high-nutrient sites (estuarine waters) decreasing seawards (maximum > 9 mg m^{-3}). Maximum Chl *a* biomass (~ 26-38 mg m^{-3}) was recorded at the intermediate site (NWN) during July at the time of the biomass increase of the autotrophic ciliate *Mesodinium rubrum*. The decrease in Chl *a* towards the mouth of estuary could be also explained by the seawards increase in the exchange rate between estuarine and Solent coastal waters on each tidal cycle (Lauria, 1998). This distribution pattern of nutrients and Chl *a* in relation to salinity distribution along Southampton Water is in good agreements with previous measurements carried out within the system (e.g. Bryan, 1979; Antai, 1989; Kifle, 1992; Kifle & Purdie, 1993, Iriarte and Purdie, 1994, Ashe, 1996; Lauria, 1998; Hydes and Wright, 1999).

5.6.2 SEASONAL SUCCESSION OF PHYTOPLANKTON SPECIES IN SOUTHAMPTON WATER

Development of phytoplankton blooms, bloom intensity and community composition in the Southampton Water estuary will be influenced by the physical conditions e.g. stability of water (Lauria et al. 1999), light availability (Holley & Hydes, 2002; Nedwell et al., 2002), species adaptation (Lauria, 1998), ambient nutrient concentrations (Fichez et al., 1992; Sanders et al., 1997) and nutrient ratios (Nedwell et al., 2002; Gieder & La Roche, 2002).

During this study, phytoplankton biomass fluctuated with relatively smaller peaks in the upper (44-213 mg C m^{-3}) and mid estuary (59-347 mg C m^{-3}) from May to end of June increasing to maximum levels of ~1000 mg C m^{-3} during July when the phototrophic ciliate *M. rubrum* was abundant. This ciliate exclusively dominated the phytoplankton community, particularly, in the upper and mid estuary (with values of Chl *a* up to 16-38 mg m^{-3}) contributing 25-75% and 60-85% of the total cell biomass during July at these sites, respectively (figure 5.13), being particularly abundant at NWN. However, *Mesodinium rubrum* contributed less (17-32%) to the total cell biomass in the lower estuary (coastal waters). The more stratified water column and nutrient levels at the intermediate site appear to favour the growth of *M. rubrum* in this region of the estuary. This distribution pattern of *M. rubrum* along Southampton Water is consistent with previous studies (Leakey, 1986; Iriarte, 1991; Kifle, 1992; Crawford, 1992; Ryan, 1994) that recorded *M. rubrum* in surface waters, particularly in the estuary (NWN) during spring-summer between 1985 and 1995. This ciliate is known to produce red tides in Southampton Water (Crawford & Purdie, 1992) and exceptional chlorophyll levels of up to 100 mg Chl *a* m^{-3}, has been reported (Crawford & Purdie, 1992).

In contrast, lower phytoplankton biomass (ranged from 90-430 mg C m^{-3}) was recorded at the coastal site with the maximum peak measured in early June and mainly dominated by diatoms (~93% total cell biomass), particularly *Guinardia delicatula* (222 mg C m^{-3}, 54% of total diatom biomass). This diatom bloom followed a noticeable increase in nutrients (measured in mid May), particularly Silicate and *G. delicatula*, has also recorded, with lower cell biomass, in the upper (60 mg C m^{-3}) and mid (150 mg C m^{-3}) estuary at the same time increasing seawards. This chain-forming diatom is a common species forming high populations along the estuary and was previously recorded in the upper Test (Proenca, 1994), mid estuary, (Antai, 1990; Kifle, 1992; Anning, 1995) during June-July and at Calshot, in coastal waters (Howard et al., 1995).

In spring (from mid May-early June) the relatively small-celled flagellate *Phaeocystis* sp., were numerically abundant only at the coastal site but not recorded at the other sites. *Phaeocystis* is

generally known to dominate phytoplankton community in coastal waters at this time of the year as previously recorded by Iriarte (1991), forming blooms (up to 16 mg Chl *a* m^{-3}).

Other small flagellates were numerically very abundant at the upper and mid sites and were coexistent with the ciliate *M. rubrum*. These flagellates, despite their lower contribution to total phytoplankton biomass contributed highly to total phytoplankton cell number comprising approximately 69% (upper estuary), 70% (mid estuary) and 67% (coastal waters) in mid July, late July and mid August respectively. The presence of a transition period of ciliates (e.g. *Mesodinium rubrum*) and flagellates (e.g. *Eutreptiella marina*) between the spring diatom bloom and summer dinoflagellate bloom is a common feature in Southampton Water (e.g. Iriarte, 1991; Kifle, 1992) and other estuarine systems (e.g. Kocum et al., 2002b). This species succession is mainly due to reduced nutrient conditions, particularly Si after periods of maximum diatom growth (i.e. spring-early summer), since these organisms (e.g. flagellates), which are not Si-requiring, cannot compete with diatoms for N and P (Peperzak, 1993; Kocum et al., 2002b).

Dinoflagellates were abundant during summer months coincident with sunny weather, good irradiance conditions and reduced water turbulence. The highest peak of dinoflagellate biomass was recorded in mid August with greatest contribution to total phytoplankton biomass in the upper (46%) and mid (56%) estuary compared to their contribution in coastal waters (22%). This is mainly due to reduced water stability at the mouth of the estuary. Dinoflagellates are generally known to bloom under calm water conditions with reduced turbulence (White, 1976; Margalef, 1978; Pollinger & Zemel, 1981; Kifle, 1992), since high turbulence negatively affect their cell growth, cell division and physiology as experimentally demonstrated (Thomas & Gibson, 1990; Berdalet, 1992; Berdalet & Estrada, 1993; Thomas et al., 1995). *Scrippsiella trochoidea* and *Prorocentrum micans* dominated the dinoflagellate bloom at all sites along the estuary during August-September 2000 with *S. trochoidea* being very abundant, particularly at the intermediate site (NWN).

5.6.3 GROWTH OF PHYTOPLANKTON AND COMMUNITY COMPOSITION UNDER NON-LIMITING LIGHT CONDITIONS

From previous nutrient data, and data collected during this research, it is evident that nutrients, particularly nitrate and silicate, are generally not limiting phytoplankton growth during the spring-summer period. In addition, results from the Southern Nutrient Study "SONUS" conducted in Southampton Water from 1995 to 1997 (Hydes & Wright, 1999) showed that nutrient concentrations were high enough to support phytoplankton growth throughout the whole year. However, phosphate may decrease to undetectable levels at salinities >34 as previously reported

by Hydes & Wright (1999) or become completely depleted, particularly, during blooms as detected in spring 1999 (see chapter 3). Therefore the phytoplankton in Southampton Water are unlikely to be nutrient-limited, particularly in the upper and mid regions of the estuary. Chlorophyll concentration however showed considerable variations during the sampling period in 2000 with episodic increases of varying magnitude. This can be mainly attributable to varying irradiation conditions (i.e. light limitation) that has previously been shown to be the main driver of phytoplankton growth in such nutrient-rich and turbid estuaries (Holley & Hydes, 2002; Nedwell et al., 2002; Kocum et al., 2002a &b).

During the incubation experiments described in this chapter, chlorophyll biomass (mg Chl *a* m^{-3}) of the natural phytoplankton community (collected from the three different sites along the salinity gradient in Southampton Water) noticeably increased from *in situ* values of 1-16, 1-24 and 2-6 mg Chl *a* m^{-3} to experimental values (i.e. upon incubation) of 146-205, 34-82 and 27-70 mg Chl *a* m^{-3} at the upper, mid and lower estuarine sites, respectively (see figure 5.25) when incubated under good light conditions, with no nutrients added. This finding, although laboratory conditions might deviate from the *in situ* situation (Underwood & Kromkamp, 1999). The hypothesis been tested that phytoplankton in Southampton Water are not nutrient-limited but at times may be light-limited. Figure 5.25 clearly shows that phytoplankton under non-limiting light conditions in the laboratory achieve much higher peak chlorophyll/carbon levels than that produced in the estuary during the same time period particularly in the upper and mid estuary.

During all incubation experiments, chlorophyll-a concentration increased to peak values (figure 5.25) after a period of incubation from 2 to 7 days (see table 5.5) according to the initial concentration of nutrients as well as the initial cell density which varied temporally (for each month) and spatially (at each site). Maximum peaks (146-205 mg Chl *a* m^{-3}) were measured in cultures of water samples collected from the upper estuary (UE culture) compared to that from the ME (34-80 mg Chl *a* m^{-3}) and LE culture (27-69 mg Chl *a* m^{-3}). This is mainly attributed to the higher initial nutrient levels available in the upper estuary enabling phytoplankton species to grow for a longer period (biomass peak started to decline after 5-8 days). In contrast, nutrients, which were initially low, became limiting for phytoplankton growth in all LE cultures (collected from coastal waters) during the four experiments with chlorophyll peaks, produced of lower magnitude, occurring earlier and lasting for a shorter period (biomass peak declined after only 3-5 days).

The phytoplankton community composition on the peak day (see figures 5.19, 5.20 & 5.22) noticeably varied from the *in situ* community initially collected (see figure 18). Changes in community composition during the incubation experiments could be attributable to the initial

community structure (on day 0), initial nutrient concentration and competitions among species for nutrients. A diverse mixture of phytoplankton species was recorded at the beginning of the experiments (day 0), but diatoms mostly dominated the biomass peak during all experiments, particularly cultures of estuarine waters (UE & ME), comprising approximately 100%, 58-93% and 16-100% of the total biomass peak in UE, ME and LE cultures, respectively (see figure 5.18). The absence, or the less contribution, of other phytoplankton groups (mainly dinoflagellates and ciliates) may be attributable to the difference in growth rate among the growing species. Diatoms in general, are known to be fast-growing organisms (Chan, 1980; Banse, 1982) and therefore can out-compete other species that known to have low growth rate, e.g. dinoflagellates (White, 1976; Brand & Guillard, 1981; Kifle, 1992; Langdon, 1993) In addition, dinoflagellates are weak competitors for nutrients (Chang & McClean, 1997; Smayda, 1997) and thereby, can not compete with other species if compared with diatoms.

Another explanation for the absence of other phytoplankton groups (i.e. dinoflagellates and/or ciliates) in on the peak day could be due to the fact that these organisms do not grow under high turbulence, particularly dinoflagellates (Berdalet & Estrada, 1993; Thomas et al., 1995) occurred during the daily mixing of culture bottles. Moreover, some of these species (particularly, *M. rubrum*) are known to be short lived in laboratory cultures (*Purdie, pers. comm.*)

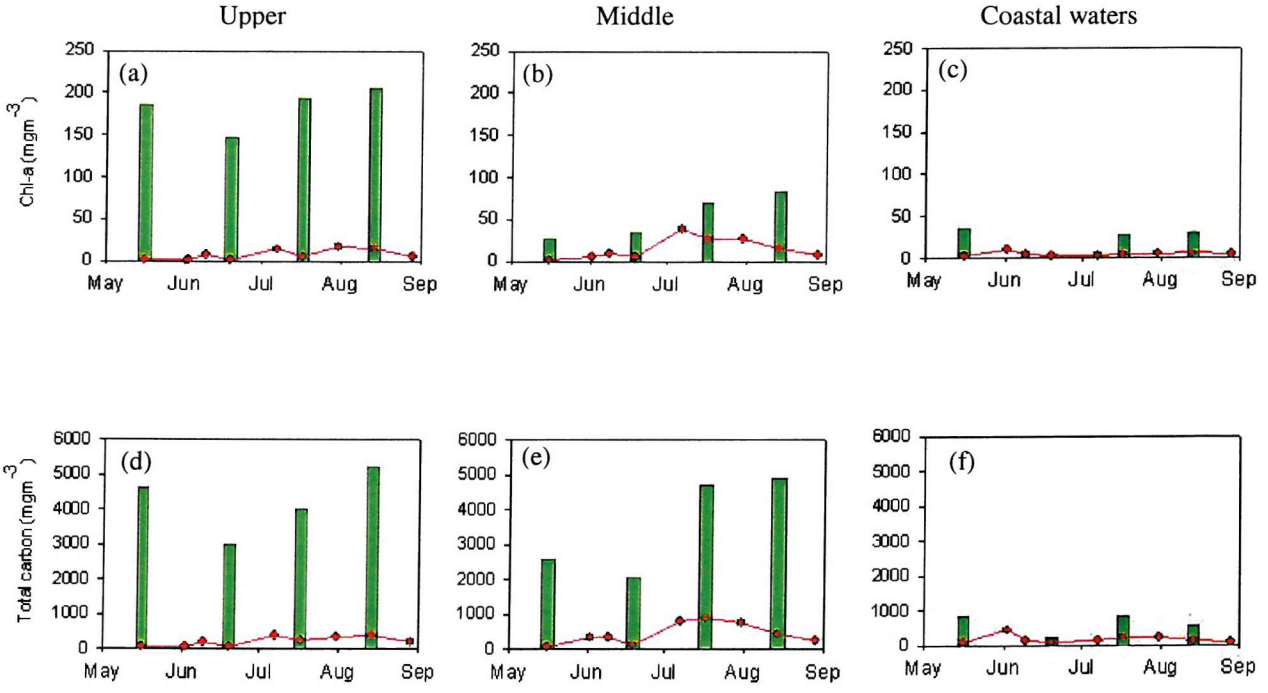


Figure 5.25. Temporal variation in chlorophyll a (mg m^{-3}) and phytoplankton biomass (mg C m^{-3}) as measured throughout the estuary (red lines) compared to peak Chl *a* and peak carbon biomass as measured during the four incubation experiments in the upper estuary (a, d), middle estuary (b, e) and coastal water (c, f). Values for total peak chlorophyll and peak carbon biomass presented as the mean of two duplicates.

Among the diverse diatom community initially recorded (day 0) the chain-forming diatoms, *Thalassiosira rotula* and *Chaetoceros* spp. dominated the peak diatom community in most experiments, particularly in the UE and ME incubations. Both these diatom species are known to have a high growth rate (Kifle 1992) and can out-compete other species, particularly large-celled diatom species, e.g. *Odontella*, *Rhizosolenia* and *Guinardia* that were initially dominant (on day 0). It was previously hypothesized (Malone, 1977) that smaller cells have higher growth rates and the dominance of these small-celled species could be also explained by their ability to grow in relatively low nutrient concentrations as recorded in the Gironde plume (Herbland et al., 1998; Labry et al., 2002) when species of smaller-sized cells were abundant in P-limited waters, i.e. when P became exhausted.

In the lower estuary cultures (LE), some diatom species e.g. *Thalassiosira cf. rotula* and *Guinardia delicatula* grew during Exp. 1 (May) and Exp. 2 (June), however these diatoms only lasted for short period (declined after 4 and 3 days in May and June experiment, respectively) due to nutrient, particularly silicate, depletion and phytoplankton peak community changed from a diatom-dominated to a flagellate-dominated (including *Phaeocystis* sp.) community. Flagellates are known to grow during periods of silicate shortage (Reid et al., 1990; Peperzak et al., 1993). Thin filamentous algae (may be cyanobacteria) were also recorded on the peak day in both experiments, being potentially abundant in Exp.2. In contrast, the biomass peak in Exp. 3 and Exp. 4 (of the LE culture) was dominated by diatoms with *T. rotula* being the most abundant. An interesting observation detected during microscopic analysis, was that cells of *T. rotula* detected in LE culture(s) were much smaller in size (10-15μm) than those recorded in the UE and ME cultures (~30μm). This might be attributed to the fact that higher nutrient levels are favoured by larger cells due to their relatively higher half-saturation and their maximum uptake rates (Dugdale, 1967). In addition, phytoplankton of smaller sized-cells (particularly, diatoms) are known to be abundant in nutrient limited waters, mainly P-limited, as recorded by Herbland et al. (1998) and Labry et al. (2002).

Guinardia delicatula and *Rhizosolenia shrubsolei* grew better in the LE culture (table 5.5) compared to other cultures (UE & ME), comprising 40% and 33% of peak diatom biomass in Exp.1 and Exp.3, respectively. This is due to the fact that both species grow better in more saline waters (Grall, 1972; Kifle, 1992; Lauria, 1998). It was previously reported that those diatom species, particularly *G. delicatula* shown to grow optimum within a salinity range of 32 (current study, chapter 3) to 34 (Grall, 1972, cited in Kifle, 1992). Some other diatoms; e.g. *Ditylum brightwellii*, which were abundant *in situ,* but did not grow at all cultures. This could be related to the day-length (light:dark cycle) as a day length of more than 16h was previously reported to be inhibitory to the growth of *D. brightwellii* (Paasche, 1968).

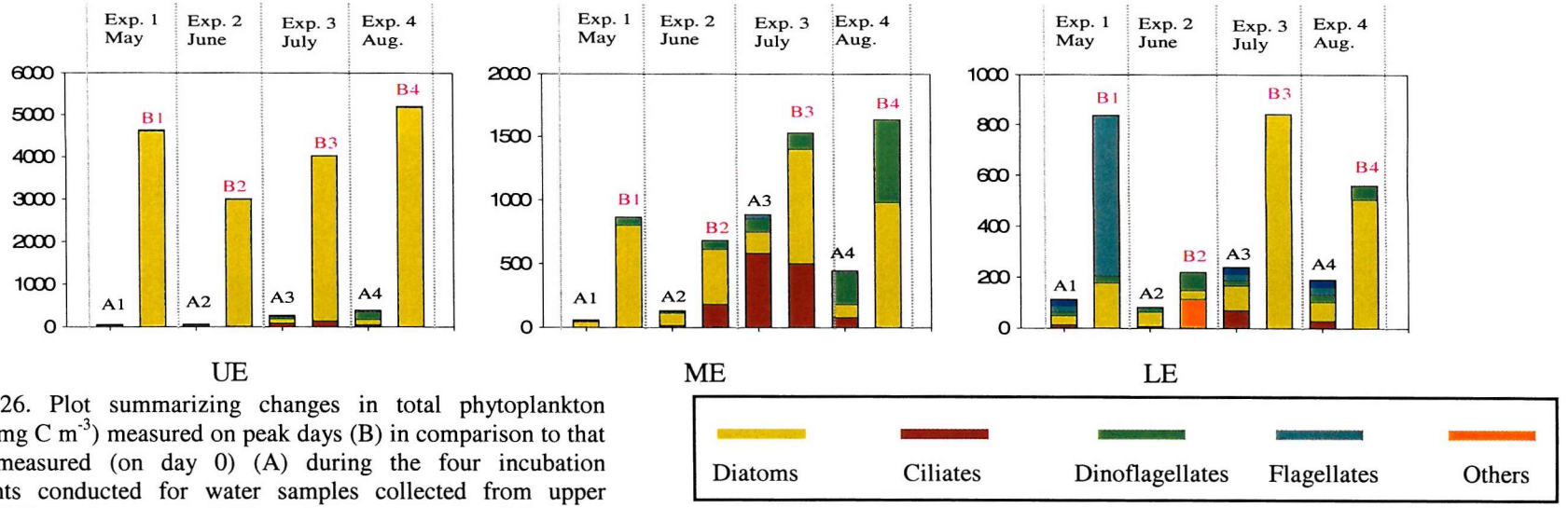


Figure 5.26. Plot summarizing changes in total phytoplankton biomass (mg C m^{-3}) measured on peak days (B) in comparison to that initially measured (on day 0) (A) during the four incubation experiments conducted for water samples collected from upper estuary (UE), mid estuary (ME) and coastal waters (LE). N.B different scale was used for each plot (i.e. for each site).

Table 5.5. Summary of results obtained from the four incubation experiments (May, June, July and August) conducted for surface water samples collected from upper estuary (UE), mid estuary (ME) and coastal waters (LE). Date included in this table are expressed as mean value (for Chl-a, carbon biomass and cell enumeration) of duplicate flasks. Nutrient ratio data refers to values before (in bold) and after (below) peak biomass in all cultures. See Appendix IV.

	Peak day			**Peak Chl *a***			**Dominant species**			**N/P**			**N/Si**			**P/Si**		
	UE	**ME**	**LE**	**UE**	**ME**	**LE**	**UE**	**ME**	**LE**	**UE**	**ME**	**LE**	**UE**	**ME**	**LE**	**UE**	**ME**	**LE**
May	6	5	4	146.5	34.1	51.8	*T. rotula*	*T. rotula*	*R. shrubsolei, G. delicatula* small flagellates + others	**17.3** 31.2	**17.1** 236.7	**18.7** 167.3	**1.6** 5.1	**0.8** 11.6	**1.4** 6.8	**0.093** 0.15	**0.049** 0.043	**0.061** 0.025
June	8	6	2	146.1	34.9	69.2	*Chaetoceros* spp.	*Chaetoceros* spp.	small flagellates	**16.4** 62.4	17.2 76	**17.2** -	**0.8** 1.4	**1.1** 3.7	**1.7** 3.4	**0.050** 0.01	**0.083** 0.02	**0.099** 0.048
July	6	6	4	192.7	69.6	26.8	*T. rotula*	*T. rotula + M. rubrum*	*T. rotula + R. shrubsolei*	**16.6** 81.6	**15.3** 332.9	**15.4** 96.8	**0.9** 1.8	**0.9** 1.9	**0.9** 1.9	**0.054** 0.023	**0.060** 0.023	**0.056** 0.014
August	6	4	4	205.2	82	30	*T. rotula*	*T. rotula + P. micans*	*T. rotula*	**15.7** 62.7	**15.3** 175.6	**15.7** 43.3	**0.8** -	**1.2** -	**1.1** -	**0.053** 0.044	**0.081** 0.021	**0.069** -

It is noticeable that the cultures from the middle site (ME) had a more diverse phytoplankton community compared to the other two cultures (figure 5.26). A mixture of different species succeeded to grow in these cultures during the incubation periods and contributed to total phytoplankton biomass on the peak day. The intermediate conditions of nutrients and salinity characterized by the water sampled from the mid estuary (NWN) seemed to favour most phytoplankton groups. For example, the autotrophic ciliate *Mesodinium rubrum*, which contributed up to 34%, 68% and 33% to the total initial biomass (during Exp.3) in the UE, ME and LE cultures, respectively, only survived in the ME culture although no increase in their biomass occurred. In ME culture(s) *M. rubrum* comprised about 27% and 30% of the total biomass peak in Exp.2 and Exp.3, respectively. This is supported by previous studies which have shown to be difficult to culture *M. rubrum* in the laboratory (Crawford, 1992).

In all experiments, nutrient concentrations gradually declined during the incubation period with increasing phytoplankton biomass. Chl *a* concentrations declined from the peak value when nutrients become limiting to growth of phytoplankton. In each culture, the phytoplankton biomass on peak days, either estimated from chlorophyll biomass or carbon biomass, varied from one experiment to the other according to the seasonal variation in nutrient availability. In cultures from the upper estuary (UE culture) phytoplankton biomass increased from initial values (1.1-15.5 mg Chl *a* m^{-3} & 50-385 mg C m^{-3}) to peak values ranging between 146-205 mg Chl *a* m^{-3} and 3470-5018 mg C m^{-3}, with minimum peak in May and maximum peak in August, in contrast to temporal changes in ambient nutrient concentrations that decreased towards summer. This might be related to the fact that the initial biomass was higher in August (Exp. 4) than in other experiments. Another possible explanation to this finding is that the summer phytoplankton species positively respond to enhanced irradiance conditions, and independently of nutrient concentrations.

The nutrient supply ratio is one of the drivers of phytoplankton growth and biomass (Gowen, 1992, Labry et al. 2001, Nedwell et al. 2002, Kocum et al., 2002a & b) and nutrient ratios (N:P, N:Si, P:Si) can indicate which nutrient may become limiting for phytoplankton growth. Changes occurred in nutrient removal ratios, N:P, N:Si, P:Si, during the incubation period of the three cultures (UE, ME and LE) as presented in figures 5.23 - 5.25. N:P removal ratios were near to the Redfield ratio (N:P=16) at the beginning of the incubation period of all four sets of experiments then the ratio increased following the peak chlorophyll day (figure 5.23) indicating P-limitation. In all experiments phytoplankton species initially take up nutrients in atom ratios very close to that recorded by Redfield, 16N:1P:16Si (Redfield, et al., 1963) for balanced growth while ratios

changed after a few days (after the peak day) indicating a degree of limitation of one or more nutrients.

For UE culture(s), phytoplankton biomass in May (Exp. 1) declined when the values of nitrate:phosphate (N:P) and nitrate:silicate (N:Si) increased to 31.2 and 5.1, respectively (see table 5.5) indicating P and Si limitation, while no N-limitation was recorded in this experiment. In the other three experiments (Exp. 2-4) involving UE culture(s), conducted in June, July and August phosphorus seemed to be the only nutrient limiting phytoplankton growth after peak day of these experiments. Silicate and nitrate were in concentrations sufficient not to limit phytoplankton growth of UE culture(s) during these three experiments.

In the mid estuary culture(s) (ME), peak biomass ranged between 34-82 mg Chl *a* m^{-3} and 682-1675 mg C m^{-3} during the four experiments with highest peak, again, in July and August. Nutrient ratios after the peak day indicated P-limitation in all experiments as the value of N:P were much higher (see table 5.5) than Redfield's. P:Si were subsequently decreased to values below the "standard" Redfield value confirming conditions of P-limitation. To some extent Si became limiting in Exp. 2 (June) after the peak growth of the diatom *Chaetoceros* spp. However, a degree of N-limitation is indicated with a decreasing N:P ratio to a value of 0.3 in the August experiment (Exp. 4) due to reduced nutrient concentrations in summer.

In LE culture(s), peak values of chlorophyll-a were of lesser magnitude compared to other sites and ranged between 27-69 mg m^{-3}, while carbon biomass reached maximum values of 221-923 mg C m^{-3}, with a minimum peak during the June experiment (Exp. 2). Comparing nutrient uptake ratios of phytoplankton growth before and after the biomass peak value in Exp. 2 in June, indicated that silicate was dramatically limiting growth of diatoms when the value of N:Si increased to >3.4. Diatoms which were abundant in the initial phytoplankton community, decreased during the incubation period due to a silicate-shortage, i.e. Si-limitation. This would be responsible for the shift from diatom-dominated community on the initial day to a flagellate-dominated community on the peak day. Other non Si-requiring species, e.g. blue-green filaments and *M. rubrum* contributed to the peak biomass, comprising 46% and 32% of total phytoplankton biomass respectively. These species increased for 2 days and then decreased due to P-limitation (table 5.5). As recorded for ME culture(s) a degree of apparent N-limitation was recorded during the August experiment (Exp. 4) indicating that the system might be nitrogen limited towards summer season particularly in the mid and lower estuary. Similar findings of changing phytoplankton growth status from P to N+P limitation during summer was previously recorded in Southampton Water (Kifle, 1992) and has also been shown in the Gironde plume waters (Labry, et al., 2002).

CHAPTER SIX

CHAPTER SIX

6- EFFECT OF N:P:Si RATIOS ON GROWTH RATE OF TWO PHYTOPLANKTON SPECIES UNDER NON-LIMITING LIGHT CONDITIONS

6.1 INRODUCTION

The availability of inorganic nutrients such as nitrate, phosphate and silicate are key factors influencing phytoplankton growth and community structure in estuarine environments. An indication of phytoplankton limiting nutrient(s) in estuaries can be estimated from their ambient concentrations and nutrient ratios. It is often assumed that marine and estuarine phytoplankton are nitrogen-limited (Hecky & Kilham, 1988), although, phosphorus limitation has also been suggested in marine coastal (Thingstad et al., 1993) and estuarine (Pennock & Sharp, 1994; Holmboe et al., 1999; Yin et al., 2000; Labry et al., 2002; Kocum et al., 2002b) waters. Higher inputs of N and P into coastal waters (Carlsson & Graneli, 1999) compared to silicate (Si) can cause all silicate to be utilized (Wulff & Rahmn, 1988) in spring by diatoms (Wulff & Rahmn, 1988; Carlsson & Graneli 1999; Kocum et al., 2002a) leaving excess N and P for flagellate growth.

NO_3^-, PO_4^{3-} and $Si(OH)_4$ are the most likely limiting nutrients in estuaries, assuming that light limitation does not occur, (Heip et al., 1995). For most of the year, phytoplankton growth in

Southampton Water, which is considered to be a hypernutrified estuary (Hydes, 2000), tends to be light-limited (see chapter 5) rather than nutrient-limited, however, nutrient ratios (N:P, N:Si and P: Si) may influence the growth rate and consequently the community composition of phytoplankton populations (Redfield et al., 1963; Nedwell et al., 2002; Kocum et al., 2002a & b).

In this chapter, results from two sets of phytoplankton culture experiments are presented with the aim of investigating the optimal nutrient ratio for phytoplankton growth and to examine the effect of different nutrient ratios (N:P:Si) on growth rate of two contrasting species isolated from the estuary and known to be dominant in Southampton Water.

6.2 EXPERIMENTAL PROCEDURE

Two-cultured phytoplankton species (isolated by the author from Southampton Water, see chapter 2), a diatom *Thalassiosira rotula* (figure 6.1, Appendix III; plate 1) and a dinoflagellate *Prorocentrum micans* (figure 6.1, Appendix III; plate 4); were chosen for these experiments. 1 ml inculum of each culture was checked for cell density (25 cells ml^{-1} for *T. rotula* and 5-7 cells ml^{-1} for *P. micans*) and placed in 3 sterile 500 ml conical flasks containing sterile ASW (Harrison et al., 1980) and supplemented (with the exception of nitrate, phosphate, silicate) with Keller's recipes additions (Keller et al., 1987, see chapter 2). Nitrate, phosphate and silicate were added to experimental cultures in specific ratios forming three different combinations as given in Table 6.1.

Table 6.1. Different N:P:Si treatments as supplemented in each flask for *T. rotula* and *P. micans* cultures during incubation experiments

	NO_3- N	PO_4- P	$Si(OH)_4$-Si
Combination I	16 µM	0.1 µM	160 µM
Combination II	16 µM	1 µM	160 µM
Combination III	16 µM	5 µM	160 µM

Cultures of combination II (16N:1P:160Si) were incubated in duplicate to determine the reproducibility of the experimental set up. Results obtained from both flasks are included in the following analysis as mean values (for Chl *a*, nutrients and cell number) of both duplicates. The four flasks (for each organism) were then incubated (see figure 6.2) for a period of 30 days for *T. rotula* and 50 days for *P. micans* at 15 –16 ^{0}C and mean irradiance level of 150 µmol m^{-2} s^{-1} (measured using Biospherical Irradiance Meter, QSL-1000/101) on a 16H Light : 8H Dark cycle.

6.2.1 CULTURE SUB-SAMPLING

Cultures were sampled and aliquots of 5 ml of each culture preserved with 1ml of acid Lugol's iodine solution and kept in dark bottles prior to cell counting. Phytoplankton cells were counted using

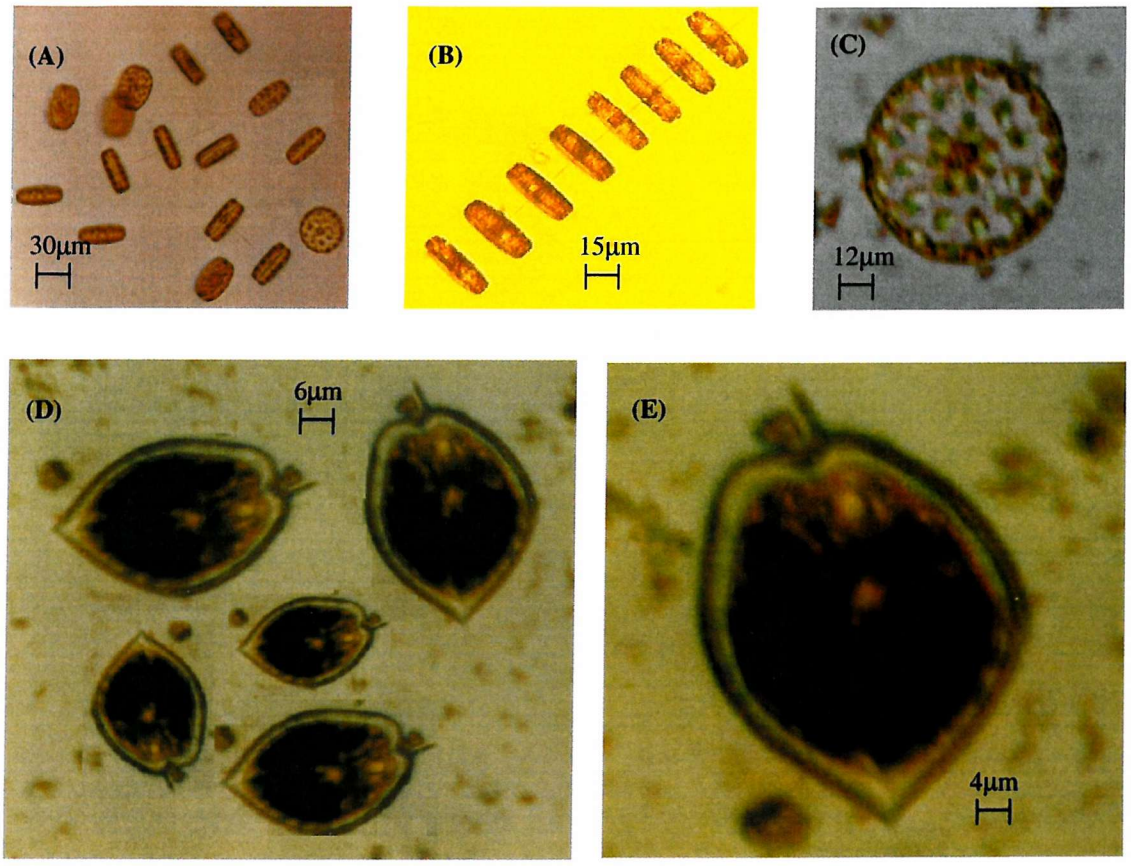


Figure 6.1. Lugol's preserved cells from the *Thalassiosira rotula* culture (**A**), a chain of cells (B), an individual cell (**C**) *Prorocentrum micans* culture (**D**) and an individual cell (**E**).

a Leitz Flouovert inverted microscope and a Sedgewick Rafter counting chamber. 10 ml (in duplicate) of each culture were filtered through Whatman 25-mm diameter GF/F filters and kept in the freezer prior to Chl *a* measurements. The aliquots of the filtrates (20 ml) were either frozen for later nitrate and phosphate analysis or kept in the fridge prior to silicate analysis (see Chapter 2). Culture flasks of both organisms were mixed (very gently for *P. micans* cultures) and their positions in the incubator changed daily to randomize the incubation conditions over the period of the experiment. Initial measurements (for Chl *a,* nutrient concentrations and cell density) were taken at the beginning of the experiment (day 0) from each flask then sub-sampled at frequent intervals. The diatom *Thalassiosira rotula* cultures were sub-sampled daily for microscopic cell enumeration and every second day for nutrient and Chl *a* measurements. The dinoflagellate *Prorocentrum micans*

cultures were sub-sampled every 2 days for microscopic cell enumeration and every 4 days for nutrient and Chl *a* measurements.

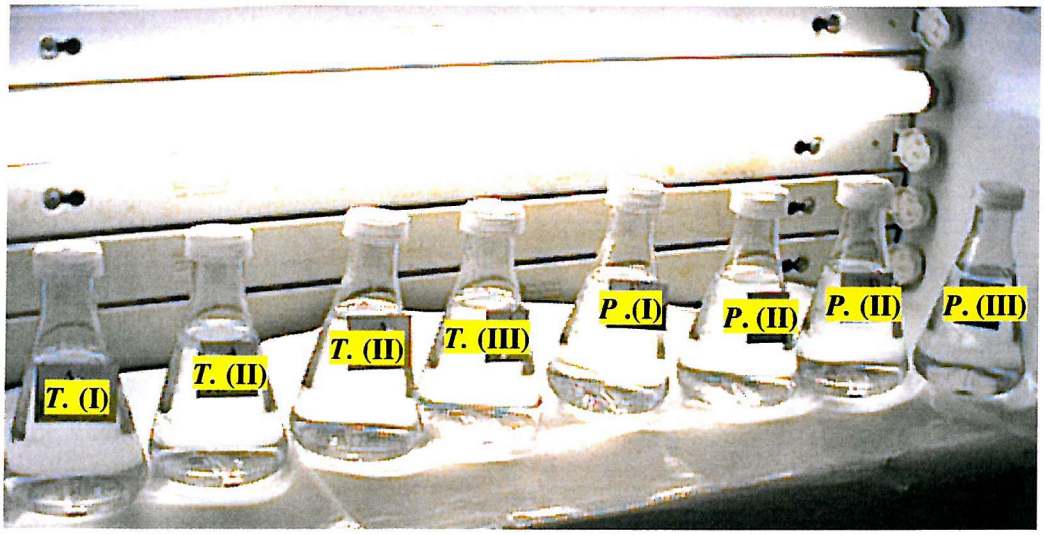


Figure 6.2. Incubated culture flasks for the two species; *Thalassiosira rotula* (***T***) and *Prorocentrum micans* (***P***) in three different nutrient combinations: 16N:0.1P (**I**), 16N:1P (**II**) and 16N:5P (**III**). N.B. 2 flasks were incubated for the nutrient-balanced culture (combination **II**).

6.2.3 DETERMINATION OF GROWTH RATE (μ)

1 ml of the preserved sub-samples (for both organisms) was placed in a Sedgewick Rafter counting chamber and left for 3-5 minutes to settle before counting. The whole chamber (includes 1000 small square) was counted in early days of the culture when cells were less numerous. However, five or ten transects across the chamber, each of which includes 20 small squares were counted in later samples. In dense cultures, particularly for *Thalassiosira* cultures, 50 small squares were randomly counted. Counts of 3-5 separate fillings of the chamber were made with typical coefficient of variations of <15%. The mean cell count (cells ml^{-1}) was plotted on a logarithmic scale against time (days) to identify the exponential growth phase and a regression line of the ln of the cell number (cell ml^{-1}) versus time (day) produced to derive the specific growth rate (d^{-1}).

6.3 THALASSIOSIRA CULTURES

6.3.1 GROWTH RATE OF T. ROTULA

The growth curves of the diatom *Thalassiosira rotula* under the three different nutrient combinations (I, II and III) are shown in figure 6.3. The diatom cells started to grow exponentially in all cultures

after 1-2 days, although the cells were initially inoculated from an exponentially growing stock. A maximum of about 9.2 x 10^3 cells ml^{-1} (mean of two flasks) was recorded on day 6 and 7 x 10^3 cells ml^{-1} on day 5 in cultures of combination II (16N: 1P) and III (16N: 5P), respectively. In contrast, a maximum of only 1.3 x 10^3 cells ml^{-1} (on day 5) was determined in the P-limited cultures (I) (table 6.2). The maximum growth rate derived from cell counts (mean value 1.74 d^{-1}) was obtained (Table 6.2) in the nutrient-balanced cultures (16N: 1P) and the lowest growth rate (0.7 d^{-1}) was estimated for cells grown in media deficient in phosphorus (combination I). In combination III with lower N:P ratio, a relatively high growth rate (1.7 d^{-1}) was obtained although, it was slightly lower than that obtained in the balanced nutrient culture (combination II) (see table 6.2).

Table 6.2. Summary of results obtained during incubation experiment of the diatom *Thalassiosira rotula* under different N:P:Si ratios.

Thalassiosira rotula	**Peak Chl *a* (mg m^{-3})**	**Peak cell number (cells ml^{-1})**	**growth rate (chlorophyll estimation) ($\mu = d^{-1}$)**	**growth rate (cell number estimation) ($\mu = d^{-1}$)**
16N: 0.1P	10.86	1.3 x 10^3	1.17	1.17
16N: 1P	56.52	9.5 x 10^3	2.25	1.73
	52.50	8.9 x 10^3	2.34	1.75
16N: 5P	45	7.0 x 10^3	2.14	1.70

Changes in Chl *a* concentration in relation to nutrient combinations (nitrate, phosphate and silicate) are shown in figure 6.4. Chl *a*, as an indicator of biomass, followed a similar pattern to cell density (cell ml^{-1}) with highest Chl *a* concentration (~ 54.8 mgm^{-3} and 45 mgm^{-3}) recorded in cultures II (16N:1P) and III (16N:5P) on day 5. A relatively smaller peak in Chl *a* of 10.86 mgm^{-3} was measured in the P-limited culture (16N:0.1P).

Chl *a* concentration (mg Chl *a* m^{-3}) dramatically decreased after day 4 in culture I and II and after day 5 in culture III. Growth rates were also estimated from changes in chlorophyll concentration (table 6.2) although these were based on fewer data points over the exponential growth period (typically 3 or 4). Chlorophyll derived growth rates were similar to those derived from cell counts in the 16N:0.1N but higher in the 16N:1P and 16N:5P.

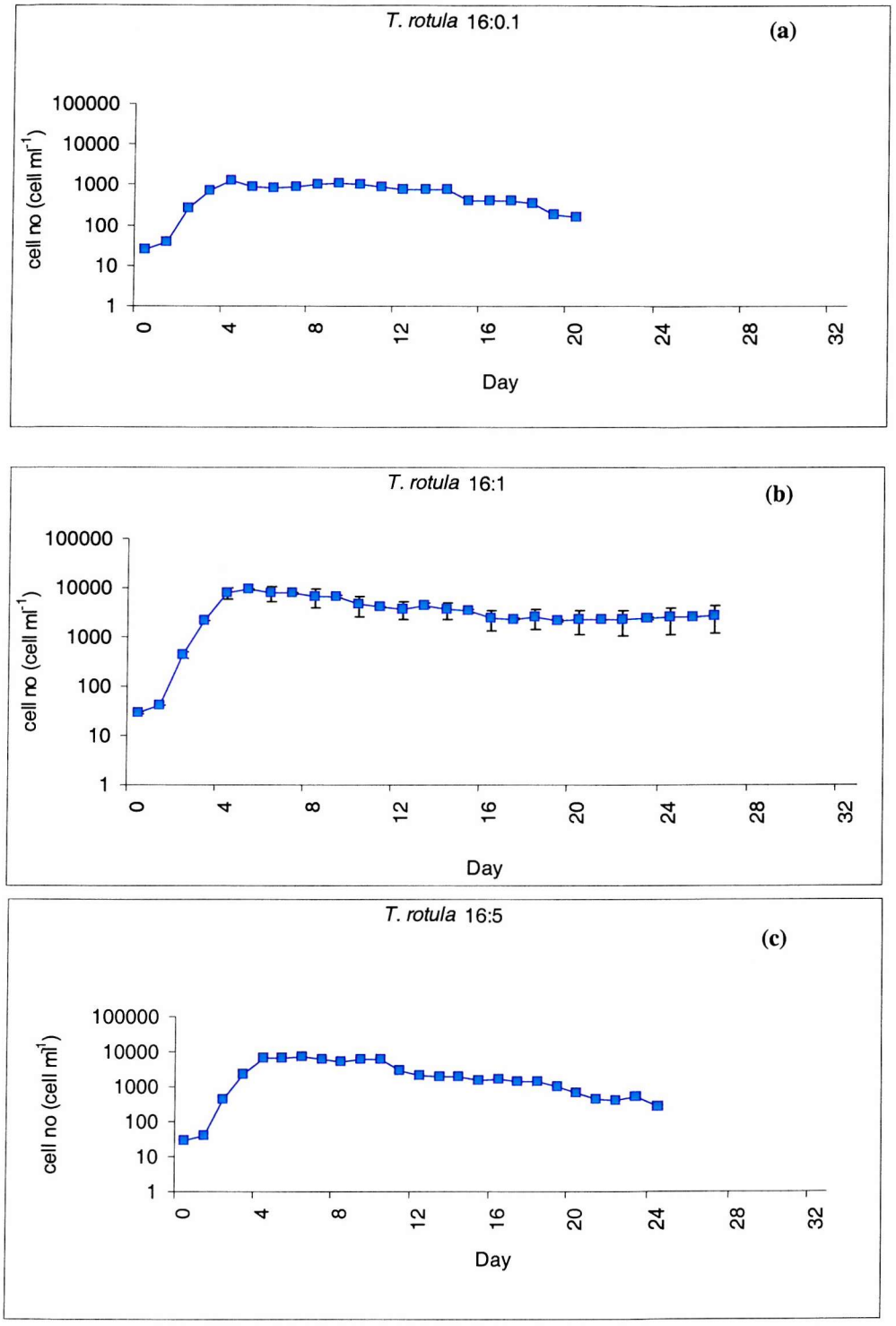


Figure 6.3. *Thalassiosira rotula* batch cultures grown under different nutrient conditions showing cell growth under N:P ratios of (**a**) 16:0.1, (**b**) 16:1 and (**c**) 16:5. Error bars in figure (**b**) represents standard difference of duplicate cultures.

Figure 6.4 shows that nitrate concentration in the phosphate limited (16N: 0.1P) culture slowly decreased and remained above 5 μM during stationary growth phase whereas phosphate became undetectable on day 8. In the balanced 16N:1P cultures nitrate and phosphate were rapidly depleted and became undetectable on day 4. In the phosphate replete culture nitrate was rapidly removed from solution and became undetectable on day 4 (figure 6.4), whereas phosphate became undetectable on day 7. Silicate concentration was non-limiting to growth in all cultures throughout the period of the incubation (figure 6.4).

In figure 6.5 nitrate, phosphate and silicate concentrations are plotted to show the different combinations of nutrient uptake ratios in the *T. rotula* cultures. A regression fit has been applied to the data in each case from day zero of the experiment to the day when one of the nutrients becomes undetectable. The slope of the regression line fit in each case is given in table 6.3. In the 16N:1P culture the diatoms took up nitrate and phosphate in a ratio (N: P = 16.2; see table 6.3) close to the Redfield ratio (N: P = 16) achieving the maximum biomass yield expressed as chlorophyll concentration as well as cell density.

The low values of N:Si and P:Si uptake ratio(s) recorded in all cultures were due to the high concentration of Si initially supplied. In contrast values of N:P ratio are well above the "standard" 16N:1P ratio (N:P > 62) during the experiment growth period of *T. rotula* under P-limited conditions (i.e. combination I) (Table 6.3, Figure 6.4). In culture of this combination (16N:0.1P) cell number decreased from day 6 onwards when phosphate declined from the initial concentration of approximately 0.13 μM to 0.05-0.08 μM (figure 6.3). Diatom cell density in this culture remained between 0.8 x 10^3 cells ml^{-1} and 1.1 x 10^3 cells ml^{-1} from day 6 to day 15 then declined to a minimum cell concentration of about 0.4 x 10^3 cells ml^{-1} when phosphate was completely depleted from the culture (figure 6.3). This indicated P-limitation (figure 6.4, Table 6.5) as nitrate and silicate concentrations were > 12 μM and 125 μM, during and after the stationary phase of growth (see table 6.3). In the P-replete culture (16N:5P), diatom cells grew exponentially between day 2-5 (see figure 6.5) with N:P uptake ratios of < 3 indicative of N-limitation (Table 6.3). Cell growth ceased when nitrated became depleted from the culture on day 5-6 although; P was still measurable until day 7-8 (figure 6.4). The value of P:Si ratio (0.061) was close to Redfield's (0.063) indicative that P was available for cells and the culture had become N-limited before P-depletion occurred.

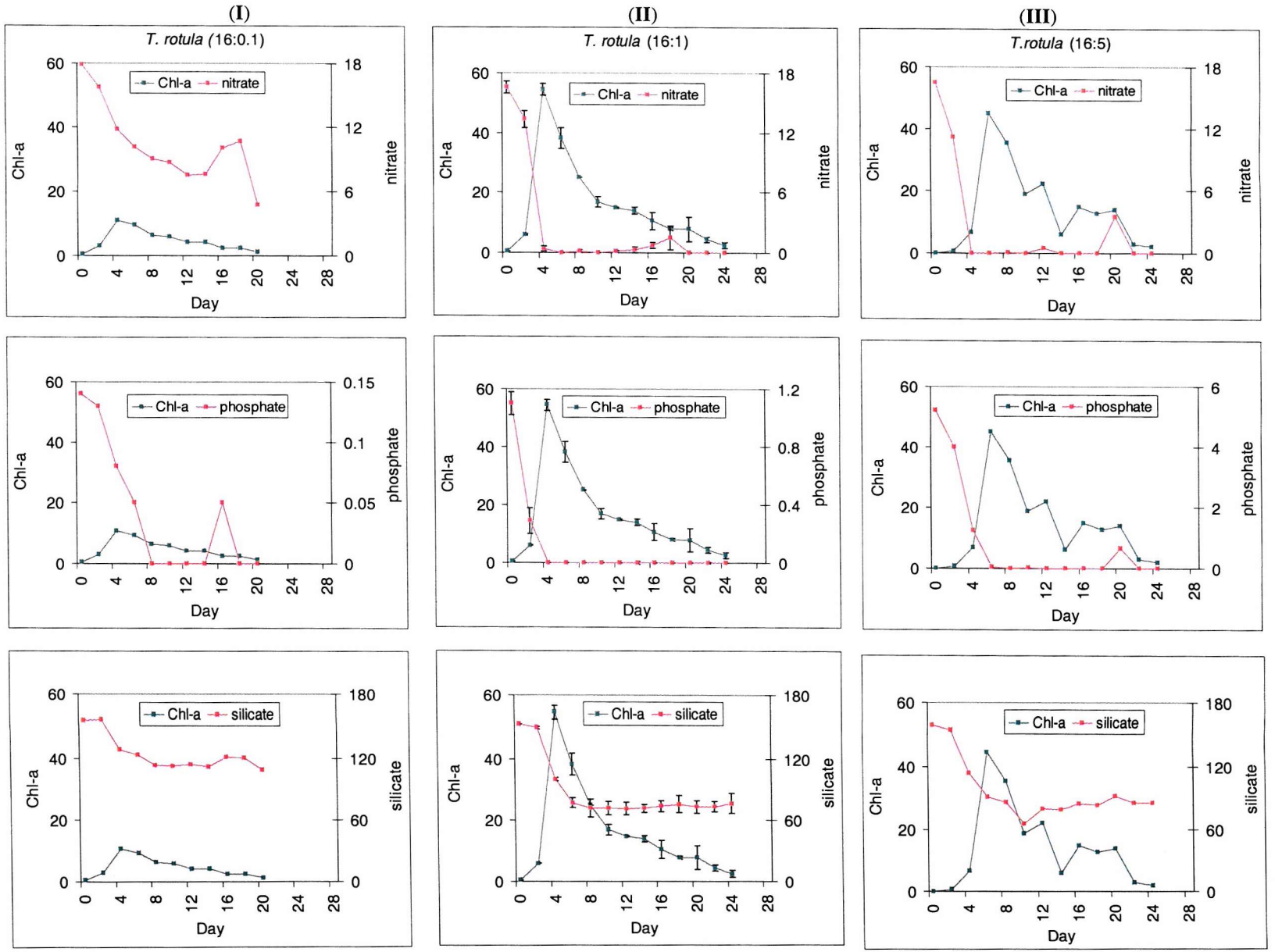


Figure 6.4. Changes in Chl *a* concentrations (mg m^{-3}) during the growth of the diatom *Thalassiosira rotula* in relation to changes in nutrient (nitrate, phosphate, and silicate) concentrations (μM) under different N:P ratios (16:0.1, 16:1 and 16:5). Error bars in culture **II** represents standard difference of duplicate cultures.

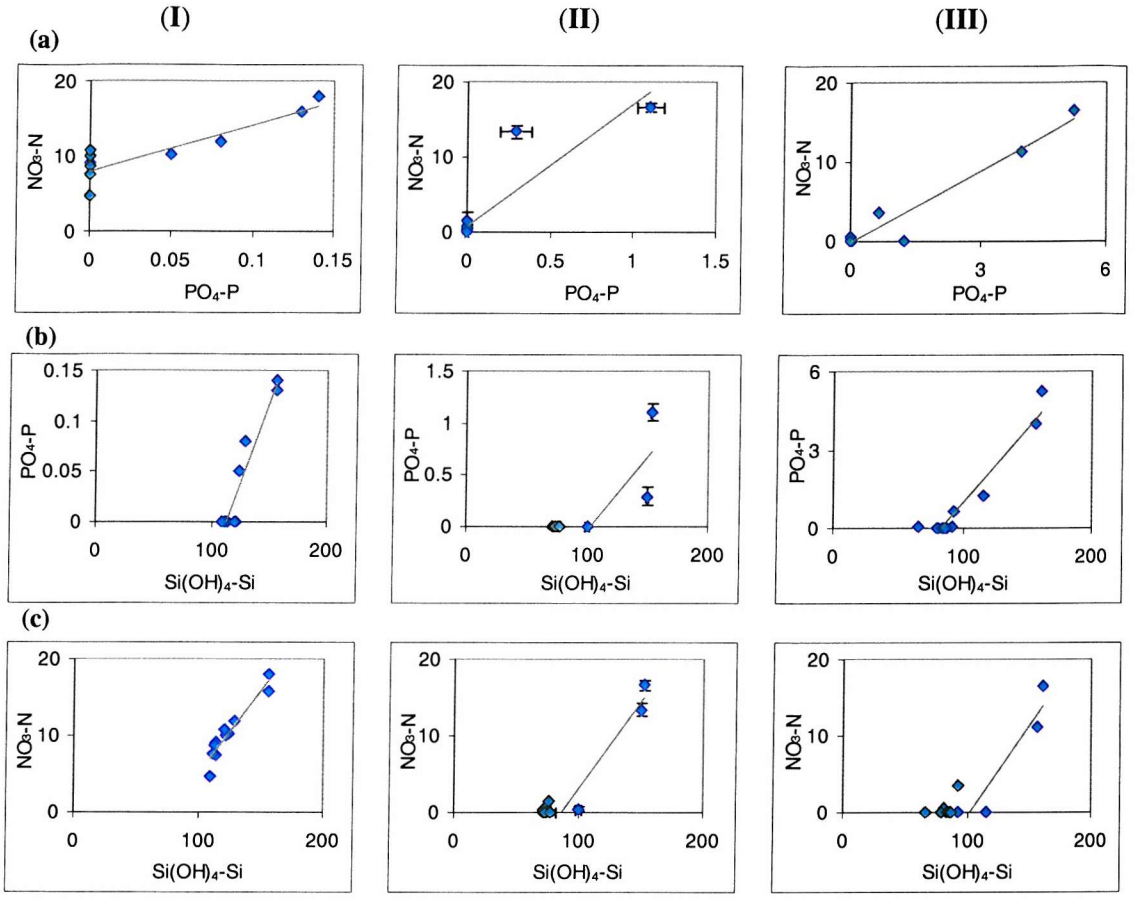


Figure 6.5. Changes in nutrient concentrations (μM) during incubation experiment of the diatom *T. rotula* under non-limited light conditions: (**a**) changes in NO_3- N versus PO_4- P, (**b**) PO_4- P versus $Si(OH)_4$-Si and (**c**) NO_3- N versus $Si(OH)_4$-Si for cultures incubated in combination **I** (16N:0.1), **II** (16N:1P) and **II** (16N:5P). Error bars in culture **II** represents standard difference of duplicate cultures.

Table 6.3. Summary of nutrient uptake ratios (N:P; N:Si and P:Si) for *T. rotula* cultures

	Thalassiosira rotula		
NUTRIENT RATIOS	**N:P**	**P:Si**	**N:Si**
Culture I (16:0.1)	62.2	0.003	0.22
Culture II (16:1)	16.2	0.014	0.23
Culture III (16:5)	2.98	0.061	0.24

6.4 PROROCENTRUM CULTURES

6.4.1 GROWTH RATE OF P. MICANS

The growth curves of the dinoflagellate *Prorocentrum micans* under the three different combinations of nutrients are shown in figure 6.6. *P. micans* showed a relatively longer lag phase compared to that of the diatom species (*T. rotula*) and cells started to grow exponentially after 6-8 days. Peak growth (maximum cell number) occurred on day 12, 14 and 16 in culture I, III and II, respectively with highest cell density achieved in culture II and III. A peak of 10.3 x 10^2 cells ml^{-1} (mean of two flasks) recorded between days 16-18 in cultures II (16N: 1P) compared to 6.3 x 10^2 cells ml^{-1} (Table 6.4, Figure 6.6) in culture III (16N: 5P).

Table 6.4. Summary of results obtained from the incubation experiment of the dinoflagellate *Prorocentrum micans* under different N:P:Si ratios.

Prorocentrum micans	**Peak Chl *a* ($mg\ m^{-3}$)**	**Peak cell number ($cells\ ml^{-1}$)**	**growth rate (chlorophyll estimation) ($\mu = d^{-1}$)**	**growth rate (cell number estimation) ($\mu = d^{-1}$)**
16N: 0.1P	3.32	1.5x 10^2	0.67	0.54
16N: 1P	15.12	10.6 x 10^2	0.74	0.75
	13.32	10.0 x 10^2	0.74	0.73
16N: 5P	11.10	6.3 x 10^2	0.67	0.67

A relatively smaller peak of 1.5 x 10^2 cells ml^{-1} was recorded (figure 6.6) in the P-limited culture (I; 16N:0.1P) on day 12 with a relatively low growth rate (0.54 d^{-1}), compared to the higher growth rates (table 6.4) of dinoflagellate cells estimated in culture(s) II (16N: 1P) of 0.74 d^{-1} (mean value) and 0.67 d^{-1} in culture III (16N:5P).

Figure 6.7 shows changes in Chl *a* concentration in relation to changes in nutrient concentrations. Chl *a* values showed a very similar pattern to that of cell concentration ($cells\ ml^{-1}$) with peaks of 3.32, 14.22 and 11.1 mg Chl *a* m^{-3} recorded in cultures I, II and III, respectively (table 6.4). Growth rates were similarly estimated from changes in chlorophyll concentration over the exponential growth period and these were almost the same as those derived from cell counts (table 6.4) in each treatment. Chlorophyll concentration showed some decrease in the 16N:1P and 16N:5P cultures during stationary growth phase although the cells in the 16N:0.1P showed little change in chlorophyll concentrations.

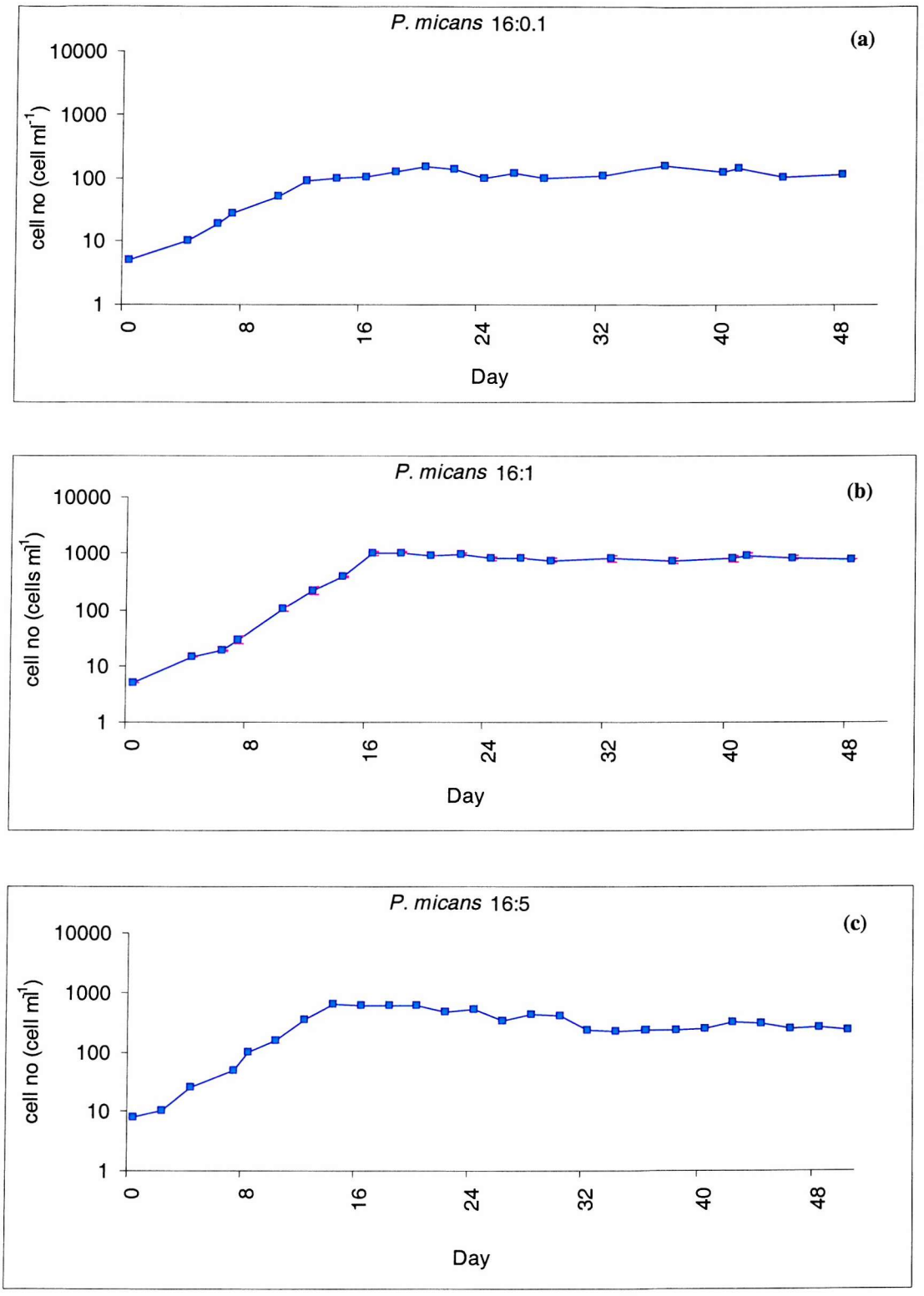


Figure 6.6. *Prorocentrum* batch cultures grown under different nutrient conditions showing cell growth under N:P ratios of (**a**) 16:0.1, (**b**) 16:1 and (**c**) 16:5. Error bars in (**b**) represents standard difference of duplicate cultures.

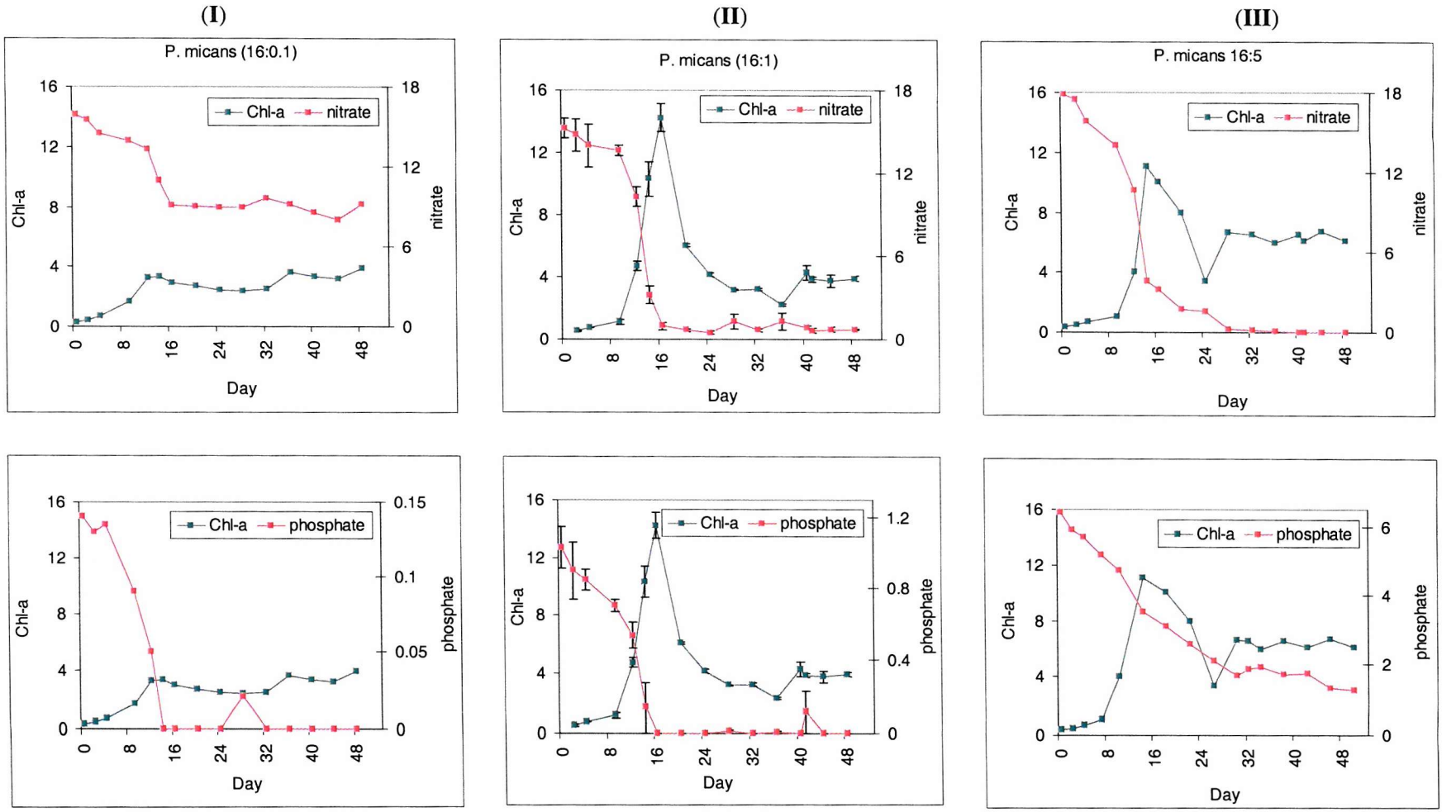


Figure 6.7. Changes in Chl *a* concentration (mg m^{-3}) during the growth of the diatom *Prorocentrum micans* in relation to changes in nutrient (nitrate, & phosphate) concentrations (μM) under different N:P ratios (16:0.1, 16:1 and 16:5).Error bars in culture **II** represents standard difference of duplicate cultures.

In figure 6.8 nitrate and phosphate concentrations are plotted to show the different combinations of N:P uptake ratios in the *P. micans* cultures. A regression fit has been applied to the data in each case from day zero of the experiment to the day when one of the nutrients becomes undetectable. The slope of the regression line fit in each case is given in table 6.5. In the 16N:1P culture the dinoflagellate took up nitrate and phosphate in a ratio close to Redfield (N: P = 15.7, see table 6.5) and reached a higher cell density than the other two treatments. In culture I the N:P uptake ratio was high (30.01) indicative of the imposed phosphate limitation. In the phosphate replete culture there appears to be two different nutrient uptake ratios (see figure 6.8) with a higher N:P ratio (4.91) during exponential growth phase up to day 14 then a lower ratio (table 6.5) from day 14 to day 28 (1.79) when nitrate became undetectable.

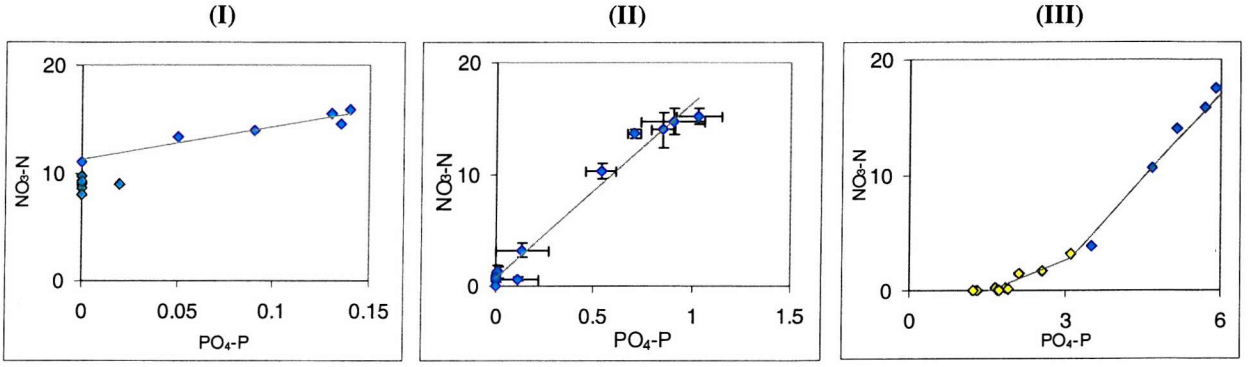


Figure 6.8. Plot showing variations in nitrate (μM) versus phosphate (μM) during growth of the dinoflagellate *P. micans* grown under different nutrient ratios, (I) 16N:0.1P, (II) 16N:1P and (II) 16N:5P the incubation period during and non-limited light conditions. Error bars in culture **II** represents standard difference of duplicate cultures.

In the phosphate limited culture nitrate concentration decreased and remained above 7 μM during stationery growth phase (figure 6.7) whereas phosphate became undetectable on day 14. In the balanced 16N:1P cultures phosphate was depleted to undetectable level on day 16 while nitrate reached a low plateau concentration also on day 16. In the phosphate-replete culture nitrate was removed from solution and becomes undetectable on day 28 whereas phosphate is slowly depleted throughout the 50 day incubation and does not reach an undetectable level (figure 6.7).

	P. micans
NUTRIENT RATIOS	**N/P**
Culture I (16:0.1)	30.01
Culture II (16:1)	15.7
Culture III (16:5)	4.91 (**1.79**)

Table 6.5. N:P uptake ratios for *P. micans* culture during incubation under different nutrient ratios. Two different uptake ratios were recorded in culture III during the incubation period. The number in bold indicates the N:P uptake ratio after the exponential phase of growth (yellow symbols in figure 6.8.

6.5 DISCUSSION

6.5.1 GROWTH RATE OF T.ROTULA & P. MICANS

Both the diatom *Thalassiosira rotula* and *Prorocentrum micans* showed similar "typical" growth curves, with an initial lag phase, which varied from one species to another, followed by an exponential growth phase and a stationary growth phase with no cell division after which cells starts to die off. Specific growth rate(s) determined in this study showed a similar range (1.17-2.34 d^{-1} for *T. rotula* and 0.54 – 0.75 d^{-1} for *P. micans*) as those reported for the same species in other studies with a range of 0.95-2.9 d^{-1} for *T. rotula* (e.g. Schöne, 1974; Kraweic, 1982; Heath, 1988; Kifle, 1992) and of 0.41-0.56 d^{-1} generally for *Prorocentrum* species (Wagey et al., 2000; Hansen, 2002).

The diatom species, *T. rotula*, had a higher growth rate (1.17-2.34 d^{-1}) and a shorter lag phase (~2 days) compared to the dinoflagellate species, *P. micans* (0.54-0.75 d^{-1}, with lag phase of 6-8 days) under different nutrient ratios. This finding is in a good agreement with previous studies involving diatoms and dinoflagellates reporting that diatoms are rapidly growing (Banse, 1982; Kifle, 1992) while, dinoflagellates are known to have substantially lower growth rates (Chan, 1980; Banse, 1982). Diatom species generally tend to divide four times as fast as dinoflagellate species (Banse, 1982; Brand & Guillard, 1981; Langdon, 1993). This fact partially explains the succession of phytoplankton communities in Southampton water with diatoms growing rapidly in spring when $Si(OH)_4$ is more available and dinoflagellates growing in summer as they can not compete for nutrients with the fast growing diatoms that dominate spring phytoplankton bloom. Dinoflagellates are generally weak competitors for nutrients compared with diatoms (Chang & McClean, 1997; Smayda, 1997); however the extremely motile dinoflagellates can compete successfully with diatoms (Broekhuizen, 1999).

6.5.2 REDFIELD RATIOS AS INDICATORS FOR NUTRIENT LIMITATIONS

The Redfield N:P ratio of 16:1 (Redfield et al., 1963) is often used as a reference indicator for determining nutrient limitation of phytoplankton growth and differentiating N-limitation from P-limitations assuming that phytoplankton under N-limited conditions shows a N:P <16 but are P-limited at N:P >16 (Geider & La Roche, 2002). The ratio of N:P may have lower values (Geider & La Roche, 2002) as determined in nutrient-replete cultures (median 9:1) or may significantly exceeded the Redfield value, reaching 25:1 in some circumstances (Broecker & Henderson, 1998). Despite this variability, Geider & La Roche (2002) showed the value of N:P ranges from 5 to 19, with most observations falling below 16, particularly under nutrient-replete conditions. They also indicated that the threshold of N:P that marks the transition between N-limitation and P-limitation is a value between 10 -30 (Boynton et al., 1982) or between 20-50 (Geider & La Roche, 2002).

Results of the present study, indicate that values of N:P ratio in both cultures (*T. rotula* & *P. micans*) were very close to the "typical" Redfield value ranging between 15.7-16.2 at the beginning of the incubation period (non- nutrient limited) when cultures supplemented with 16μM N : 1μM P. However the ratio significantly deviated form Redfield values, with higher N:P ratios (60.6 & 49.9) in P-limited cultures (combination I) and lower values (3 & 3.81) in P-replete cultures (combination III).

A nutrient becomes limited for the growth of any species if its concentration is below the critical nutrient concentration of that species (Stewart & Levin, 1973; Tilman, 1977; Armstrong & McGehee, 1980). Critical nutrient concentration is a physiological parameter that sets a lowest level of nutrient needed for phytoplankton growth (Sommer, 1989; Tilman 1990; Carignan & Planas, 1994) which can be predicted unlike other limiting factors, e.g. irradiance (Kirk, 1983; Han et al., 1999); this parameter is however, independent of nutrient supply.

6.5.3 GROWTH CONDITIONS: COMPARISON BETWEEN ESTUARINE AND CULTURE CONDITIONS

Taking into account the fact that phytoplankton species in Southampton Water are not generally nutrient-limited (Holley & Hydes, 2000; Nedwell et al., 2002) and nutrients within the estuary are high enough to support their growth throughout the whole year (Wright et al., 1997), the reason for frequent blooms not developing could be attributed to the irradiance conditions or some nutrient availability at the time of the bloom (nutrient ambient ratio). Competition of phytoplankton species, in estuaries, for nutrients can be predicted by the critical nutrient concentration (Armstrong & MacGehee 1980; Sommere, 1983) and/or the cellular content (i.e. cell quota, Q) of a single (N or P) limiting nutrient (Q_N or Q_P). In contrast to nutrient competition, prediction of phytoplankton competition for light seems to be more difficult (Tilman, 1990; Carignan & Planas, 1994) as light follows daily changes and can not be cycled like nutrients and is never distributed in a homogenous way, as nutrients are, in aquatic environments (Carignan & Planas, 1994; Han et al., 1999).

Although, *T. rotula* has been shown to be an abundant species throughout the estuary (maximum = $0.3x10^3$ - $0.7x10^3$ cells ml^{-1} of the same cell size (40-55 μm) and $0.6x10^3$cells ml^{-1} with much smaller cells (10-20 μm) during the period 1999-2000, the same species did not reach the cell density that occurred during the culture experiment density ($1.3x10^3$ - $9.5x10^3$ cells ml^{-1}), with the higher cell density in nutrient "balanced" cultures (16 μM nitrate : 1 μM phosphate). The smaller sized-cells recorded for this species along the estuary during the period July-August 2000 could be an indication

of a degree of P-limitation, particularly towards summer months, as suggested by Labry and co-authors (Labry, et al. 2002). They demonstrated that species of smaller sized-cells (3-20 μm) dominated the spring diatom bloom in the Bay of Biscay as a consequence of the early P-limitation recorded. The highest cell density of *T. rotula* recorded in Southampton water in 1999 and 2000 (current study) always occurred after a bloom of large-celled diatoms (e.g. *Guinardia delicatula*), which caused dramatic decline in P concentration after collapsing (current study, see chapter 3) and consequently led to P-limitation. Nitrate concentration was about 1.8 μM after the bloom of *G. delicatula* terminated in May 1999 however phosphate declined to undetectable level. The value of the N:P ratio was 16.2 before the bloom of *G. delicatula* in May 1999 and 21.6 before the bloom of *T. rotula* in June 1999 at the Dock-Mooring site.

A similar finding recorded for the dinoflagellate *P. micans* that had a maximum cell density of < $0.7x10^2$ cells ml^{-1} along the estuary during the period from May 1999 to April 2001 compared with 1.5-10.6 $x10^2$ cells ml^{-1} during the incubation experiment with, again, highest cell concentration in cultures with N:P of a value of 16. This could indicate that, beside the enhanced effect of the good light conditions provided to cultured cells during incubation period compared to the *in situ* conditions, the ambient concentrations of nutrients, particularly nitrate, phosphate and silicate within the estuary might not be available in the standard ratios favored by phytoplankton for better growth. *T. rotula* attained its maximum cell concentration in spring/summer 1999 and 2000 (current study) when nutrient ratios of N:P, N:Si and P:Si were falling between the standard levels with values of 16 (or 25), 1 and 0.063, respectively as previously suggested for phytoplankton, in general (Redfield et al., 1963; Broecker & Henderson, 1998) and for diatoms (16, 2.3 and 0.14), in particular (Peeters & Peperzak, 1990).

In a similar way, nutrient ratios, particularly N:P was close to this level when *P. micans* archived a noticeable increase in cell number. However environmental conditions (e.g. nutrient load light conditions and tidal mixing) in Southampton Water, as a highly changeable system, were continuously varying within short periods of time preventing the cells from forming frequent blooms with high biomass. This could be an explanation for the fact that phytoplankton blooms in Southampton water episodically occurred and are short-lived (less than a week) as recorded within the estuary in spring-summer 1999 from discrete analyses of water samples as well as by a continuous monitor (current study; chapter 3 and Holley & Hydes, 2002).

CHAPTER SEVEN

CHAPTER SEVEN:

7- COMPOSITION AND BIOMASS OF PHYTOPLANKTON ASSEMBLAGES USING HPLC PIGMENTS ANALYSIS

7.1 INTRODUCTION

Phytoplankton pigments can be used as quantitative biomarkers of some classes and/or species allowing an insight into the natural community composition of phytoplankton and their dynamics (Barlow et al., 1997, 1998; Breton et al., 2000; Pinckney et al., 2001). HPLC analysis has been used quantitatively to verify the use of pigments as chemotaxonomic biomarkers in lakes (Descy et al., 2000; Trees et al., 2000), oceans (Higgins & Mackey, 2000; Trees et al., 2000), estuaries and coastal waters (Brunet et al., 1996a; Pickney et al., 1998; Breton et al., 2000; Schluter et al., 2000; Trees et al., 2000; Ansotegui et al., 2001) as well as in the Antarctic environment (Rodriguez et al., 2002; Garibotti et al., 2003).

The aim of results presented in this chapter is to assess the usefulness of pigment chemotaxonomy as a tool to estimate phytoplankton composition and biomass in the temperate macrotidal estuary, Southampton Water. Results from seasonal studies conducted in Southampton water during productive months in 1999 and 2000 are presented to show the temporal and spatial distribution of phytoplankton biomass, community structure and species succession along the estuary using HPLC chemotaxonomy of specific photosynthetic and photoprotectant accessory pigments in comparison with microscopic enumeration (cell number and cell carbon).

Samples were collected temporally (through the main productive months) and spatially (along the Test estuary, the Itchen estuary and Southampton Water) in 1999 and 2000 to determine the temporal and spatial variations in phytoplankton community structure and species seasonal succession throughout the Southampton Water Estuary. For the temporal study, weekly (every 5-7 days) surface water samples were collected at the Dock-Mooring site (see figure 2.1) from April-September 1999, and during 2000 a biweekly sampling interval was undertaken (at 3 different sites) during the period from mid May to early September. For the spatial study, two one-day surveys were conducted throughout the estuary in 1999 (10th June and 22nd July) and two in 2000 (16th May and 15th August) to investigate the changes in phytoplankton composition along the Southampton Water Estuary (including 13-16 sites along the estuary).

7.1.1 PIGMENT DETECTION

Approximately 20 pigments were detected from the HPLC chromatogram, among which (in addition to Chl *a*) 7 important pigments (concentrations mostly > 0.1 mg m^{-3}) were selected; fucoxanthin (Fuc), peridinin (Peri), alloxanthin (Allo), diadinoxanthin (Diad), chlorophyll-b (Chl *b*), chlorophyll c1+c2 (Chl *c1+c2*), and chlorophyll c3 (Chl *c3*) as primary taxonomic markers of the dominant phytoplankton groups and/or species. Small traces (< 0.1 mg m^{-3}) of 19'-hexanoyloxyfucoxanthin (19-Hex), 19'butanoyloxyfucoxanthin (19-But), violaxanthin (Viol), brasinoxanthin (Bras) were also found in some samples. Representative absorbence chromatograms of some field samples are illustrated in figure 7.1. The chlorophyll breakdown products, phaeophorbides *a1* & *a2*, phaeophytin *a1* & *a2* were also detected by HPLC but not included in the following data analysis. Three other unidentified carotenoid pigments were found in the analyzed samples; one of these carotenoids was found in relatively high concentrations in some samples and may be a transformation product from the sediment. Some non-pigment compounds however are known to give similar optical responses to that given by plant pigments (R. Barlow, *pers com.).*

7.1.2 PIGMENT CONCENTRATIONS AND INTERNAL RELATIONSHIPS

I- Fluorometric and HPLC Measurements of Chl a

Comparisons of total Chl *a* concentration from acetone extracts analyzed by HPLC and fluorometrically showed good agreement between both methods (see figure 7.2) although the later was always overestimated by a value between 22%-41%. This could be due to the interference of other pigment and chlorophylls according to the method used (Trees et al., 1985, Jeffrey et al., 1997)

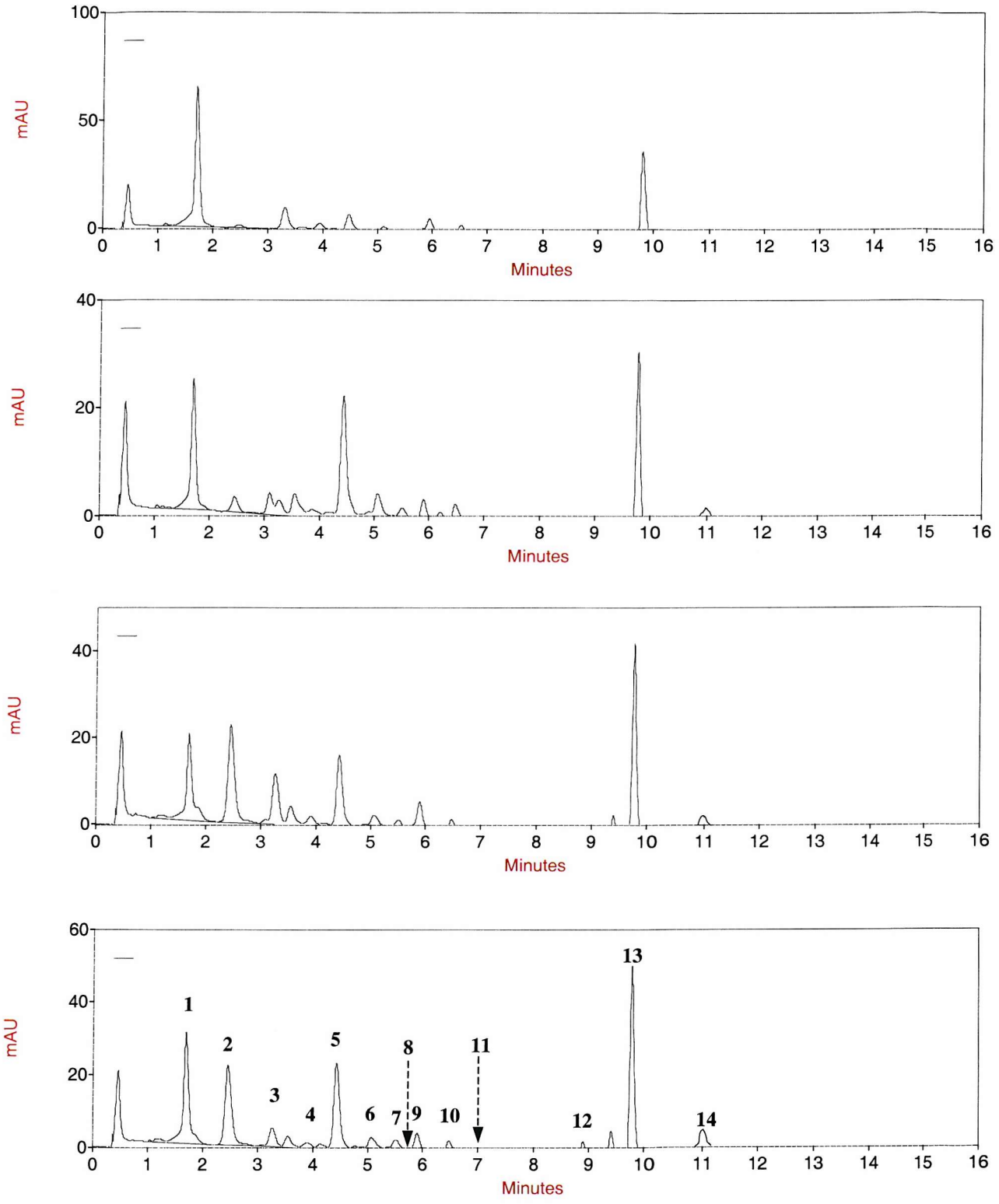


Figure 7.1. Four representative absorbence chromatograms of some surface water samples collected from Southampton Water in 1999 and 2000.

(**1** = Chl *c3*, **2** = Chl *c1+c2*, **3** = Peri, **4** = 19-But, **5** = Fuc, **6** = 19- Hex, **7** = Pras, **8** = Viol, **9** = Diad, **10** = Allo, **11** = Zea, **12** = Chl *b*, **13** = Chl *a*, **14** = β-caroten).

as the HPLC method individually separates and quantifies pigments by absorption and/or fluorescence and hence minimize pigments overlapping that could have occurred (e.g. fluorometric method). A fluorometric adaptation for the Welschmeyer method (Welschmeyer, 1994) was not available during the field work component of this research.

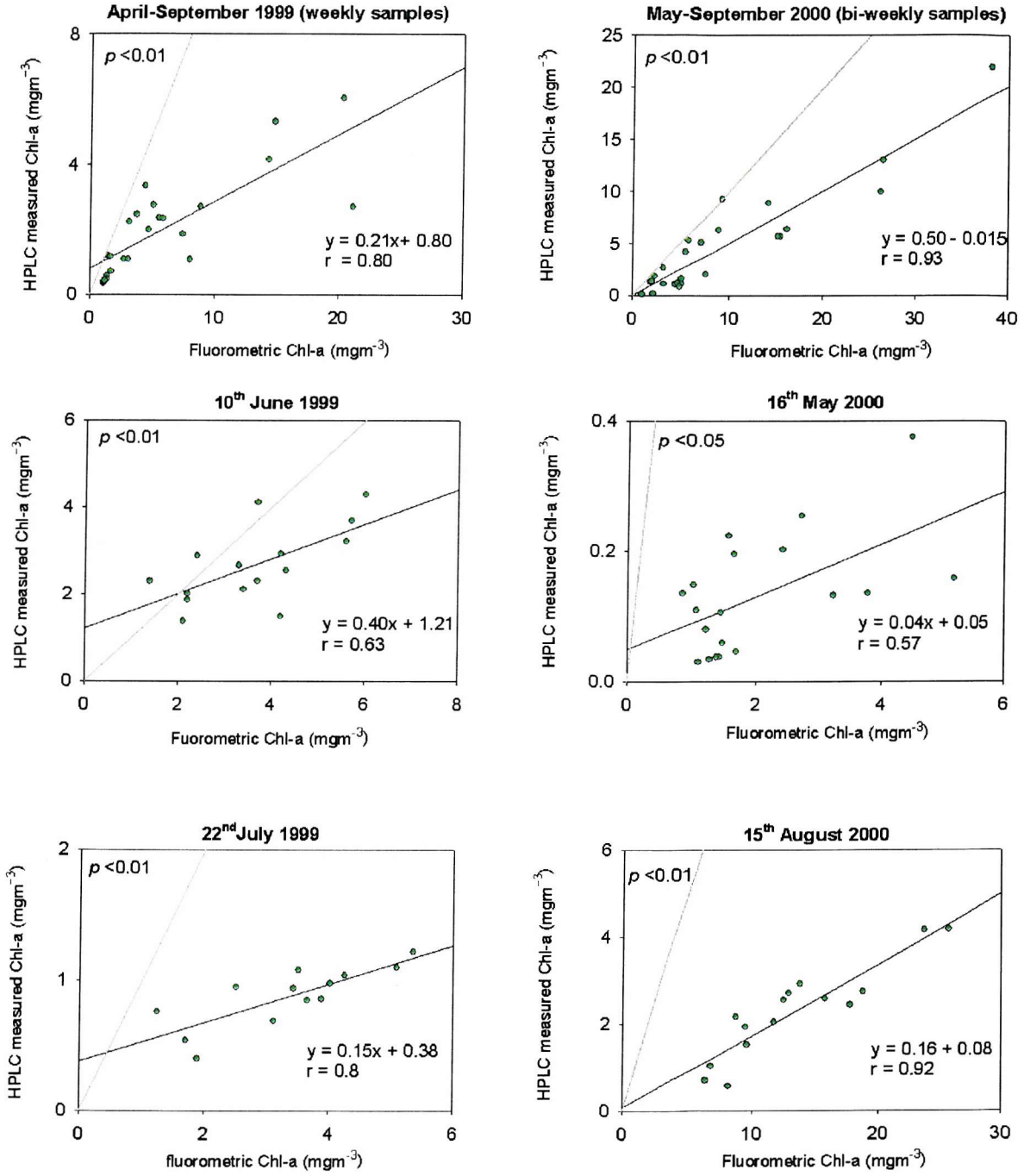


Figure 7.2. Comparisons of fluorometric and HPLC measurements of Chlorophyll-a throughout the period of study in 1999 and 2000. Regression equations, correlation coefficient (r) and significance (p) are also shown.

II- Total Chl a and Total Accessory Pigments relationship

Total Chl *a* measured by the HPLC method correlated significantly ($p< 0.01$) to the total accessory pigments (total carotenoids, Chl *c* and Chl *b*) in most samples (see figure 7.3) during the period of study (temporal data) and at different sites (spatial data) with a mean value of correlation coefficient (r) of 0.86, indicating that Chl *a* concentrations are related to the total amount of accessory pigments and can be used as internal comparison of HPLC measurements of other pigments (Trees et al., 2000; Poulton, 2002). The gradient of the regression line (figure 7.3) ranged between 0.23-0.89, which was, for some samples, less than the value estimated by Trees et al. (2000).

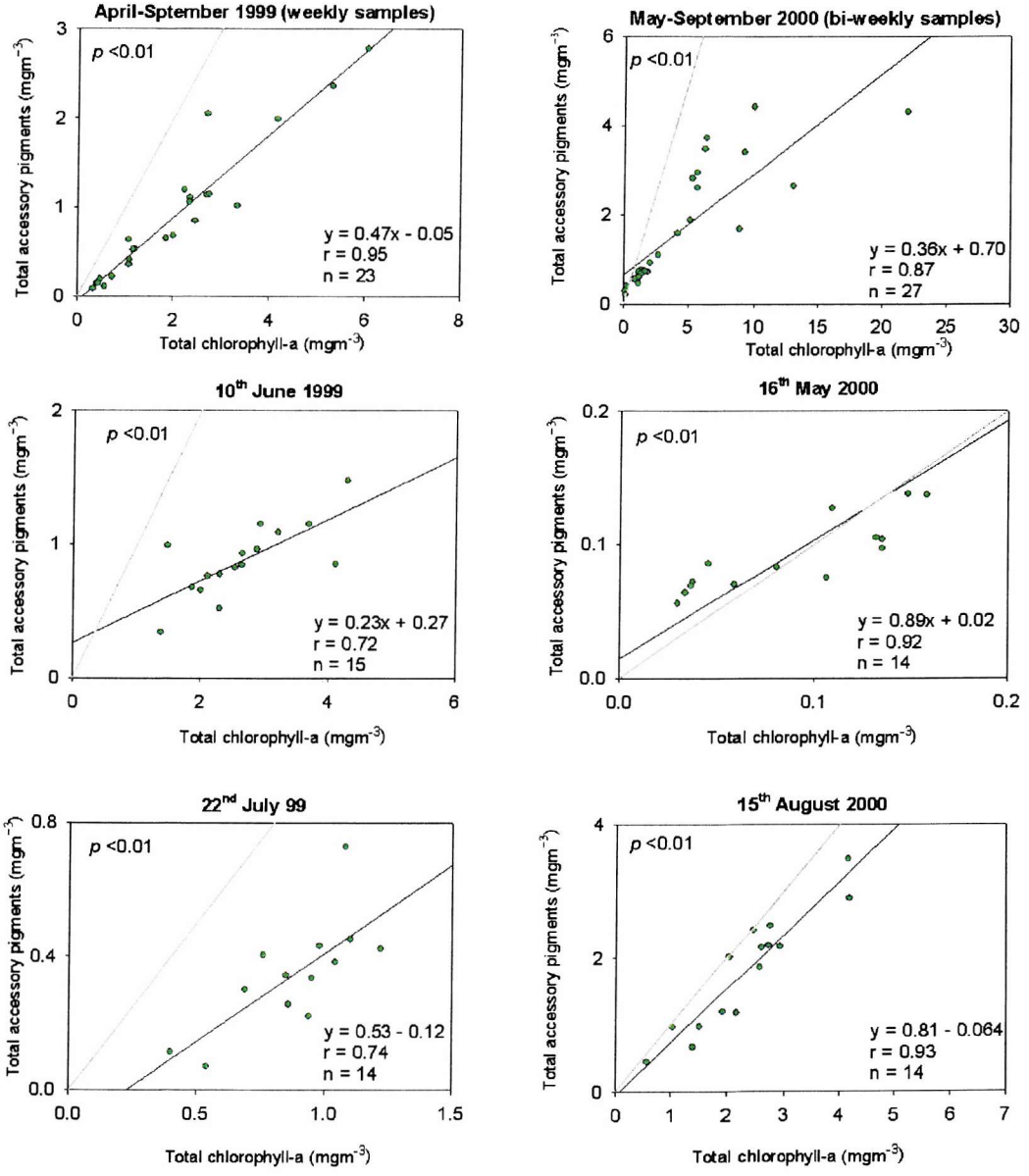


Figure 7.3. Comparisons of total Chlorophyll-a (mg m^{-3}) and total accessory pigments (mg m^{-3}) throughout the period of study in 1999 and 2000. Regression equations, correlation coefficient (r) and significance (*p*) are also shown.

7.2 TEMPORAL CHANGES IN PHYTOPLANKTON COMMUNITY COMPOSITION AND PIGMENT SIGNATURES IN SOUTHAMPTON WATER

7.2.1 TEMPORAL CHANGES FROM APRIL-SEPTEMBER 1999

HPLC pigment analysis was used to assess the phytoplankton pigments in surface water samples collected from the Dock-Mooring site in 1999. A consistent pattern of these pigments as specific species biomarkers was recorded during the sampling period (April-September 1999).

A significant correlation ($r = 0.85$, $n = 23$, $p < 0.01$) was found (figure 7.4) between Chl *a* (mg m^{-3}) estimated by the HPLC technique and total phytoplankton biomass (mg C m^{-3}), estimated from cell counts, in these samples. Maximum peaks in Chl *a* (ranged between ~4-6 mg m^{-3}) was recorded during the spring diatom blooms (19th May and 3rd June) and the summer dinoflagellate bloom (mainly *Scrippsiella trochoidea*) on 28th July.

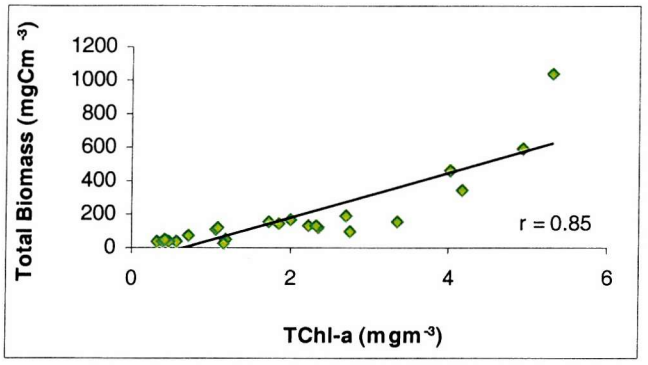


Figure 7.4. Plot of HPLC measured chlorophyll-a (mg m^{-3}) versus total phytoplankton biomass (mg C m^{-3}) of surface water samples collected from the Dock-Mooring site from April-September 1999.

During the spring diatom bloom, Chl *a* varied according to the phytoplankton species composition present (see figure 7.5) with a value of < 2 mg m^{-3} when the relatively small-celled diatom *Skeletonema costatum* was numerically abundant and increased to a value of ~4 mg m^{-3} and ~5.6 mg m^{-3} when the chain forming diatoms, *Guinardia delicatula* and *Thalassiosira rotula*, respectively were dominant. A Chl *a* peak was also detected in July (1st July & 28th July) when dinoflagellates (mainly *S. trochoidea*) were abundant.

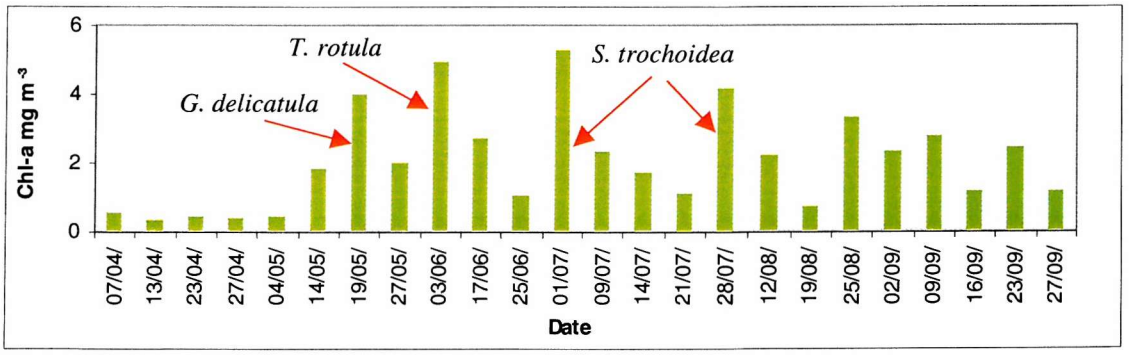


Figure 7.5. Seasonal variation in HPLC measured Chl *a* in surface waters at the Dock-Mooring site from April-September 1999.

Seasonal distribution of 7 important pigments detected during the period of study is presented in figure 7.6 and changes in the ratio of 5 biomarker pigments to Chl *a* in relation to the total biomass of the relevant phytoplankton group are presented in figure 7.7. Fuc showed large seasonal variation with high concentrations (up to 1.15 mg m^{-3}) detected in spring whenever diatoms were exclusively dominant with a strong correlation ($r = 0.74$, $p < 0.01$) between both variables (see table 7.1). Higher concentrations of Fuc (1.15, 0.9 & 1.15 mg m^{-3}) were measured (19th May, 3rd June and 28th July) on sampling dates when relatively larger diatoms, *Guinardia delicatula* (201 mg m^{-3}), *Thalassiosira rotula* (260 mg m^{-3}) and *Odontella sinensis* (65 mg m^{-3}) were very dominant. Similar finding was reflected in the Fuc/ Chl *a* ratio (see figure 7.7) with higher values (0.2-0.3) measured in May when diatoms were most abundant. The value of Fuc/ Chl *a* ratio was relatively high during the first diatom bloom (mainly *Guinardia delicatula*) compared to the ratio estimated when the centric diatom *T. rotula* dominated the diatom community. Chl *c1+c2* occurred in lower concentrations (0-0.3 mg m^{-3}) compared to Fuc, but showed a similar seasonal distribution (figure 7.6) as well as the ratio to the Chl *a* (not shown).

Figure (7.6) shows a temporary very high increase in chlorophyll *b* concentration (1.14 mg m^{-3}) as well as the Chl *b*/Chl *a* ratio (> 0.2) on the day (3rd June) of the maximum peak of the flagellate *Eutreptiella marina* (250 cells ml^{-1}, 97 mg C m^{-3}). Although, the flagellate species may have been underestimated in some field samples (using light microscopy), due to their smaller-sized cells, a strong correlation was found between the total biomass of the *E. marina* and Chl *b* concentration (see table 7.1).

Table 7.1. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered at the Dock-Mooring from April to September 1999.

Pigment biomarker	Phytoplankton group	Linear regression equation (mg C/mg pigment)	r	n	*p*
Fucoxanthin	Bacillariophyceae	Y= 0.003x + 0.19	0.74	23	*<0.01*
Peridinin	Dinophyceae	Y= 0.002x + 0.01	0.98	23	*<0.01*
Chl *b*	Eutreptiella marina	Y= 0.01x + 0.093	0.62	23	*<0.01*
Alloxanthin	Cryptomonas sp.	Y= 0.03x + 0.01	0.76	23	*<0.01*
Diadinoxanthin	Bacillariophyceae	Y= 0.0003x – 0.03	0.46	23	*<0.05*
	Dinophyceae	Y= 0.0003x – 0.03	0.70	23	*<0.01*

Seasonal variation of Peri at the Dock-Mooring site from April-September 1999 is shown in figure 7.6. Highest concentrations in Peri were measured during the summer dinoflagellate bloom, mainly *Scrippsiella trochoidea* (58-99 % of the total dinoflagellate carbon) and *Prorocentrum micans* (1- 37

% of the total dinoflagellate biomass) with a very strong correlation between both variables (see table 7.1).

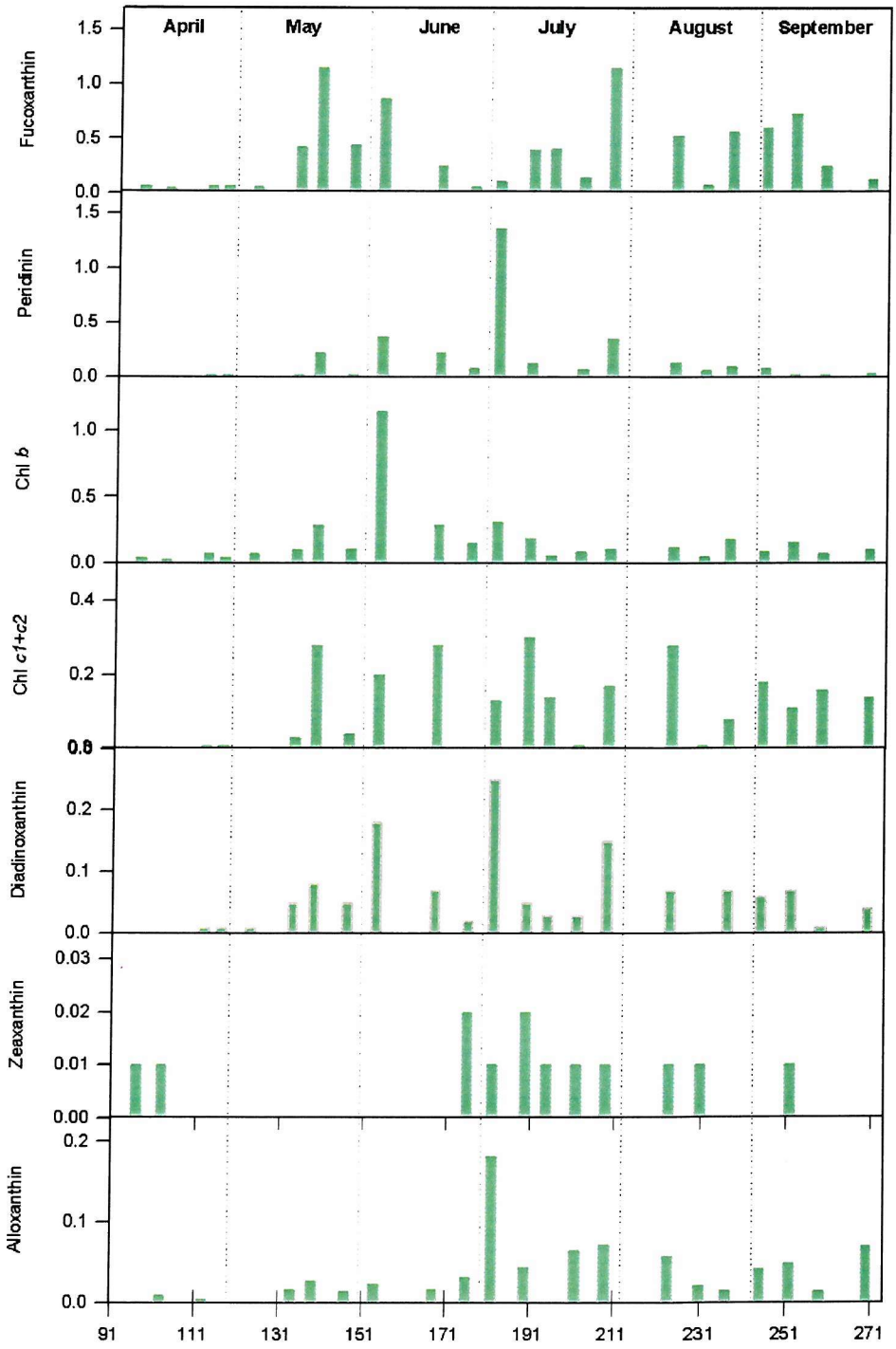


Figure 7.6. Seasonal changes in concentration (mg m^{-3}) of some specific pigments in surface waters at the Dock-Mooring from April-September 1999.

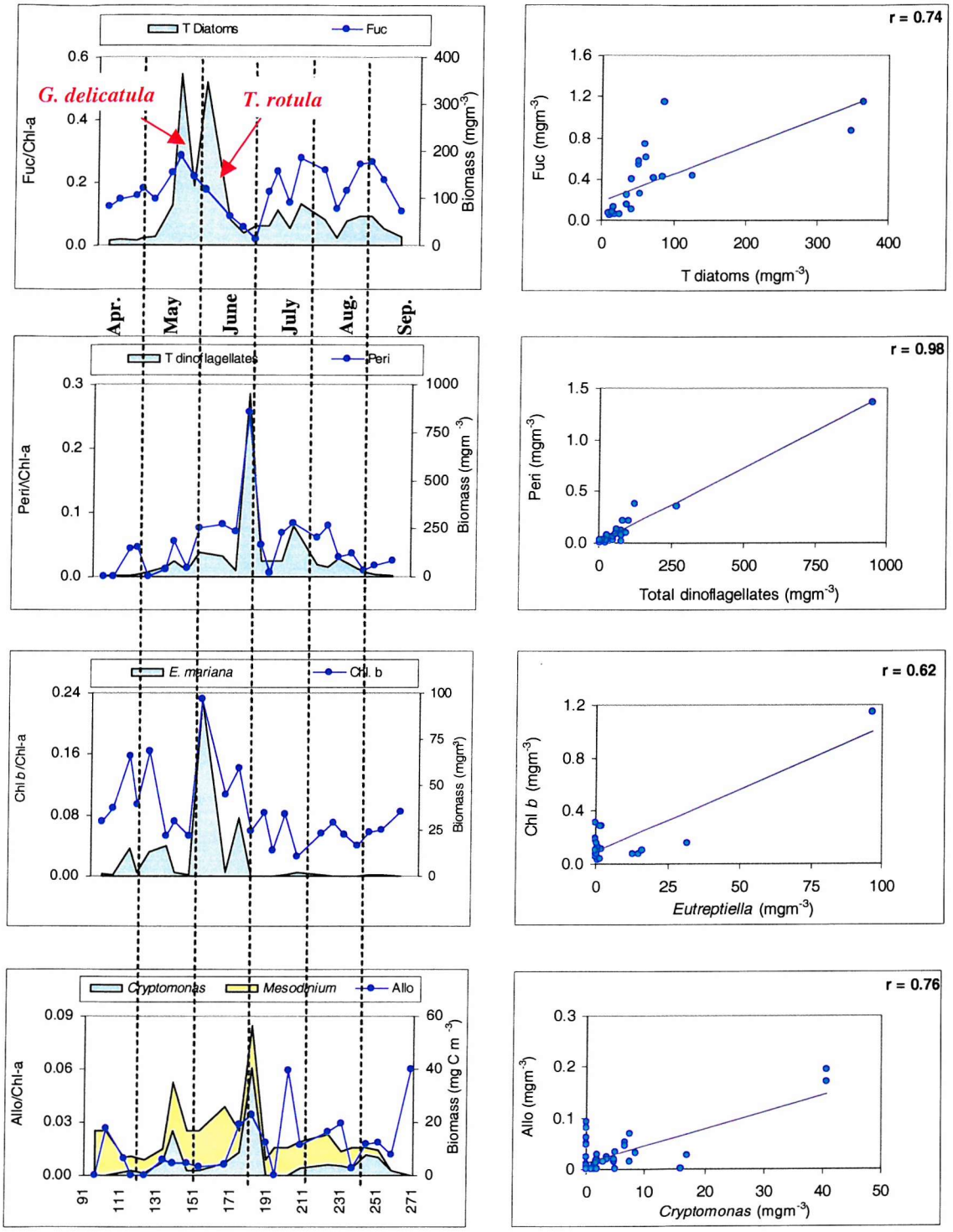


Figure 7.7. Seasonal changes in the ratio of some specific pigments (fucoxanthin, diadinoxanthin, peridinin, Chl *b*, alloxanthin) to Chl *a* in relation to the biomass of the relevant phytoplankton group and/or species at the Dock-Mooring from April-September 1999.

Maximum Peri/ Chl *a* ratio (0.28) was recorded in early July during the bloom of *Scrippsiella trochoidea* (figure 7.7) and the maximum peak in Peri concentration coincided with the maximum carbon biomass of *Scrippsiella trochoidea.*

Small traces (<0.02 mg m^{-3}) of Zea (figure 7.6) were detected on some sampling dates, although no Cyanophyceae were detected by light microscopy. Similarly, no *Phaeocystis* was seen by light microscopy, while it is suspected the presence of this flagellate and/or other small flagellate at some sites along the estuary (see section 5.4.3 chapter 5), particularly in May when the chromatogram peak of Chl *c3* started to increase (figure not included as Chl *c3* was not fully quantified as mg m^{-3}). *Phaeocystis* sp. is known to sometimes occur in Southampton Water at this time of the year (Iriarte, 1991) and was seen in surface water samples collected in 2000.

Results of plotting concentrations of some specific phytoplankton biomarkers (Fuc, Chl *c1+c2*, Peri, Allo, Chl *b*, Diad and Zea), each as a dependent variable with Chl *a* as an independent variable (figures not presented) are summarized in table 7.2. Strong correlations were recorded between Fuc (r = 0.71, p <0.01) and Chl *a* throughout the period of study (April-September 1999) indicating that diatoms were highly contributed to the total phytoplankton community with relatively much higher contribution (r = 0.91, $p < 0.01$) during spring and early summer. Peri also showed clear covariation to Chl *a* (r = 0.72) during the sampling period with strong correlation (r = 0.83, $p < 0.01$) in summer when dinoflagellates were exclusively abundant. Similar findings could be mirrored by the strong correlation (r =0.93, $p<0.01$) estimated between Diad, the dark induced pigment present in most Bacillariophyceae and Dinophyceae, and Chl *a* (table 7.2), indicating that both classes (diatoms and dinoflagellates) almost dominated the phytoplankton community during the sampling period.

Pigment Biomarker	r	*p*
Fucoxanthin	**0.71**	*p <0.01*
Chl C1+c2	**0.61**	*p <0.01*
Peridinin	**0.72**	*p <0.01*
Alloxanthin	**0.60**	*p <0.01*
Chl *b*	**0.65**	*p <0.01*
Diadinoxanthin	**0.93**	*p <0.01*
Zeaxanthin	**0.18**	*p = 0.43*
n	**23**	

Table 7.2. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable); table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, *p* are italic.

A significant correlation ($p < 0.01$) was recorded between Chl *b* and Chl *a* and indicates that Chlorophyceae significantly contributed to phytoplankton community recorded during the sampling period. Although, with the exception of *E. marina* (figure 7.6) and few cells of *Scenedesmus* sp, no other green algae were identified at the Dock-Mooring site during the sampling period. *E. marina*

appeared in low concentrations (<35 cells ml^{-1}) on all sampling dates with a maximum peak (~250 cells ml^{-1} & ~100 mg m^{-3}) in early June (3rd June).

Microscopic analysis did not detect any Cyanophyceae at the Dock-Mooring site during the sampling period; this could be due to their generally smaller-sized cells. However, the weak correlation between Zea and total Chl *a* (see table 7.2), indicating that blue-greens were not significantly contributing to the phytoplankton community recorded at the Dock-Mooring site in spring/summer 1999.

7.2.2 TEMPORAL CHANGES FROM MAY TO AUGUST 2000

During the productive period (16thMay – 29thAugust) in year 2000 the HPLC technique was used for pigment analysis of surface water samples collected at 3 sites in Southampton Water. HPLC measured Chl *a*, as a universal indicator of phytoplankton biomass, showed a very similar spatial and temporal variation to total phytoplankton biomass, estimated as cell carbon, (figure 7.8) with a significant correlation (figure 7.9, r = 0.76, n = 27, p <0.01). A maximum concentration of Chl-a was measured during the first week of July in estuarine waters [8.9 & 21.9 mg m^{-3} at the upper (SG6) and mid (NW Netley) estuary, respectively] during a bloom of the ciliate *Mesodinium rubrum* (figure 7.8 & 7.11). At the coastal site (Calshot, CA) a diatom bloom (mainly, *Guinardia delicatula* and *Thalassiosira rotula)* was recorded in early June with Chl *a* value of 9.3 mg m^{-3}.

HPLC pigment data indicated that Fuc and Chl *c1+c2* were the most abundant taxonomic pigments, particularly in spring and early summer (figure 7.10), indicating that diatoms dominated the phytoplankton assemblages during this time of the year. Fuc showed temporal variations during the sampling period (figure 7.10) with high peaks recorded in early June with values of 0.85, 1.15 and 2.76 mg m^{-3} in the upper estuary, middle estuary and coastal water, respectively (figure 7.10 & 7.11) at the time of the diatom bloom of *Guinardia delicatula* (see chapter 4).

Other relatively high peaks of Fuc (2.19, 1.77 mg m^{-3}) were measured at the end of July particularly at the upper and middle estuary stations (figure 7.10). These peaks coincided with a bloom of the small pennate diatom *Nitzschia closterium* A strong correlation (table 7.3) was shown between Fuc concentrations and Chl *a* in the coastal water (r = 0.97, p <0.01) but weaker correlations were shown between both variables at the upper (r = 0.46, p = 0.22) and mid estuary (r = 0.29, p = 0.46); this was, partially, due to the dominance of the autotrophic ciliate *Mesodinium rubrum* and greater presence of dinoflagellates at these two sites.

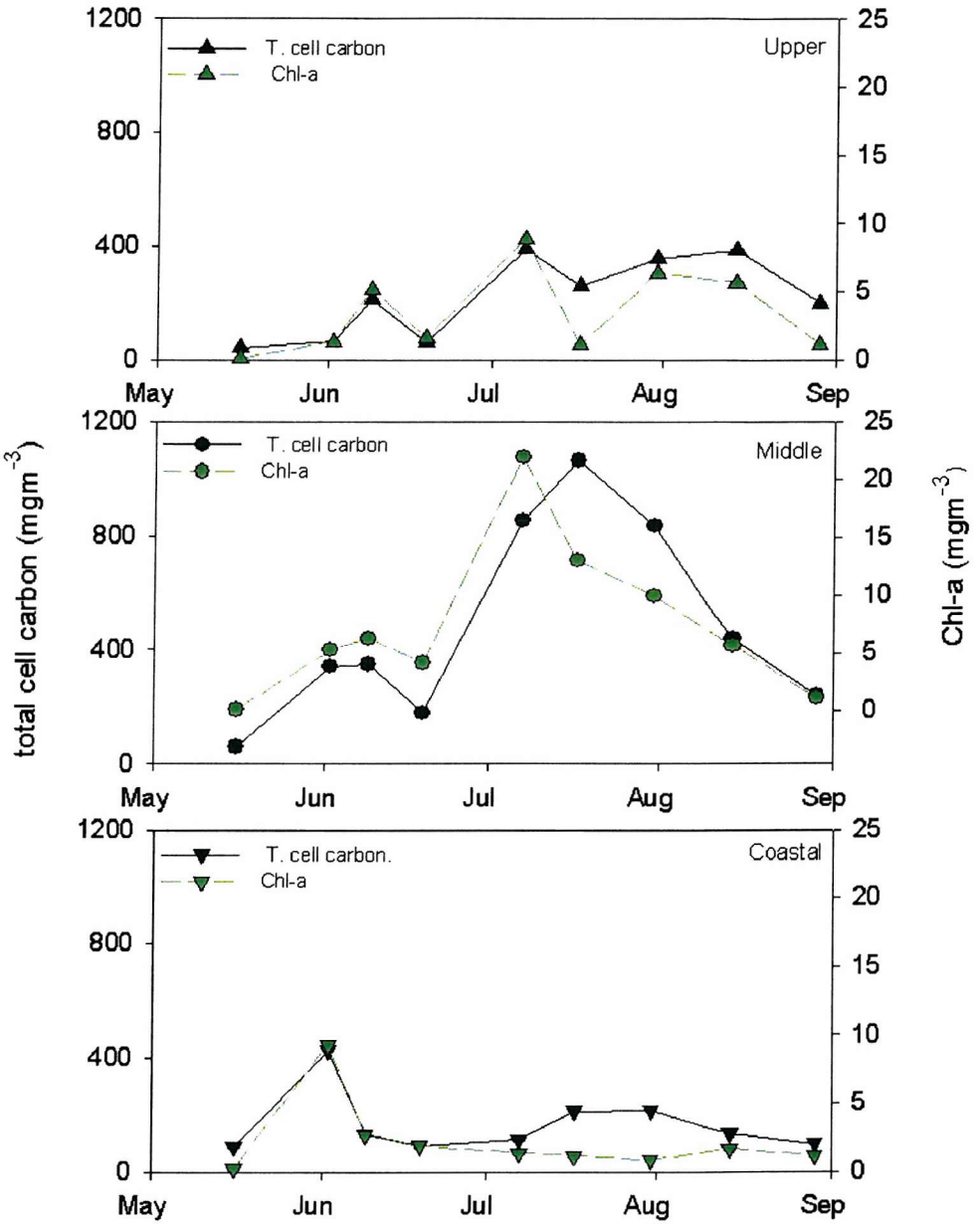


Figure 7.8. Distribution of HPLC derived Chl *a* concentration in relation to total phytoplankton biomass in water samples collected from upper estuary, mid estuary and coastal waters from May to August 2000.

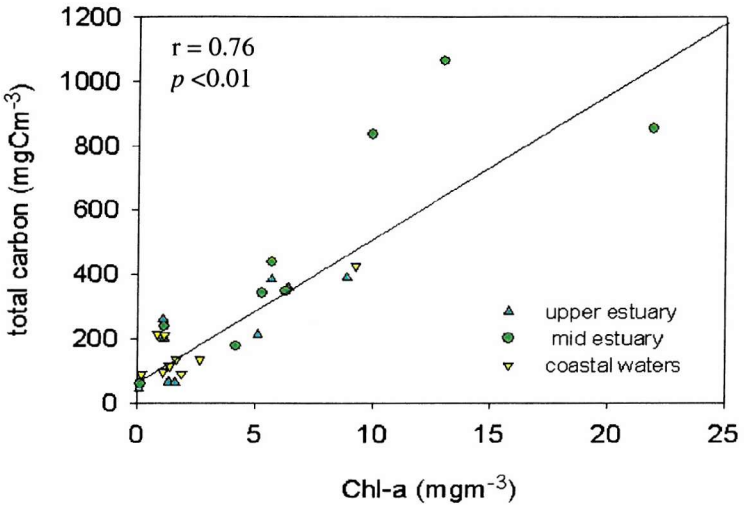


Figure 7.9. Plot of HPLC measured chlorophyll-a (mg m^{-3}) versus total phytoplankton biomass (mg C m^{-3}). Data for surface water samples collected from upper estuary, mid estuary and coastal waters are grouped for the regression analysis

Concentrations of Chl *c1+c2* occurred in lower concentrations than Fuc, but showed a similar spatial and temporal variation to that of Fuc (figure 7.10) and a similar high correlation to Chl *a* at Calshot (coastal waters) (table 7.3) but no significant correlation at NW Netley and SG6. Highest concentrations of Chl *c1+c2* (up to 0.9 mg m^{-3}) were measured during the spring bloom of *Guinardia delicatula.* At all sampling sites, ratios of Fuc and Chl *c1+c2* to Chl *a* increased in spring and early summer (figure 7.12) during the bloom period and decreased over the summer period, indicating that the relative contribution of diatoms to the total phytoplankton biomass was highest during spring. A further increase in the Fuc/ Chl *a* ratio was recorded on 14th August at all sites and coincided with the numerical increase in some small diatoms (*Thalassiosira rotula;* 20–30 μm and *Chaetoceros* spp.) with a relatively higher value (figure 7.12) at the coastal site, Calshot (CA), compared to the other two estuarine sites (upper, SG6 and mid, NWN estuary). This indicates that the relative contribution of diatoms to the total biomass was higher in the coastal waters at this time of the study period.

Alloxanthin (Allo) was detected in high concentration in July (figure 7.10) and was coincident with the extensive growth of the ciliate *Mesodinium rubrum* during this time of the year (figure 7.10 & 7.11). A maximum concentration of Allo (3.11 mg m^{-3}) was measured in the middle estuary during the first week of July. There was a strong correlation between alloxanthin concentration and Chl *a*, particularly in the upper (r = 0.84, p <0.01) and middle (r = 0.79, p <0.05) estuary (table 7.3), indicating that *Mesodinium rubrum* dominated the phytoplankton community at these sites. Higher Allo/ Chl *a* ratios were exclusively found in July at all sites at the time of the peak growth of the ciliate *Mesodinium rubrum* (figure 7.12).

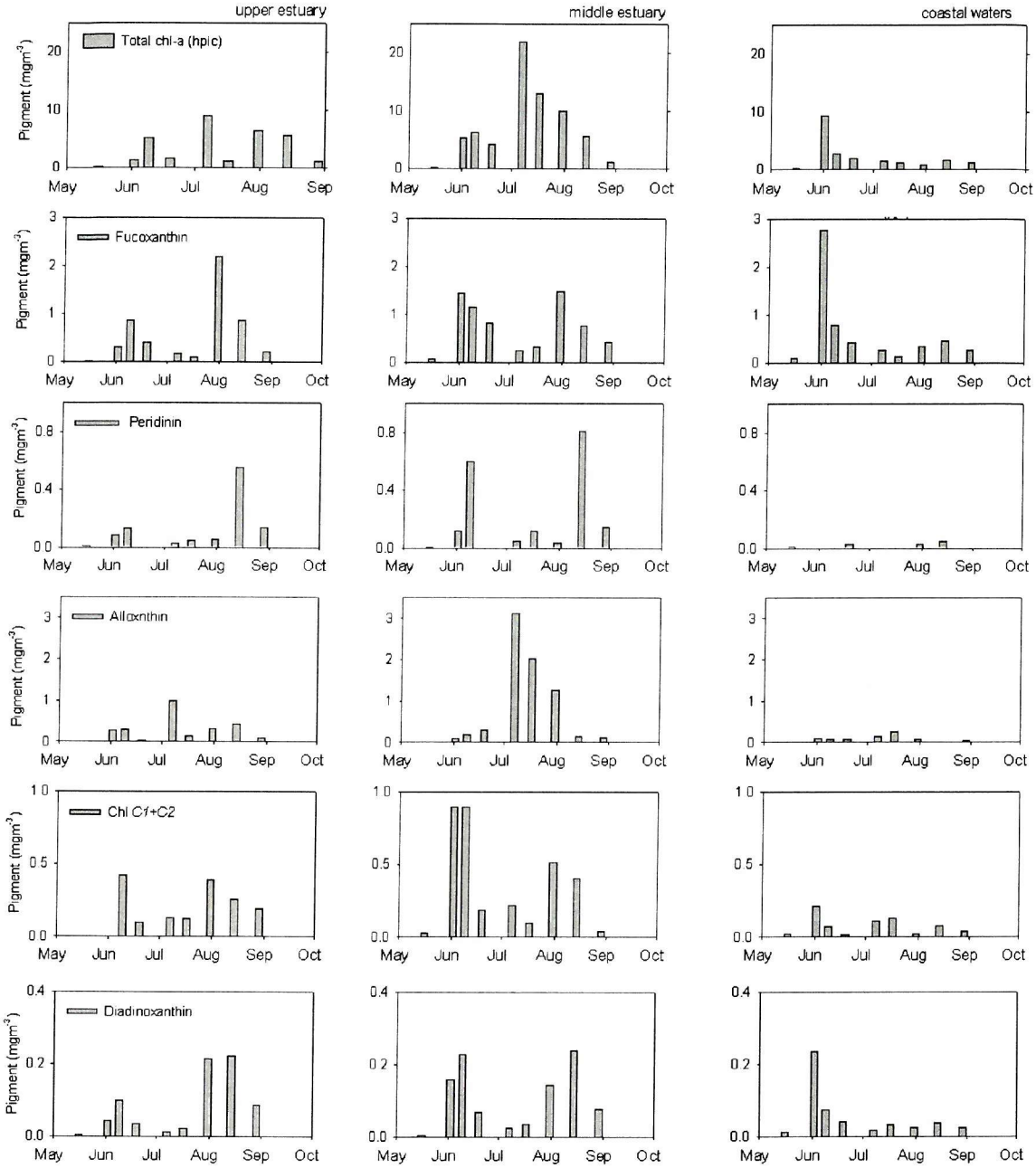


Figure 7.10. Distribution of Chl *a* concentration and 5 biomarker pigments (Fuc, Peri, Allo, Chl *c1*+c2, and Diad) as detected in surface water samples (1m) collected from upper estuary, mid estuary and coastal waters from May to August 2000.

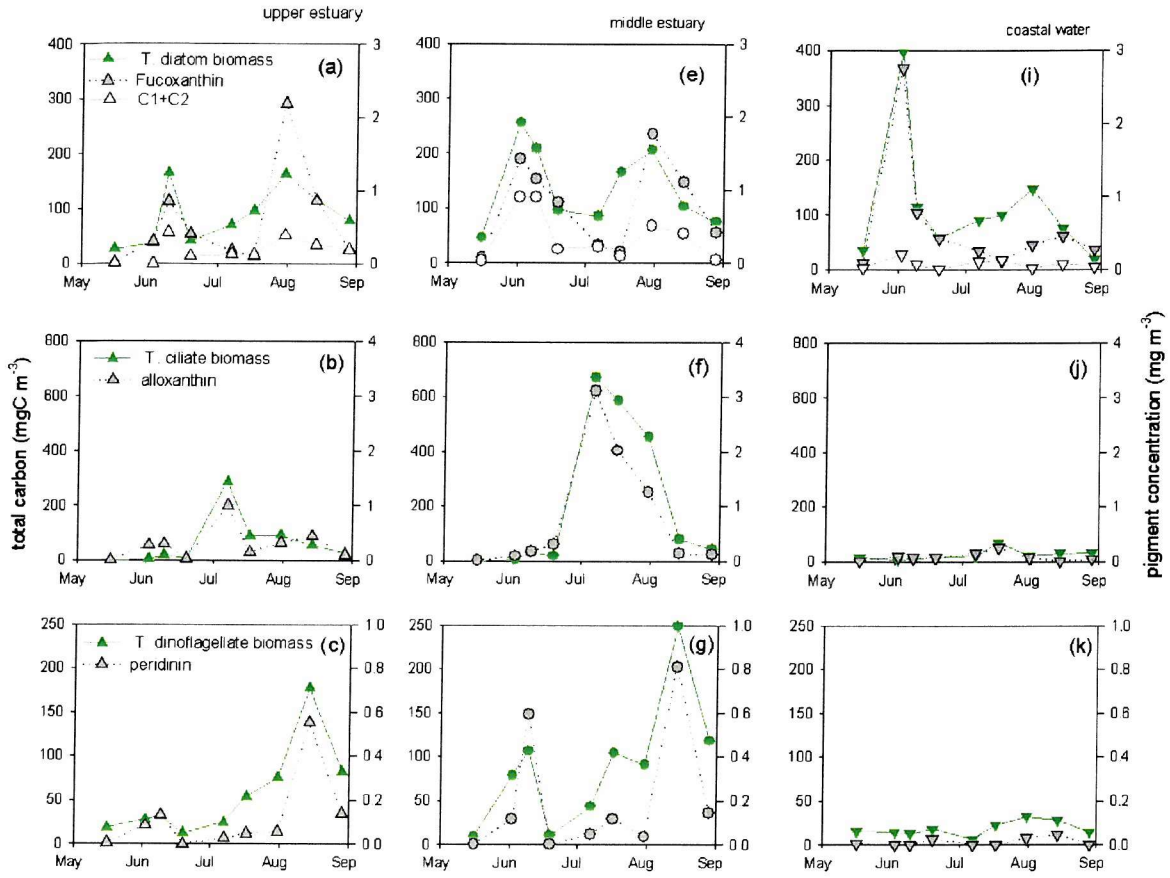


Figure 7.11. Spatial distribution of 4 biomarker pigments concentration in relation to total biomass of the relevant phytoplankton group in water samples collected from upper estuary (a, b, c), mid estuary (e, f, g)and coastal waters (i, j, k) from May to August 2000.

A relatively high ratio of this carotenoid to Chl *a* was also found earlier in spring at the upper estuarine site, when small cryptomonad-like flagellates were abundant.

Chl *c3* concentration was not quantitatively determined (as mg m^{-3}) during May–June; however its fluorescence peak increased gradually to reach a maximum level late in July, particularly in the upper and middle part of the estuary (not shown). This increase significantly coincided with the peak in growth of small flagellates ($r = 0.63$, $n = 27$, $p < 0.05$). In mid May, the Chl *c3* peak showed an increase, particularly in coastal waters coinciding with the growth of *Phaeocystis* sp., which is known to bloom in Southampton Water in some years (mostly in coastal waters) during May (Iriarte, 1991). In addition, an obvious increase in Chl *c3* was also found in late July and was coincident with the numerical increase of other small flagellates (2 – 3 μm) at the three sampling sites.

Table 7.3. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable); Table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, p are italic.

	Upper estuary		Middle estuary		Coastal water	
	r	*p*	r	*p*	r	*p*
Fucoxanthin	**0.46**	*p = 0.22*	**0.29**	*p = 0.46*	**0.97**	*p < 0.01*
Chl *c1+c2*	**0.49**	*p = 0.18*	**0.31**	*p = 0.42*	**0.71**	*p < 0.05*
Peridinin	**0.10**	*p = 0.68*	**0.19**	*p = 0.63*	**0.23**	*p = 0.55*
Alloxanthin	**0.84**	*p < 0.01*	**0.79**	*p < 0.01*	**0.06**	*p = 0.89*
Chl *b*	**0.44**	*p = 0.24*	**0.37**	*p = 0.33*	**0.16**	*p = 0.69*
Diadinoxanthin	**0.22**	*p = 0.57*	**0.01**	*p = 0.98*	**0.97**	*p < 0.01*
n	**18**		**18**		**18**	

Low concentrations of Peri were measured at all sites in spring (figure 7.10) but increased towards the end of the sampling period with highest concentrations (0.55–0.81 mg m^{-3}) found in mid August during the summer dinoflagellate bloom (figure 7.11). Peri contributed less to Chl *a* (table 7.3) throughout the period of this study with insignificant correlation between both variables at all sites. However, Peri/ Chl *a* ratio was low during spring, it increased over summer with highest ratios in mid August (figure 7.12) at the time of summer dinoflagellate bloom (figure 7.11); the ratio was much higher in the upper and mid estuary (0.23 & 0.3) in August-early September.

Chl *b* occurred in lower concentrations (maximum = 0.42 mg m^{-3}) compared to other pigments (figure 7.10), although it was significantly correlated (table 7.4) to the biomass of the flagellate *Eutreptiella marina*. This may suggest that this flagellate dominated the chlorophycean community in Southampton Water during the period of study. Chl *b* did not correlate strongly to Chl *a* (table 7.3) at the 3 sites during the period of study.

The dark induced pigment, diadinoxanthin was found with variable concentrations and ranged between 0.03 – 0.24 mg m^{-3}. The temporal and spatial variations in diadinoxanthin concentration (figure 7.10) and correlation to Chl *a* (table 7.4) were similar to those of Fuc and Chl *c1+c2*, being strongly correlated ($r = 0.77$, $n = 27$, $p < 0.01$) to the later two pigments (figure not presented).

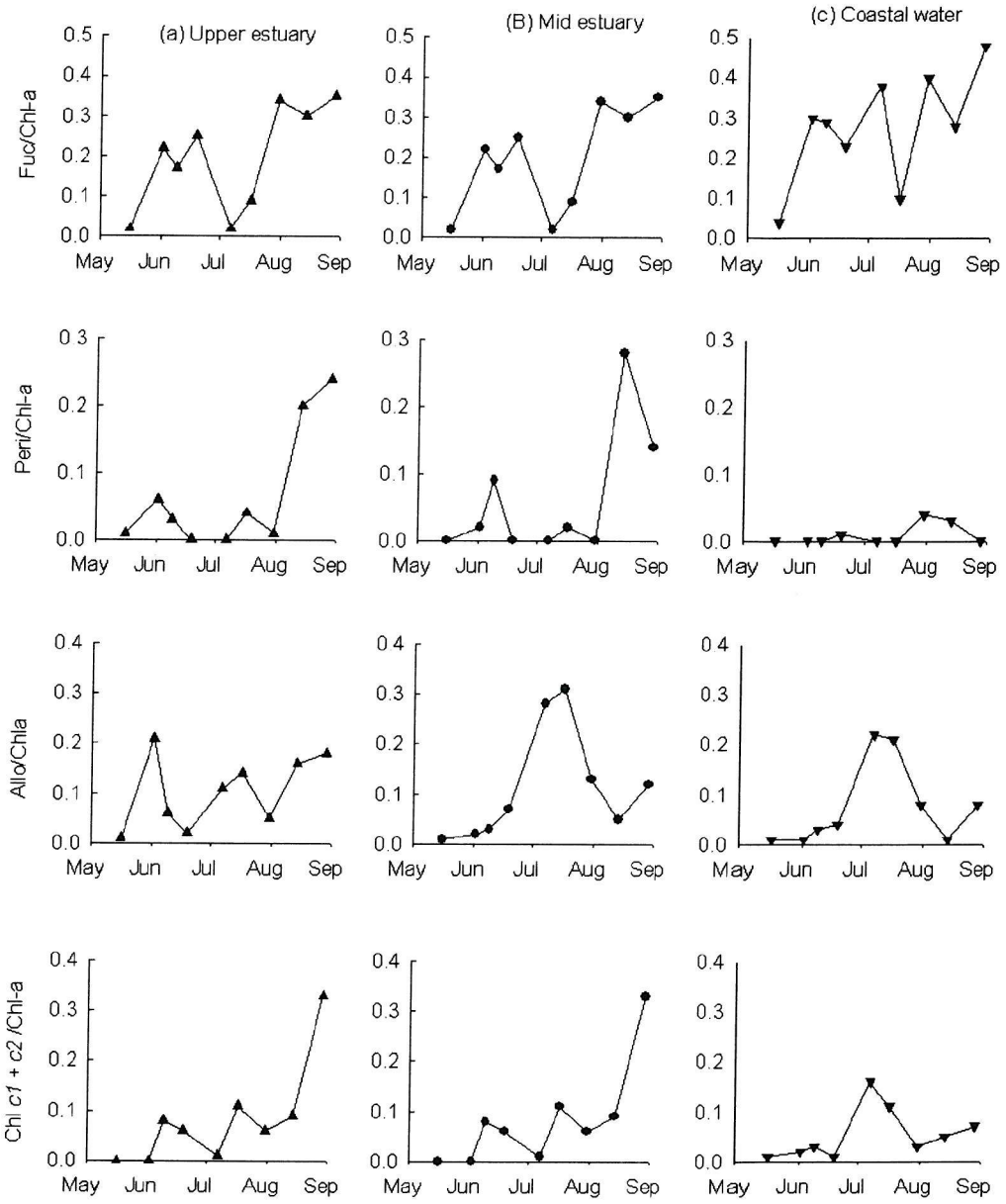


Figure 7.12. Spatial distribution of ratios of 4 specific pigments to Chl *a* as determined throughout the estuary (upper estuary, mid estuary and coastal waters) from May-August 2000.

Table 7.4. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water from May to August 2000. Data from the upper, & middle estuary and coastal water are grouped for the regression analysis.

Pigment biomarker	Phytoplankton group	Linear regression equation (mg C/mg pigment)	r	n	*p*
Fucoxanthin	*Bacillariophyceae	Y= 0.007x - 0.0147	0.77	27	<0.01
Peridinin	Dinophyceae	Y= 0.003x - 0.028	0.86	27	<0.01
Chl *b*	*Eutreptiella marina*	Y= 0.026x - 0.063	0.65	27	<0.01
Alloxanthin	*Mesodinium rubrum*	Y= 0.037x - 0.033	0.97	27	<0.01

* Data corresponding to the presence of the large-celled diatom *Odontella sinensis* are not included (see text)

7.3 SPATIAL CHANGES IN PHYTOPLANKTON COMMUNITY COMPOSITION AND PIGMENT SIGNATURES ALONG SOUTHAMPTON WATER

7.3.1 SPATIAL DISTRIBUTION IN 1999

I- 10th June Data

Chl *a* (HPLC measured) on 10th June ranged between 1.0-4.3 mg m^{-3} throughout the whole estuary with maximum concentrations in the lower Test estuary and the intermediate sites in Southampton Water. Figure 7.13 show that HPLC measured Chl *a* (mg m^{-3}) varied along the estuary with clearly similar trend to the spatial distribution of total phytoplankton biomass (mg C m^{-3}) on 10th June 1999.

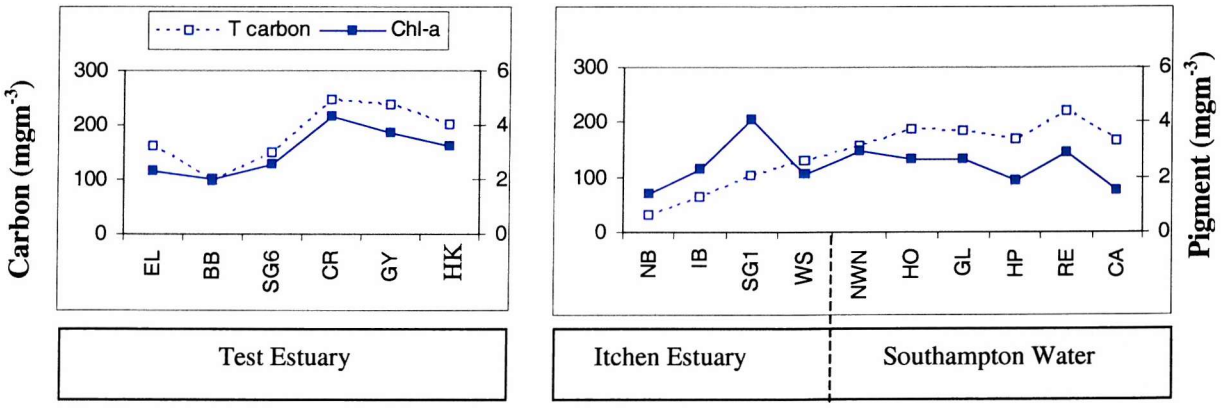


Figure 7.13. Spatial distribution of Chl *a* concentration in relation to total phytoplankton biomass through the estuary on 10th June 1999.

A significant correlation (r = 0.56, n = 16, p <0.05) was shown between both variables (figure 7.14), however carbon biomass was apparently overestimated at some sites, particularly where large-celled diatoms were abundant. This was the case at CR, GY, HK (lower Test) and WS (lower Itchen) and other sites in Southampton Water where *Odontella sinensis* and *Guinardia flaccida* were quite abundant at these sites, respectively.

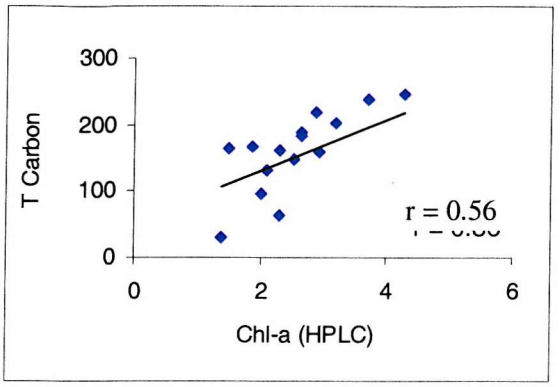


Figure 7.14. Plot of HPLC measured chlorophyll-a (mg m^{-3}) versus total phytoplankton biomass (mg C m^{-3}) of surface water samples collected from Southampton Water on 10th June 1999.

Fuc was the most abundant pigment (up to 1.0 mg m^{-3}) compared to other accessory pigments (less than 0.2 mg m^{-3}). A significant correlation (r = 0.71, n = 16, p <0.01) was shown between Fuc concentrations (mg m^{-3}) and total diatom biomass (mg C m^{-3}) but was only found (table 7.5) when the biomass of the few cells of relatively large-sized diatom *Guinardia flaccida* was not taken into account. Maximum Fuc concentration (figure 7.15) was detected at the lower sites of the Test estuary, from Cracknore (CR) as well as in the upper Itchen estuary, from Itchen Bridge (IB) to Western Shelf (WS). This was coincident with a biomass increase of the diatom *Ditylum brightwellii* (30.6 and 70.9 mg C m^{-3} at each site, respectively).

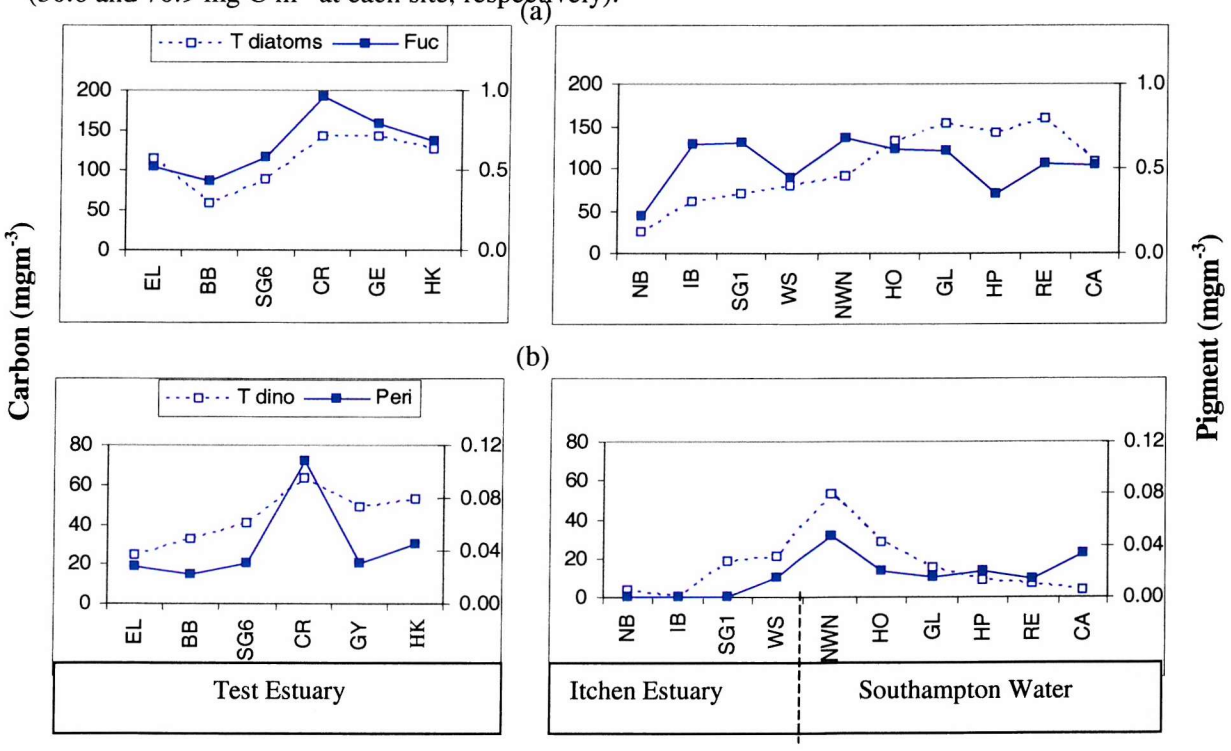


Figure 7.15. Spatial distribution of: (a) Fuc concentration in relation to total diatom biomass and (b) Peri concentration in relation to total biomass of photosynthetic dinoflagellates as detected throughout the estuary on 10th June 1999.

Peridinin was found in relatively low concentrations (maximum = 0.11 mg m^{-3}) throughout the estuary and no peridinin was detected at the two stations above the Itchen bridge (figure 7.10) although, some dinflagellates were recorded at these sites (figure 7.15). The discrepancy in the plot of both variables may have resulted from the microscopic confusion between the heterotrophic and autotrophic dinoflagellate species (e.g. *Gymnodinium* sp., which was recorded at some sites). Despite the low concentrations of peridinin, it showed a significant correspondence ($r = 0.80$, $n = 16$, $p < 0.01$) with the biomass of the photosynthetic dinoflagellates (table 7.5). Maximum Peri concentration was measured at Cracknore (CR) where the dinoflagellate *S. trochoidea* was most abundant. Similar observations were also mirrored by the high Peri/ Chl *a* ratio (figure 7.16) at this site. Peri contributed less to the phytoplankton community, particularly at coastal sites (table 7.5).

The ratio of Fuc/Chl-a was relatively constant at all sampling sites with higher values only in the lower estuary, indicating that diatoms were dominating the phytoplankton community at these sites (figure 7.15). Fuc was strongly correlated with Chl *a* (table 7.6) along the estuary ($r = 0.89$, $p<0.01$ in estuarine waters; $r = 0.66$, $p < 0.05$ in coastal waters) indicating that diatoms were exclusively contributed to the total phytoplankton community. Spatial distribution of Diad, which found in diatoms and dinoflagellates, was similar to that of fucoxanthin and peridinin and was clearly contributed to the total phytoplankton community.

Allo ranged between 0-0.083 mg m^{-3} (not shown) with highest values of 0.083 and 0.067 at Cracknore (CR) and Calshot (CA), respectively. Allo showed a relationship (not shown) with the total biomass of Cryptophyceae (*Cryptomonas* sp in the current study) and was contributed more to T Chl *a* (table 7.6) estimated in estuarine waters ($p < 0.05$), however no significance observed between both variables in coastal waters ($p = 0.8$).

Chl *b* concentration during the sampling day (10th June) ranged from 0.04-0.19 mg m^{-3} along the estuary (figure 7.17) with maximum concentration of Chl *a* as well as ratio of Chl b/ Chl *a* (figure 7.16) at the mouth of the estuary (coastal waters). A very week correlation was found between Chl *b* and biomass green algae (represented only by *E. marina* on the sampling day). This could be due to the small-sized cells of green algae that were difficult to observe by light microscopy.

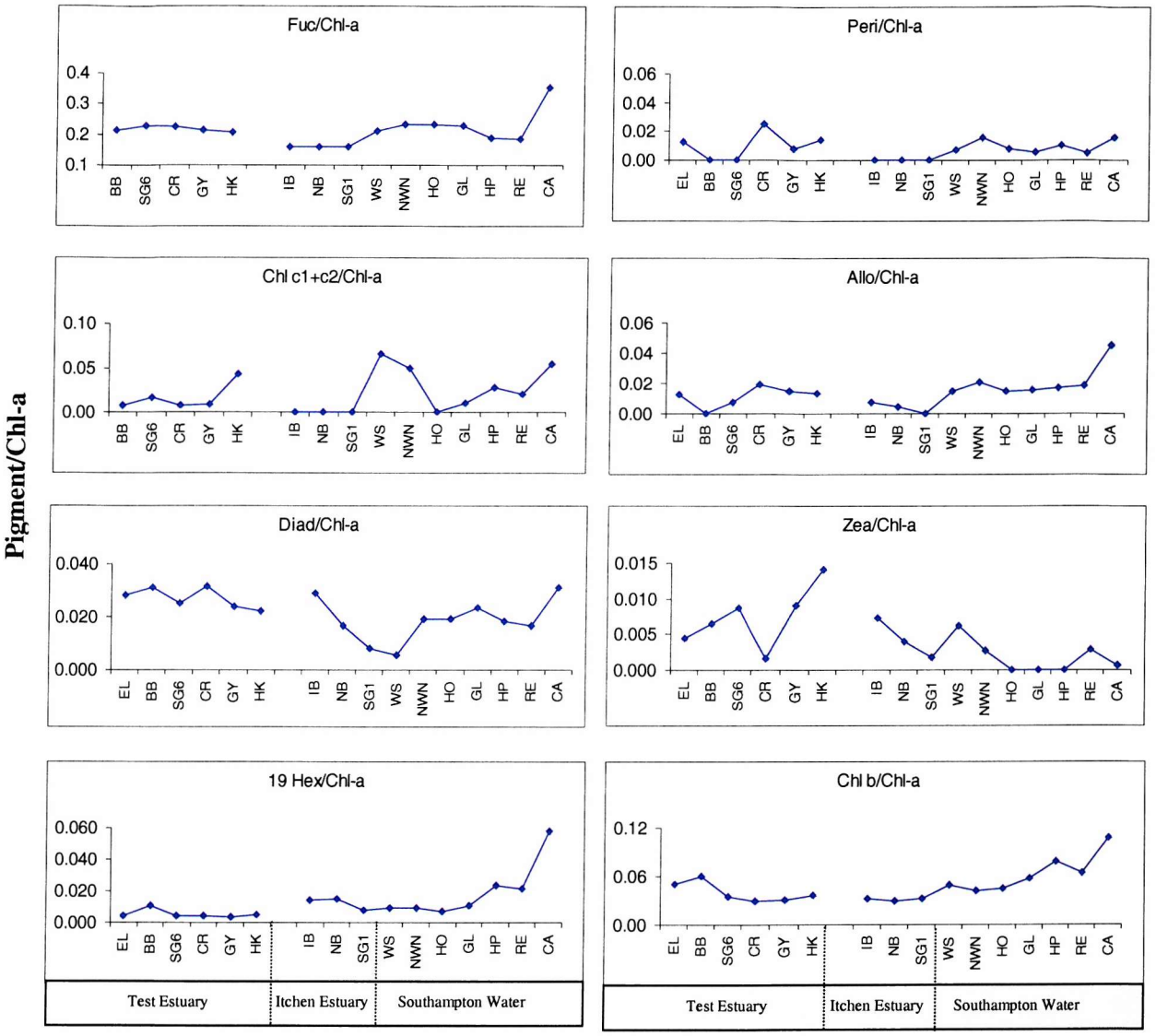


Figure 7.16. Spatial distribution of ratio of some specific pigments to Chl *a* as determined through the estuary on 10th June 1999.

Table 7.5. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water from on 10th June 1999.

Pigment biomarker	**Phytoplankton group**	**Linear regression equation (mg C/mg pigment)**	**r**	**n**	***p***
Fucoxanthin	*Bacillariophyceae	Y= 0.003x + 0.25	0.71	16	<0.01
Peridinin	Dinophyceae	Y= 0.001x + 0.001	0.80	16	<0.01
Chl *b*	*Eutreptiella marina*	Y= 0.002x + 0.12	0.12	16	= 0.61
Zeaxanthin	Cyanophyceae	Y= 0.012x – 0.01	0.36	16	= 0.18

* Carbon biomass of the relatively large-celled diatom, *Guinardia flaccida* is included.

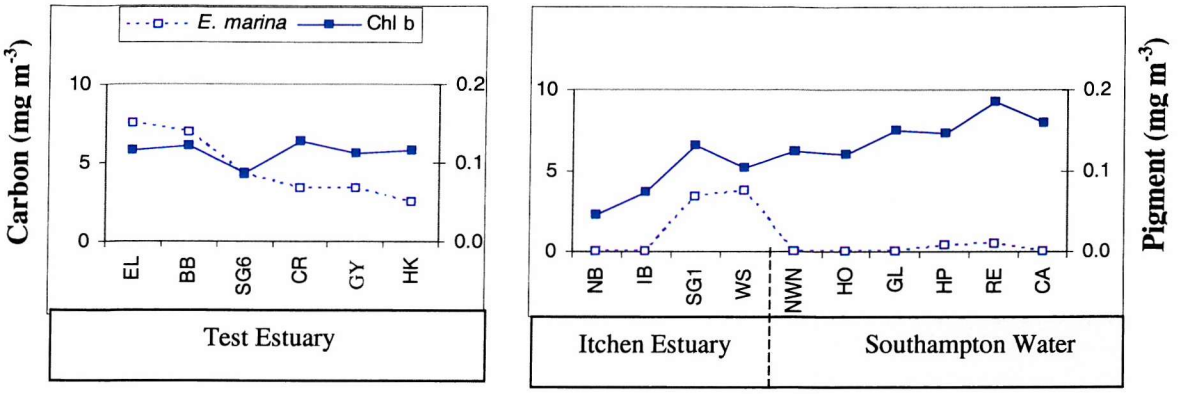


Figure 7.17. Spatial distribution of Chl *b* concentration in relation to total the biomass of *Eutreptiella marina* as detected along the estuary on 10th June 1999.

Traces of other pigments (e.g. Zea, 19 Hex) were detected in some samples (figure 7.18) with relatively low concentrations (<0.1 mg C m^{-3}). No Cyanophyceae or 19-Hex containing coccolithofords were detected in microscopic analysis. These pigments were less important as they were less contributed to the total phytoplankton community as mirrored from the correlation between

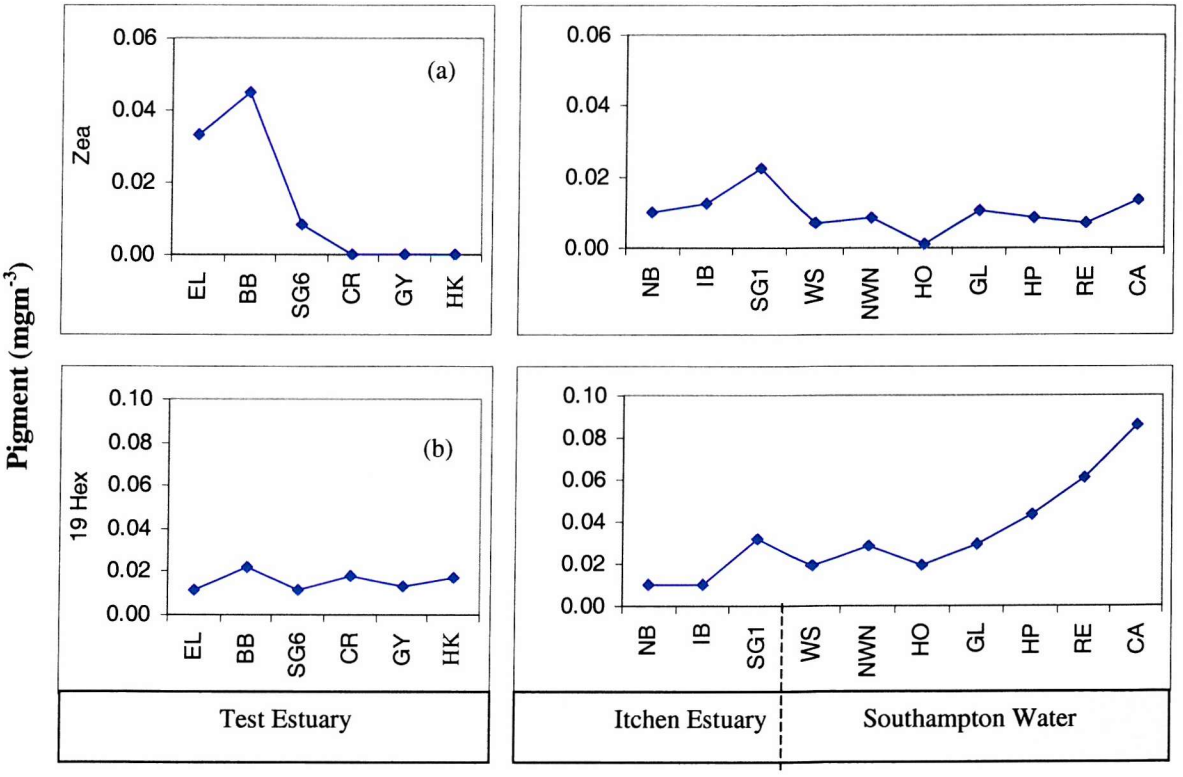


Figure 7.18. Spatial distribution of (a) zeaxanthin and (b) 19-hexanoyloxyfucoxanthin concentration as detected throughout the estuary on 10th June 1999.

Table 7.6. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable); Table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, *p* are italic.

	Estuarine waters		Coastal waters	
Pigment Biomarker	**r**	***p***	**r**	***p***
Fucoxanthin	**0.89**	$p < 0.01$	**0.66**	$p = 0.05$
Peridinin	**0.56**	$p < 0.10$	**0.10**	$p = 0.90$
Alloxanthin	**0.63**	$p < 0.06$	**0.10**	$p = 0.80$
Chl b	**0.71**	$p < 0.05$	**0.10**	$p = 0.90$
n	**20**		**12**	

II- 22nd July Data

HPLC measured Chl *a* along the estuary on 22nd July varied from 0.4-1.22 mg m^{-3} (figure 7.19) with maximum concentrations (1.22 mg m^{-3}) in the upper Itchen estuary (IB site). Figure 7.19 shows that the spatial distribution of Chl *a* (mg m^{-3}) along the estuary was clearly similar to that of the total photosynthetic phytoplankton biomass (mg C m^{-3}). A good agreement (r = 0.75, n = 13, $p < 0.01$) was found between both variables (figure 7.20). Phytoplankton biomass was apparently overestimated when the large-celled diatoms (*Odontella sinensis & Rhizosolenia styliformis*) are abundant however it was underestimated compared to Chl *a* biomass, in some samples, particularly towards the mouth of the estuary. This could be a result of underestimating some of the relatively small-celled diatoms, for example, *Skeletonema costatum* that was numerically dominant at these sites (see chapter 4).

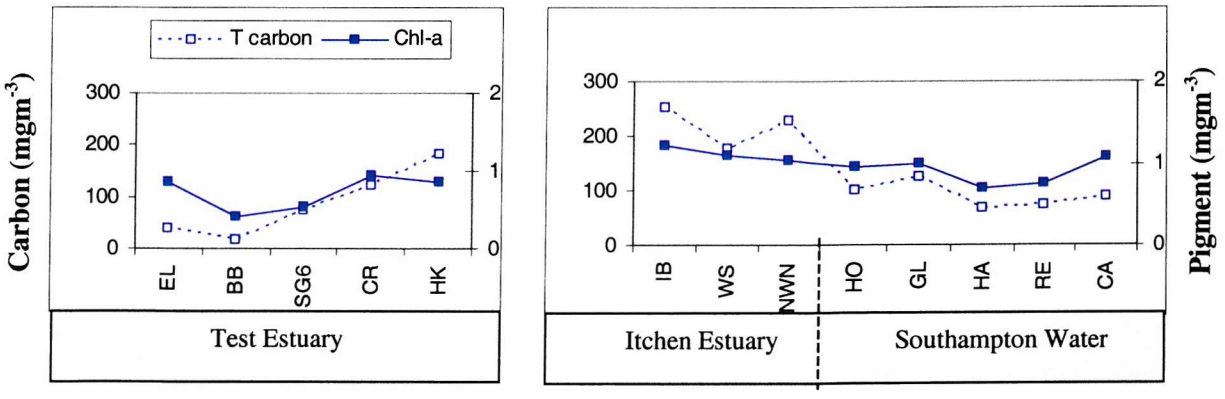


Figure 7.19. Spatial distribution of Chl *a* concentration in relation to total phytoplankton biomass throughout the estuary on 22nd July 1999.

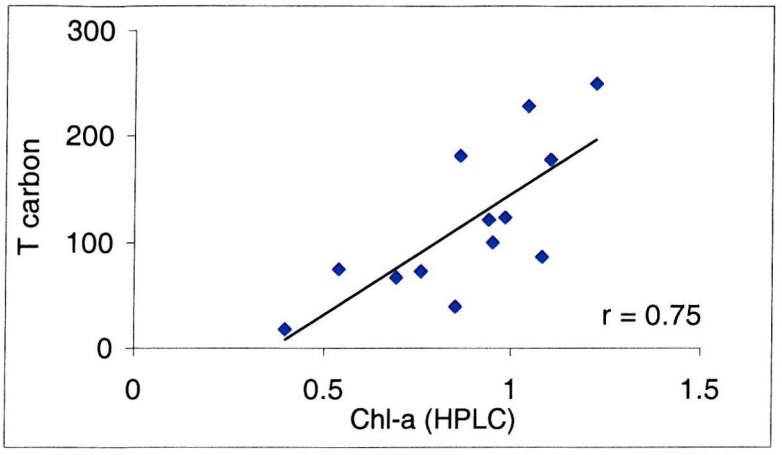


Figure 7.20. Plot of HPLC measured chlorophyll-a (mg m^{-3}) versus total phytoplankton biomass (mg C m^{-3}) of surface water samples collected from Southampton Water on 22nd July 1999.

Fuc (0.06-0.16 mg m^{-3}) and Peri (up to 0.13 mg m^{-3}) were the most abundant pigments at most sites. Peri was most abundant in estuarine and intermediate sites however Fuc dominated the pigment content at coastal sites (figure 7.21). A significant correlation ($r = 0.80$, $n = 13$, $p < 0.01$) was found between fucoxanthin concentrations (mg m^{-3}) and total diatom biomass (mg C m^{-3}) only when the biomass of the relatively large-sized diatom *Odontella sinensis & Rhizosolenia styliformis* were not included (table 7.7). Maximum fucoxanthin levels (0.12, 0.14 and 0.16 mg m^{-3}) were measured (figure 7.21) in the lower estuary (Reach, Green land and Calshot, respectively). This was coincident with the numerical increase in the diatom *Skeletonema costatum* (27.5, 34.5 and 64.7 cells ml^{-1} at each site, respectively) as well as the biomass increase in the diatom *Rhizosolenia styliformis.*

Peri concentrations ranged between 0 and 0.13 mg m^{-3} in Southampton water during the sampling day. No Peri was detected (or found in relatively lower concentration; maximum = 0.02 mg m^{-3}) at coastal sites however highest concentrations were detected in estuarine (figure 7.21) water with maximum values (0.13 mg m^{-3}) at the top end of the Itchen Estuary (IB). This coincided with the biomass increase in *Scrippsiella trochoidea* and *Protoperidinium minutum.* Although, Peri was apparently underestimated at some sites in the Test Estuary, particularly at BB and SG6 (figure 7.21). A significant correspondence ($r = 0.75$, $n = 13$, $p < 0.01$) was estimated between Peri concentrations and the biomass of photosynthetic dinoflagellates (table 7.7).

Fuc was strongly correlated to Chl *a* (see table 7.8) only at coastal sites (from NWN seawards to Calshot) indicating that diatoms were dominating the phytoplankton community at these sites (table 7.7), however they contributed less to the total phytoplankton community at estuarine sites as indicated also from Fuc/ Chl *a* ratio (figure 7.24). With the exception of the higher Fuc/ Chl *a* ratio at BB (0.15), which was coincided with the increase in the centric diatom *Cyclotella*, Fuc/ Chl *a* ratio increased from <0.1 to >0.16 towards the coastal waters (figure 7.24).

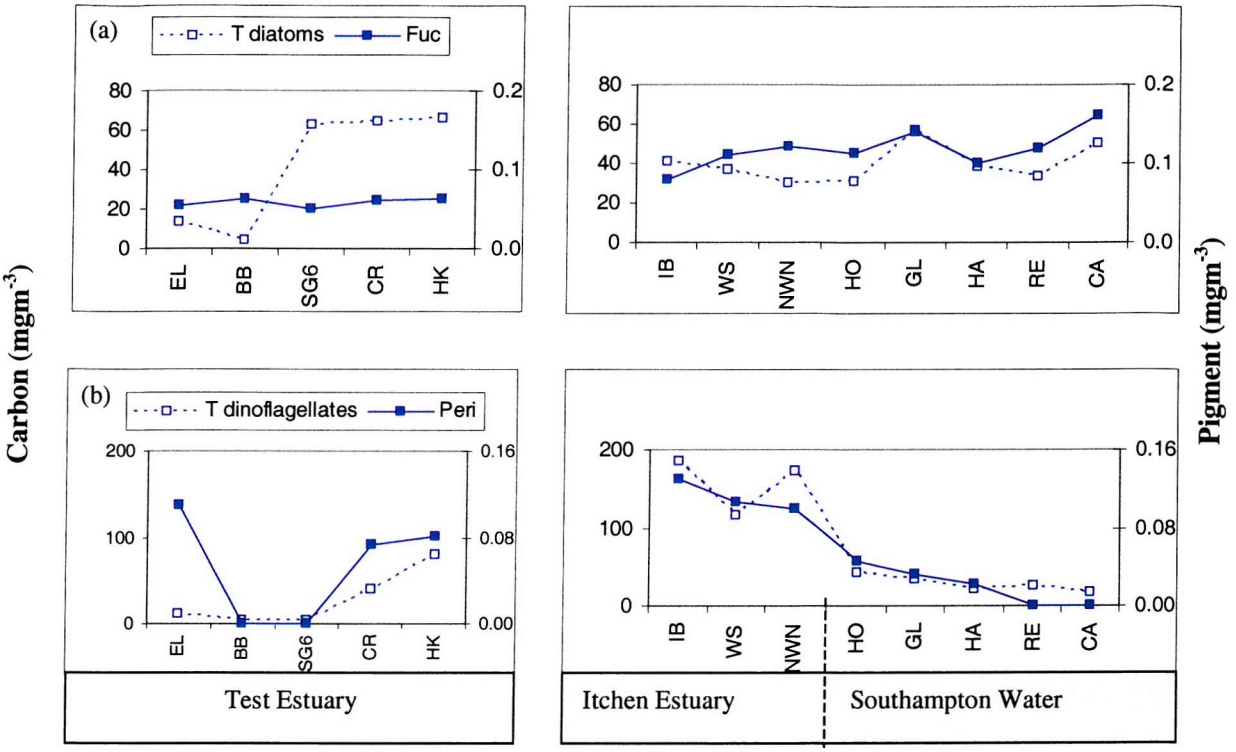


Figure 7.21. Spatial distribution of (a) fucoxanthin concentration in relation to total diatom biomass and (b) peridinin concentration in relation to total biomass of photosynthetic dinflagellates as detected throughout the estuary on 22nd July 1999.

In contrast, Peri was a small component of total pigments in coastal waters (table 7.8) compared to its contribution at the upper (along the Itchen and Test estuaries) and mid (intermediate between estuarine and coastal) estuarine sites on the sampling day (22nd July). Figure 7.24 shows the spatial changes in Peri/ Chl *a* ratio throughout the estuary. Maximum Peri/ Chl *a* ratios were estimated at estuarine and intermediate sites with highest contribution to Chl *a* at these sites (table 7.8) and decreased towards the mouth of estuary (coastal waters). Spatial distribution of Diad (not shown) as well as its ratio to Chl *a* were quite similar to that of Fuc (figure 7.24) along the whole estuary on the sampling day and strongly correlated ($r = 0.95$, $n = 13$, $p > 0.01$) to Fuc.

Allo, the biomarker pigment of Cryptophyceae, was detected in relatively low concentrations, from 0.02-0.55 mg C m^{-3}, along the estuary (figure 7.22) and was not detected at some sites. *Cryptomonas* sp. was recorded at most sampling sites with considerable concentration (up to 320 cells ml^{-1}& 13.2 mg m^{-3}) however; a very week correlation was estimated between biomass of *Cryptomonas* and Allo concentration (table 7.7).

Table 7.7. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water on 22nd July 1999.

Pigment biomarker	Phytoplankton group	Linear regression equation (mg C/mg pigment)	r	n	*p*
Fucoxanthin	*Bacillariophyceae	Y= 0.004x + 0.05	0.80	13	< 0.01
Peridinin	Dinophyceae	Y= 0.001x + 0.03	0.75	13	< 0.01
Chl *b*	Eutreptiella marina	Y= 0.012x + 0.06	0.67	13	< 0.05
Alloxanthin	Cryptomonas sp.	Y= 0.003x + 0.028	0.07	13	= 0.34
	M. rubrum	Y= 0.002x + 0.018	0.59	13	< 0.05

*Carbon biomass of the relatively large-celled *Odontella sinensis and Rhizosolenia styliformis* is not including in the total biomass of Bacillariophyceae.

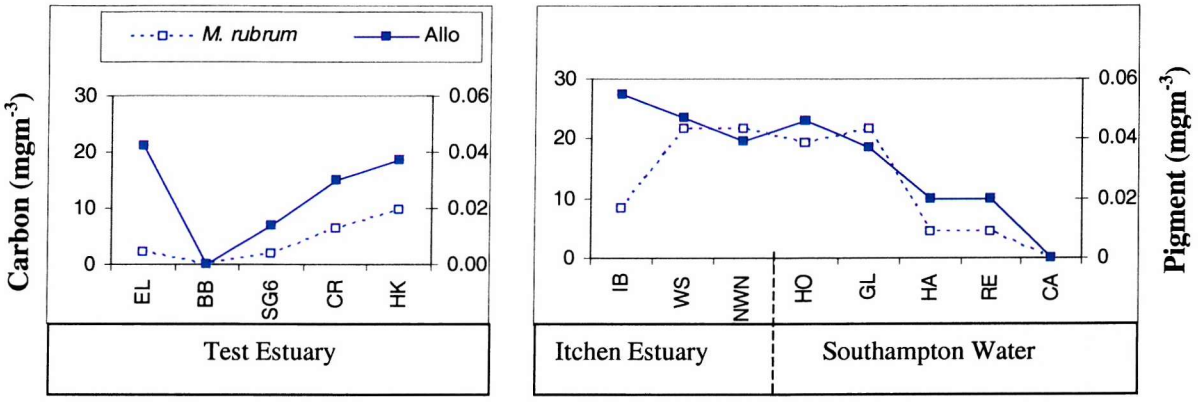


Figure 7.22. Spatial distribution of alloxanthin concentration in relation to total biomass of the autotrophic ciliate *Mesodinium rubrum* as detected through the estuary on 22nd July 1999.

The spatial distribution of Allo (figure 7.22) as well as Allo/ Chl *a* (figure 7.24) were significantly correlated (p <0.05) with the biomass of the photoautotrophic ciliate *M. rubrum* (figure 7.22). Allo significantly contributed to the total Chl *a* at estuarine sites (p < 0.01) on 22nd July indicating that alloxanthin-containing organisms (i.e. *M. rubrum* and/or *Cryptomonas* sp.) were greatly contributed to the phytoplankton community recorded at these sites (table 7.8).

Chl *b* concentration ranged from 0.02-0.29 mg m^{-3} along the estuary (data not presented) with no significant relationship with Chl *a*. *Eutreptiella marina* was the most abundant green algae reported on 22nd July and showed a good correlation with Chl *b*. This small flagellate was not recorded at all sampling sites and could be missed during microscopic analysis due to the small-sized cells and this could be an explanation of the insignificant correspondence between total *Eutreptiella* biomass and the T Chl *a*. Higher concentrations of Chl *b* as well as ratio of Chl *b*/ Chl *a* ratio recorded (figure 7.24) in the lower estuary (from GL down to CA).

Table 7.8. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable). Table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, *p* are italic.

	Estuarine waters		Coastal waters	
Pigment Biomarker	**r**	***p***	**r**	***p***
Fucoxanthin	**0.63**	*p = 0.13*	**0.72**	*p < 0.05*
Peridinin	**0.93**	*p < 0.01*	**0.37**	*p = 0.47*
Alloxanthin	**0.96**	*p < 0.01*	**0.10**	*p = 0.89*
Chl *b*	**0.91**	*p < 0.01*	**0.61**	*p = 0.20*
n	**14**		**12**	

Other traces of pigments were detected in relatively low concentrations (<0.1 mg m^{-3}) along the estuary. For example, 19-Hex was recorded (0.01-0.05 mg C m^{-3}) in most samples (figure 7.23) with highest levels recorded at seaward stations. 19-Hex was significantly correlated ($p < 0.05$) to total Chl *a*, however, no 19-Hex-containing coccolithofords were detected from microscopic analysis. In a similar way, Zea was found (up to 0.02 mg C m^{-3}) in some samples, particularly in the upper estuary (figure 7.23). This pigment was less important to the total phytoplankton community as it contributed little (r = 0.2) to the total Chl *a*.

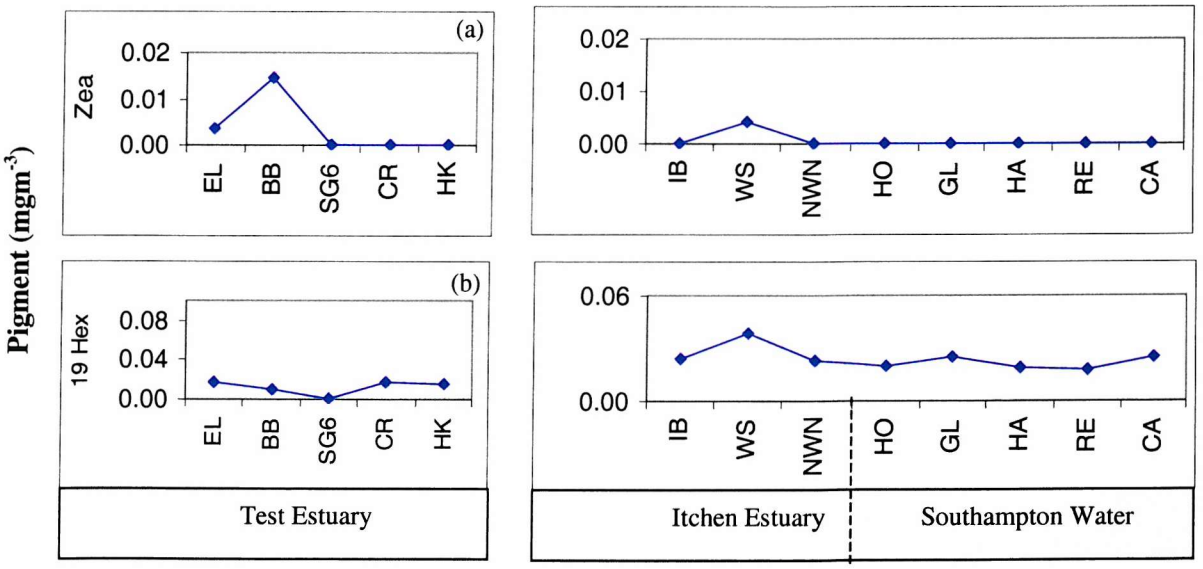


Figure 7.23. Spatial distribution of (a) zeaxanthin and (b) 19-Hex concentration as detected throughout the estuary on 22nd July 1999.

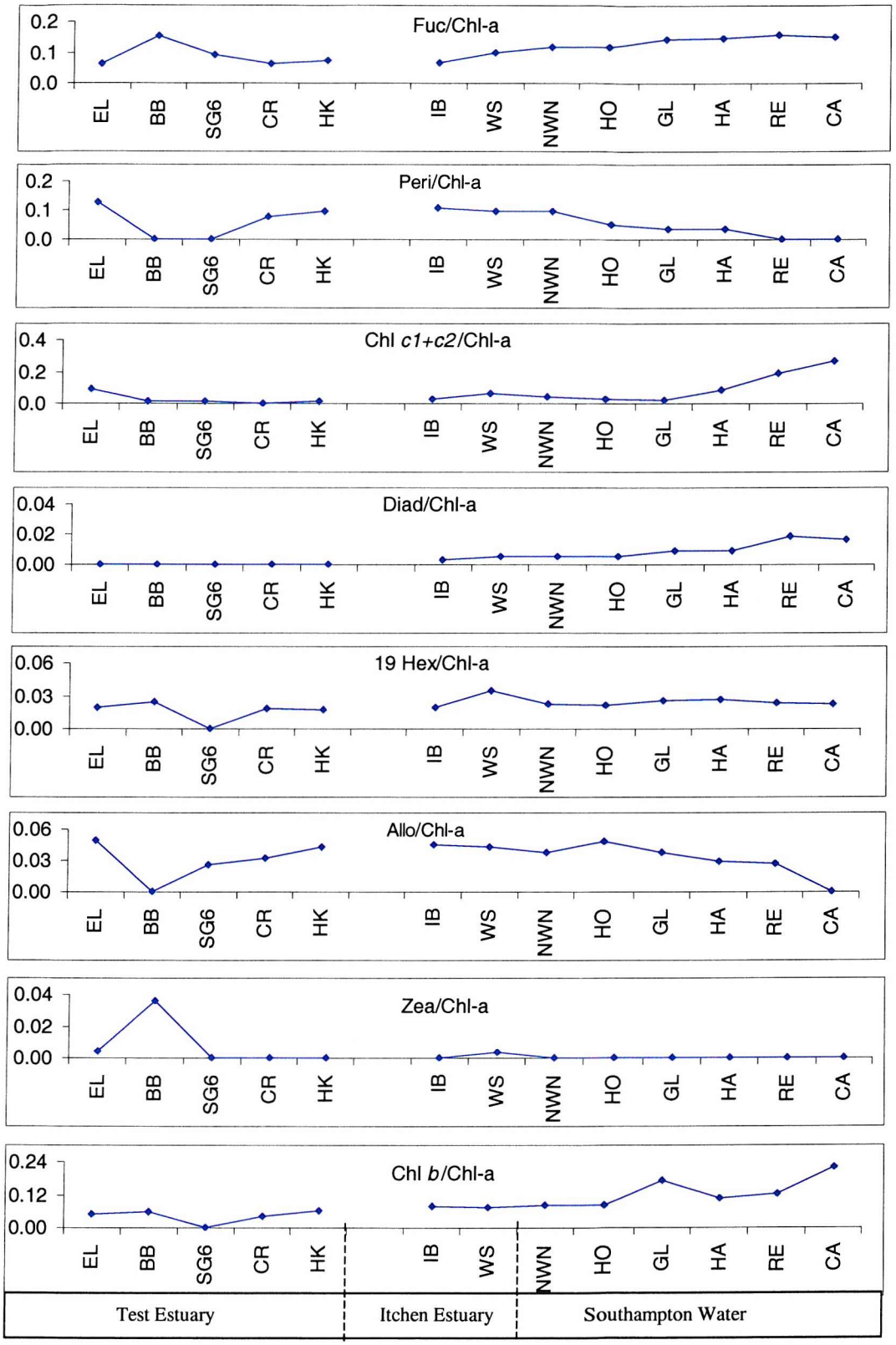


Figure 7.24. Spatial distribution of the ratio of some specific pigments (mg m^{-3}) to Chl *a* (mg m^{-3}) as determined throughout the estuary on 10th June 1999.

7.3.2 SPATIAL DISTRIBUTION IN 2000

I- 16th May Data

Samples for enumeration of phytoplankton collected on 16th May were accidentally lost, however the spatial distribution of the HPLC measured pigments are presented to show, in part, the quantitative changes in phytoplankton abundance in surface and deep water samples along Southampton Water.

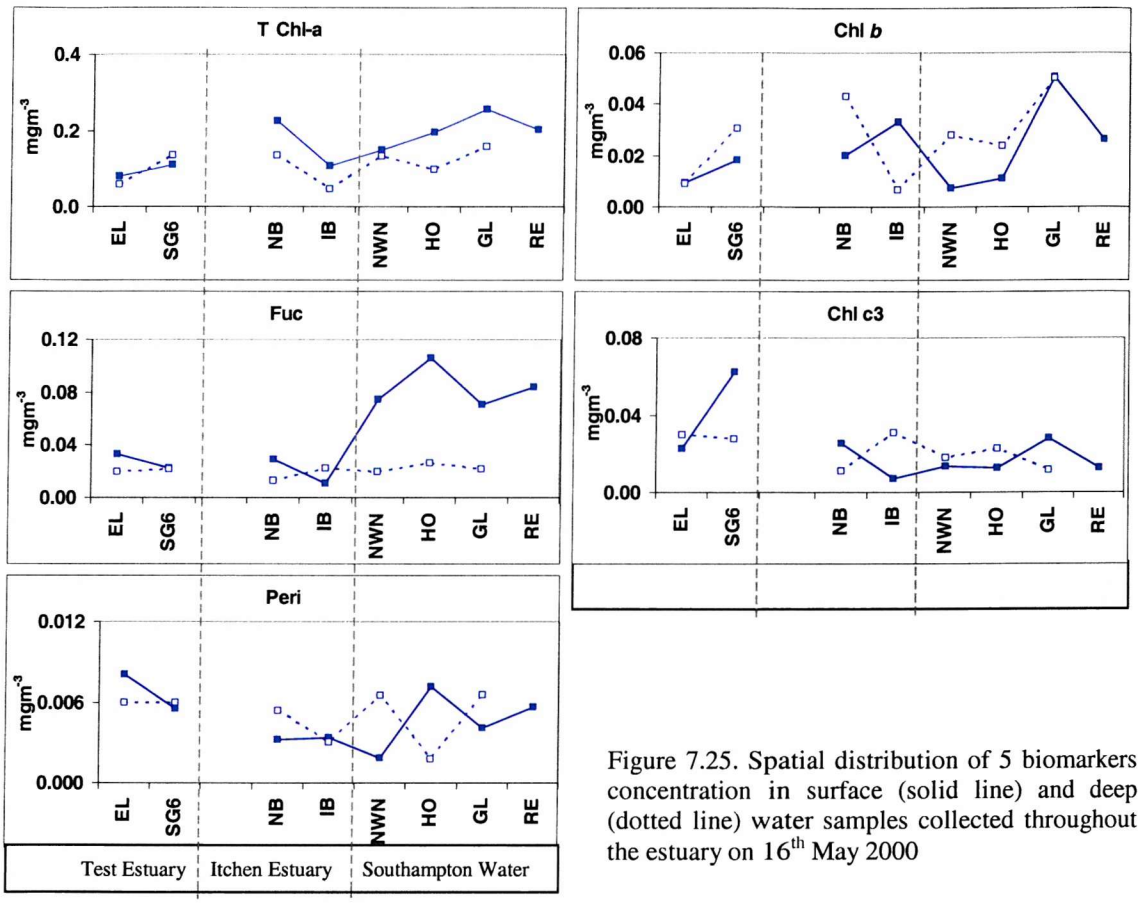


Figure 7.25. Spatial distribution of 5 biomarkers concentration in surface (solid line) and deep (dotted line) water samples collected throughout the estuary on 16th May 2000

Chl *a* varied from 0.8 to 0.25 mg m^{-3} in surface waters (1 meter depth) throughout the estuary on 16th May (figure 7.25) with high concentrations (0.14-0.25 mg m^{-3}) towards the coastal waters (from NW Netley down to Reach). A higher level of Chl *a* (0.22 mg m^{-3}) was recorded at the top of the Itchen Estuary (Northern Bridge, NB). Fuc (0.1-0.11 mg m^{-3}) was the most abundant pigment throughout the estuary indicating that diatoms were the most numerous group in Southampton Water during the sampling day. Plotting the concentration of Fuc as a dependent variable with Chl *a* as an independent variable (figures not presented) indicated that Fuc was clearly contributing ($r = 0.56$, $p < 0.05$) to the total Chl *a* biomass in surface water samples collected on 16th May (table 7.9)

	Surface		Bottom	
Pigment Biomarker	**r**	***p***	**r**	***p***
Fucoxanthin	**0.56**	*p < 0.05*	**0.28**	*p = 0.45*
Chl *c1+c2*	**0.78**	*p < 0.05*	**0.01**	*p = 0.83*
Peridinin	**0.26**	*p = 0.35*	**0.55**	*p = 0.20*
Alloxanthin	**0.72**	*p < 0.05*	**0.20**	*p = 0.67*
Chl *b*	**0.49**	*p = 0.22*	**0.94**	*p < 0.01*
Diadinoxanthin	**0.46**	*p = 0.25*	**0.92**	*p < 0.01*
n	**16**		**14**	

Table 7.9. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable). Table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, p are italic.

Two other important (concentration up to ~0.05-0.07 mg m^{-3}) accessory pigments (Chl *b* and Chl *c3*) were detected on the sampling date. Chl *b* varied from 0.01 to 0.05 mg m^{-3} with highest concentrations (figure 2.25) of 0.03 and 0.05 recorded at Green Land (GL) and Reach (RE), this could indicate that green algae were abundant in coastal waters. However Chl *c3* varied from 0.01 to 0.06 mg m^{-3} with highest concentrations 0.03 and 0.06 recorded in estuarine water at Northern Bridge (NB) and SG6, respectively.

Peri the biomarker pigment of photosynthetic dinoflagellates occurred in relatively lower concentrations (<0.01 mg m^{-3}) compared to the other pigments detected on the sampling day (figure 7.25), this might indicate that dinoflagellates were contributed less to the total chlorophyll biomass (see table 7.9) in surface water (r = 0.26) compared to its contribution in deep water (r = 0.55), however correlations were insignificant in surface and deep water.

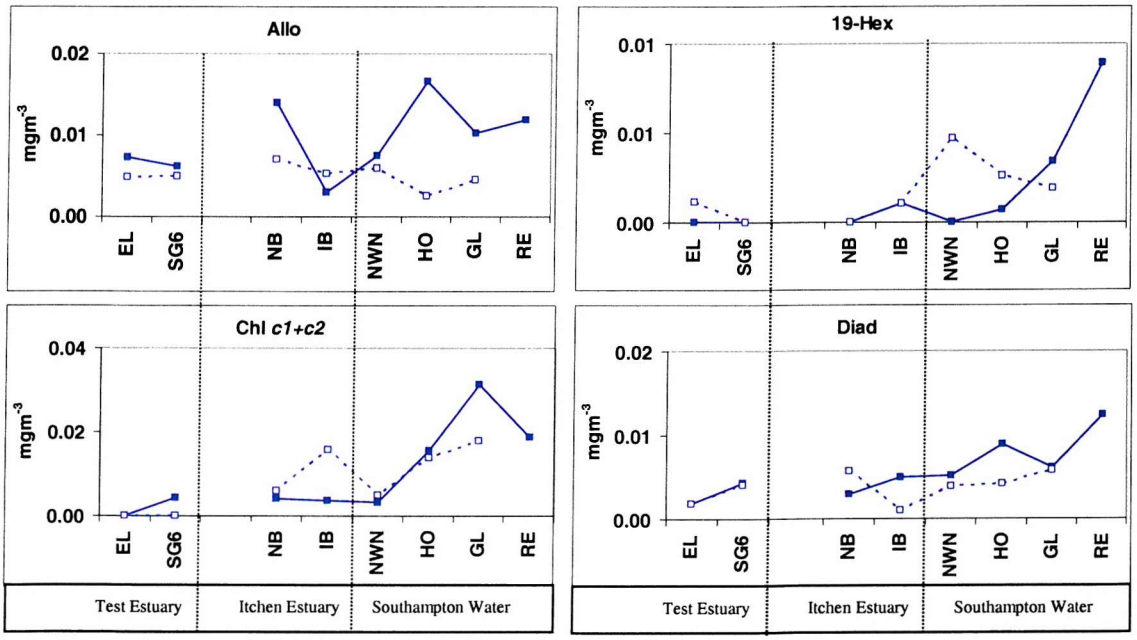


Figure 7.26. Spatial distribution of 4 biomarkers concentration in surface (solid line) and deep (dotted line) water samples collected throughout the estuary on 16th May 2000.

Other less important (<0.04 mg m^{-3}) pigments were detected on the sampling day (16th May). Spatial distribution of Allo, Chl *c1+c2*, 19-Hex and Diad throughout the estuary is shown in the figure 7.26.

From the pigment vertical profile (2 depths, surface and bottom), it was recorded that Chl *a* in deep water occurred in lower concentrations (0.05-0.16 mg m^{-3}) compared to that recorded in surface waters (figure 7.25). Fuc showed a unique distribution on the sampling day with a similar concentration (~ 0.02 mg m^{-3}) in deep waters throughout the whole estuary. Fuc in deep water was contributed less to the total chlorophyll biomass (table 7.9) however the dark induced pigment, Diad that found in both diatoms and dinoflagellates were significantly contributed to the total chlorophyll biomass. Similarly Chl *b* was significantly correlated to Chl *a* in deep water samples ($r = 0.94$, $p < 0.01$) than that in surface water samples ($r = 0.49$, $p = 0.22$) collected during the sampling day.

Spatial distribution of the ratio of some specific pigments (Fuc, Chl *c1+c2,* Peri, 19-Hex, Chl b and Diad) to Chl *a* is shown in figure 7.27. Fuc/ Chl *a* ratio in deep water showed lower values compared to the values recorded in surface waters. The spatial distribution of Fuc/ Chl *a* ratio was however similar in surface waters as well as in deep waters, with the exception of the higher deep value (~0.5) at Itchen Bridge (IB) site. A higher Chl c1+c2/ Chl *a* ratio was also estimated at the same sampling site.

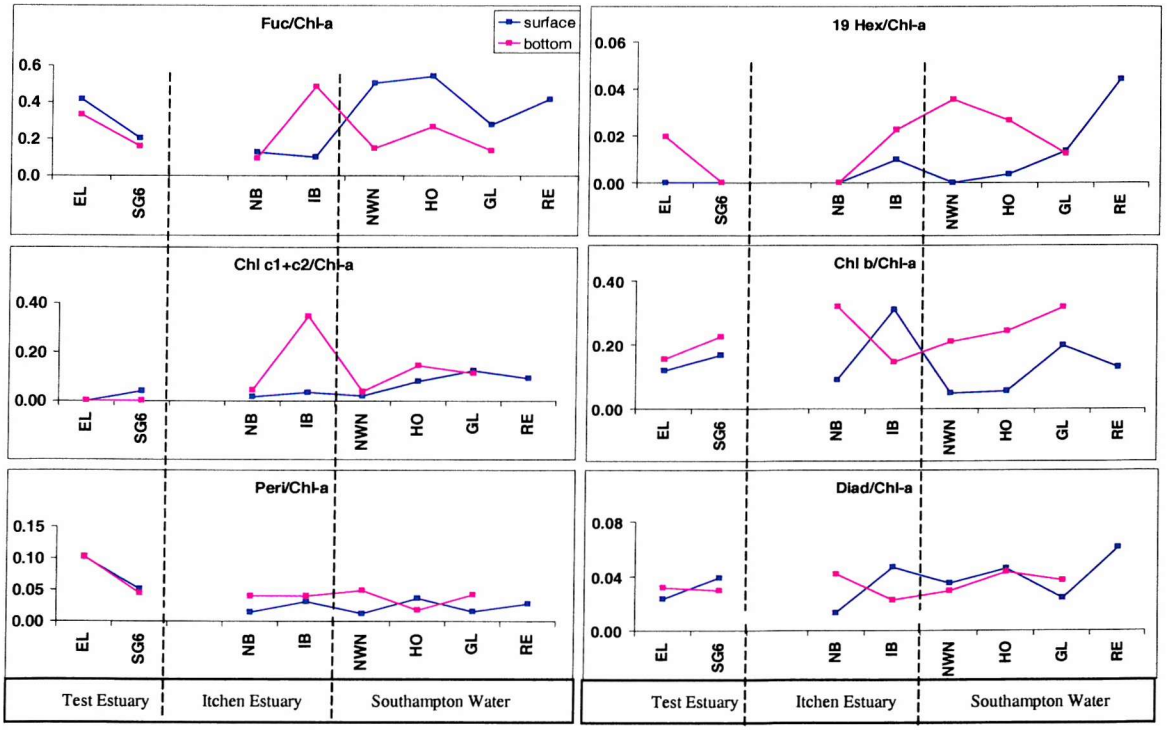


Figure 7.27. Spatial distribution of ratios of 6 biomarkers to Chl *a* in surface (blue line) and deep (pink line) water samples collected throughout the estuary on 16th May 2000.

II- 15th August Data

Figure 7.28 shows a similar spatial distribution of HPLC measured Chl *a* (mg m^{-3}) to that of the total phytoplankton biomass (mg C m^{-3}) on 15th August. A good agreement (r = 0.79, n = 15, $p < 0.01$) between both variables (figure 7.29).

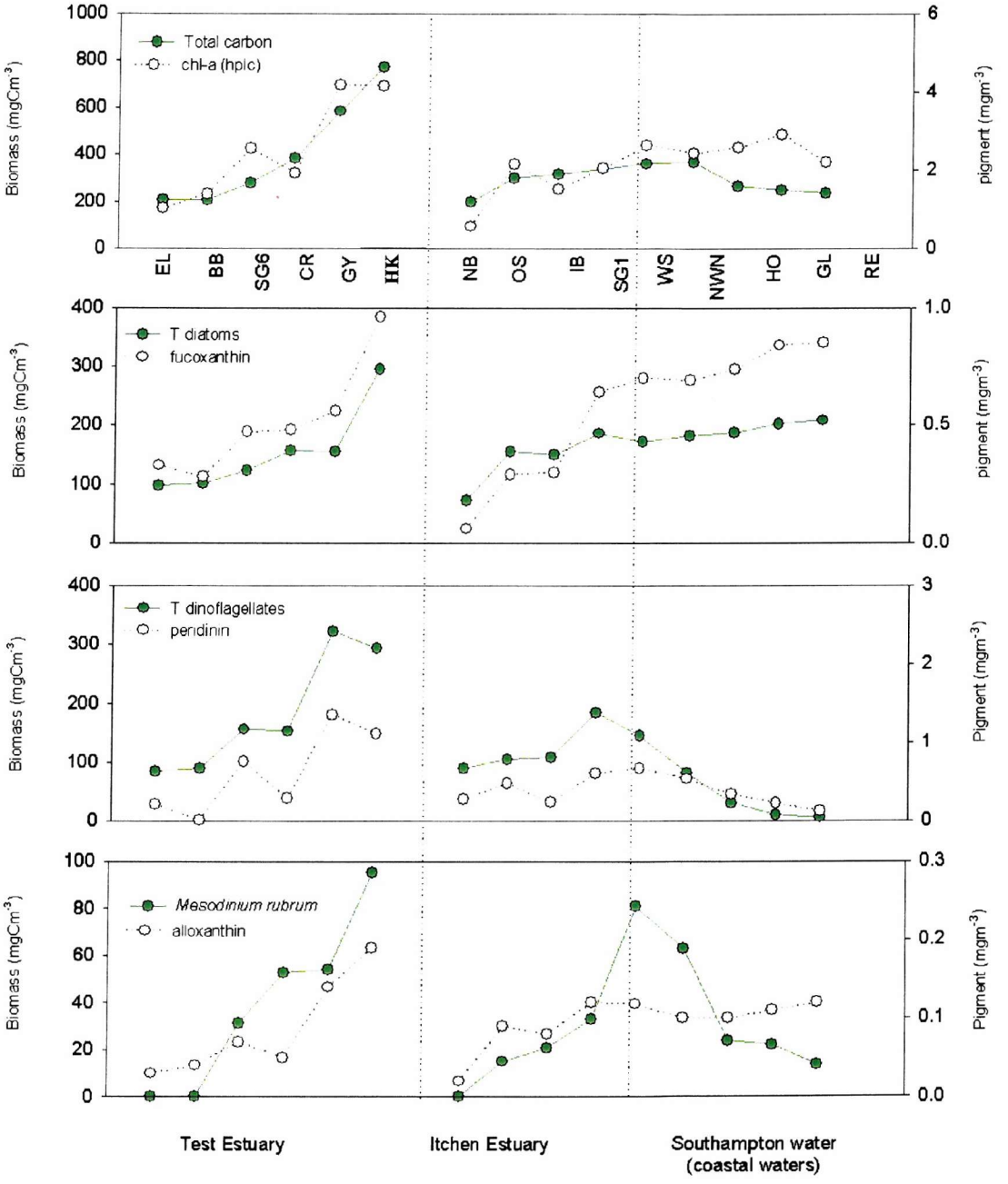


Figure 7.28. Spatial distribution of 4 biomarkers concentration in relation to total biomass of their relevant phytoplankton group of water samples collected along the estuary on 15th August 2000.

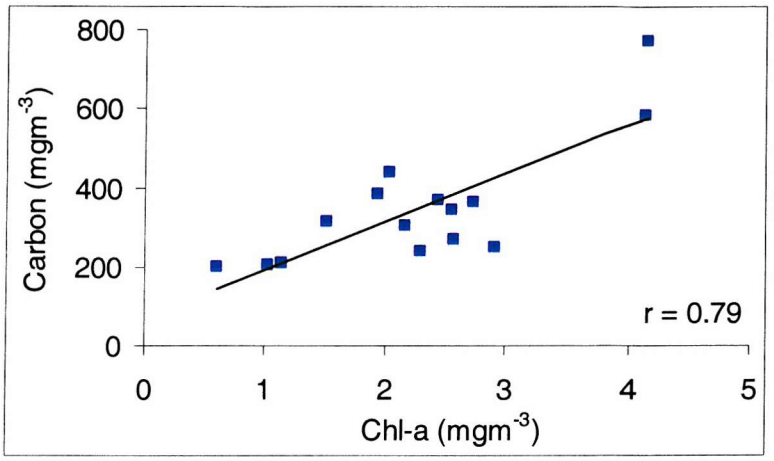


Figure 7.29. Plot of HPLC measured Chl *a* (mg m^{-3}) versus total phytoplankton biomass (mg C m^{-3}) of surface water samples collected throughout the estuary on 15th August.

Peri (0.23-1.36 mg m^{-3}) was the most abundant pigment in Southampton Water (see figure 7.28) in mid August (15th August) due to the summer dinoflagellate bloom that is known to occur in Southampton Water (e.g. Kifle, 1992) at this time of the year. Microscopic analysis of water samples revealed that phytoplankton community composition was mainly dominated by dinoflagellates (up to 54% of total carbon biomass), particularly at the upper and intermediate sites, and their importance to the total biomass declined towards the coastal waters due to the more turbulent waters seawards.

Three autotrophic species, *Scrippsiella trochoidea*, *Prorocentrum micans* and *Protoperidinium minutum*, exclusively dominated this dinoflagellate bloom. Peri showed spatial variations during the sampling day (figure 7.28) with maximum values (0.7-1.36 mg m^{-3}) at the mid estuary sites. Peri concentrations declined towards coastal waters to a minimum level (0.13 mg m^{-3}) at Reach (RE). A minimum contribution of dinoflagellates to the total phytoplankton carbon (~6%) was also recorded at Reach (RE).

Diatoms were considered to be the second dominant phytoplankton group along the estuary on the sampling day and contributed (up to 68%) to the phytoplankton community in coastal waters. Lower concentrations in Fuc were recorded at the upper sites (upper Test and upper Itchen) and increasing downwards (figure 7.28). Fucoxanthin was mostly abundant in the coastal water sites at which dinoflagellate growth declined (see figure 7.30) with a mean value of 0.76 mg m^{-3}. A higher value (0.96 mg m^{-3}) of fucoxanthin was estimated at Hythe Knock (HK). A strong correlation (table 7.10) was found between fucoxanthin and Chl *a* along the estuary (r = 0.82-0.89) on the sampling day,

indicating that diatoms were the exclusive abundant species at all sites. The correlation between peridinin and Chl *a* was (table 7.10) however much higher (r = 0.93, p <0.01) in upper sites (estuarine sites) and decreased towards the coastal waters (r = 0.28, p = 0.2). This indicated that diatom species replaced the dinoflagellate community with increasing water mixing (see figure 7.30).

Table 7.10. Results of linear regression analysis of chlorophyll-a (as independent variable) versus pigments (as dependent variable) during the spatial study on 15th August 2000; Table includes the Pearson's moment correlation coefficients, r printed in bold and significance of the correlation, p are italic.

	Estuarine Waters		Coastal Waters	
	r	*p*	r	*p*
Fucoxanthin	**0.82**	*p < 0.01*	**0.89**	*p < 0.05*
Chl C1+c2	**0.69**	*p < 0.05*	**0.68**	*p = 0.21*
Peridinin	**0.93**	*p < 0.01*	**0.28**	*p = 0.20*
Alloxanthin	**0.89**	*p < 0.01*	**0.65**	*p = 0.35*
Chl b	**0.86**	*p < 0.01*	**0.74**	*p = 0.17*
Diadinoxanthin	**0.95**	*p < 0.01*	**0.16**	*p = 0.84*
n	**20**		**10**	

Chl *c1+c2* showed a similar spatial distribution to that of fucoxanthin (not shown) with higher concentrations in coastal waters. A week correspondence was however found between Chl *c1+c2* and Chl *a* at the lower estuary (coastal waters) compared to that at estuarine sites (table 7.10). Diatom community composition in coastal water was clearly different from that in the upper estuarine sites with the large-sized diatoms, *Odontella* (*O. sinensis* and *O. aurita*) and the centric species (*Cyclotella* and *Cosinodiscus*) being mostly dominant the upper sites, however the relatively small-celled diatoms (mainly *Thalassiosira* and *Skeletonema*) were the most dominant at the lower sites.

The ratio of some specific biomarker pigments to Chl *a* showed good agreement with the total biomass of the relevant species and/or class during the one-day spatial survey conducted in August 2000. For example, Fuc to Chl *a* ratio showed relatively higher values in coastal water sites compared to the other estuarine sites (Figure 7.32), indicating that the relative contribution of diatoms to the total biomass was higher in the coastal water at this time. When the dinoflagellates population declined seawards, the community transferred from a dinoflagellate-dominated community to a diatom-dominated community (figure 7.30). Lowest Peri/ Chl *a* ratios were also estimated in coastal waters on 15th August (figure 7.32), this could be due to the reduced tendency of dinoflagellates to grow in turbulent and highly mixed water column. Chl c1+c2/ Chl *a* ratio showed the same pattern as that of Fuc/ Chl *a* ratio.

Alloxanthin (figure 7.28) showed a spatial distribution throughout the estuary with a maximum daily average concentration (~0.3 mg m^{-3}) measured at Gymp Elbow (GY) and Hythe Knock (HY) and at the middle site of the estuary (NWN). Microscopic analysis for surface water samples collected on the sampling day revealed that Cryptophyceae was represented only by one species, *Cryptomonas* sp. throughout the whole estuary. However, there was a degree of confusion during the microscopic identification between *Cryptomonas* and the small flagellates that were numerous (up to 1400 cell/ml) in most water samples collected along the estuary on 15th August, a weak relationship was recorded the biomass of *Cryptomonas* and Allo (table 7.11). Allo distribution along the Southampton Water on 15th August had a similar distribution pattern to that of the autotrophic ciliate *Mesodinium rubrum* biomass (figure 7.28) with a significant correlation ($r = 0.72$, $p < 0.001$) between both variables (table 7.11). This ciliate was known to contain cryptomonad-like endosymbionts, which changed its pigment pattern (Ansotegui et al., 2001). Alloxanthin was highly correlated ($r = 0.89$) to Chl *a* in the estuarine water compared to its correlation ($r = 0.65$) towards the coastal waters (table 7.10).

Values of Allo: Chl *a* ratio showed a consistent spatial variations on 15th August with clearly higher values (>0.1) at sites located in upper Itchen Estuary (figure 7.32) wherever the small-sized *Cryptomonas* sp. was numerically abundant (>17 % of the total cell number) compared to that (< 0.1) reported at sites located in the Test Estuary and Southampton Water as this flagellate was less contributed to the phytoplankton community (< 9% of the total cell number).

No relationship was found between Chl *b* and biomass of green algae, however higher concentrations of Chl *b* (up to 0.53 mg m^{-3}) were estimated towards coastal water sites. The lack of correspondence between both variables could be explained by the small size (Breton, et al., 2000) of the existent green algae along the estuary.

Diadinoxanthin however occurred in lower concentrations (0.06-0.43 mg m^{-3}), showed a very similar spatial distribution (not shown) to that of Fuc and Peri with maximum levels at Gymp (GY) and Hythe (HK). Diadinoxanthin is known to be present in diatoms, euglenoids and dinoflagellates (Barlow et al., 1993; Jeffery, 1997; Gibb et al., 2000). It was significantly (p <0.01) correlated to Chl *a* (table 7.10) due to the coexistence of both classes throughout the estuary particularly at the upper sites.

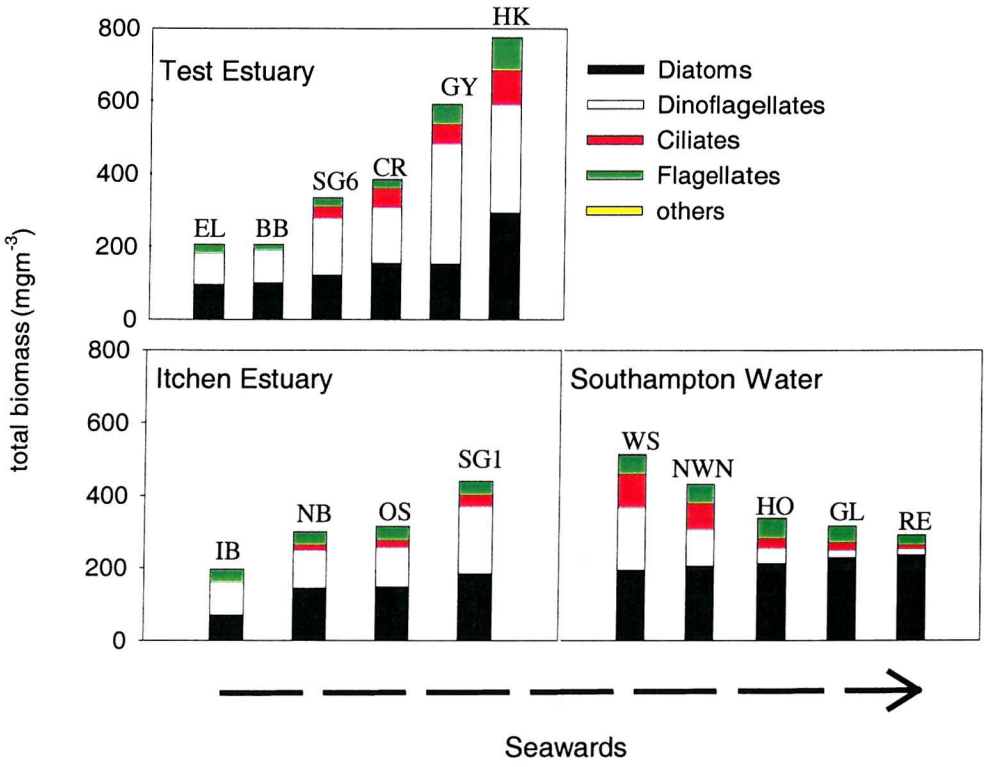


Figure 7.30. Spatial distribution of phytoplankton community composition (microscopic estimation) along Southampton Water on 15th August.

Table 7.11. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water on 15th August 2000. Data of all sampling sites along the estuary are grouped for the regression analysis.

Pigment biomarker	Phytoplankton group	Linear regression equation (mg C/mg pigment)	r	n	p
Fucoxanthin	Bacillariophyceae	Y= 0.004x - 0.015	0.90	15	< 0.01
Peridinin	Dinophycea	Y= 0.004x - 0.014	0.88	15	< 0.01
Chl b	*Eutreptiella marina*	Y= 0.049x - 0.18	0.20	15	= 0.21
Alloxanthin	*Cryptomonas* sp.	Y= 0.002x – 0.043	0.45	15	= 0.17
	Mesodinium rubrum	Y= 0.001x – 0.045	0.72	15	< 0.001
Zeaxanthin	Blue green filaments	Y= 0.009x - 0.025	0.03	15	
Chl *c3*	Small flagellates	Y = 0.00x-0.034	0.60	15	< 0.05

Brasinophyceae were never observed by light microscopy, while brasinoxanthin (Bras) was detected along the estuary (figure 7.31). Moreover, 19 But, which is found in some Chrysophyceae (Wright & Jeffrey, 1997), was measured in lower concentrations (up to 0.05) while no Chrysophyceans were

identified in microscopic analysis. In a similar way, few numbers of filamentous blue-green algae were seen by light microscopy, although, Zea was clearly detected in some samples with highest level (0.05 mg m^{-3}) seawards (figure 7.31). No correlation was found between the biomass of the detected blue green algae and the concentration of Zea (table 7.11). 19-Hex showed a slight increase (up to 0.09 mg m^{-3}) at Gymp and Hythe but no Prymnisiophytes or coccolithophord-containing 19-Hex were identified.

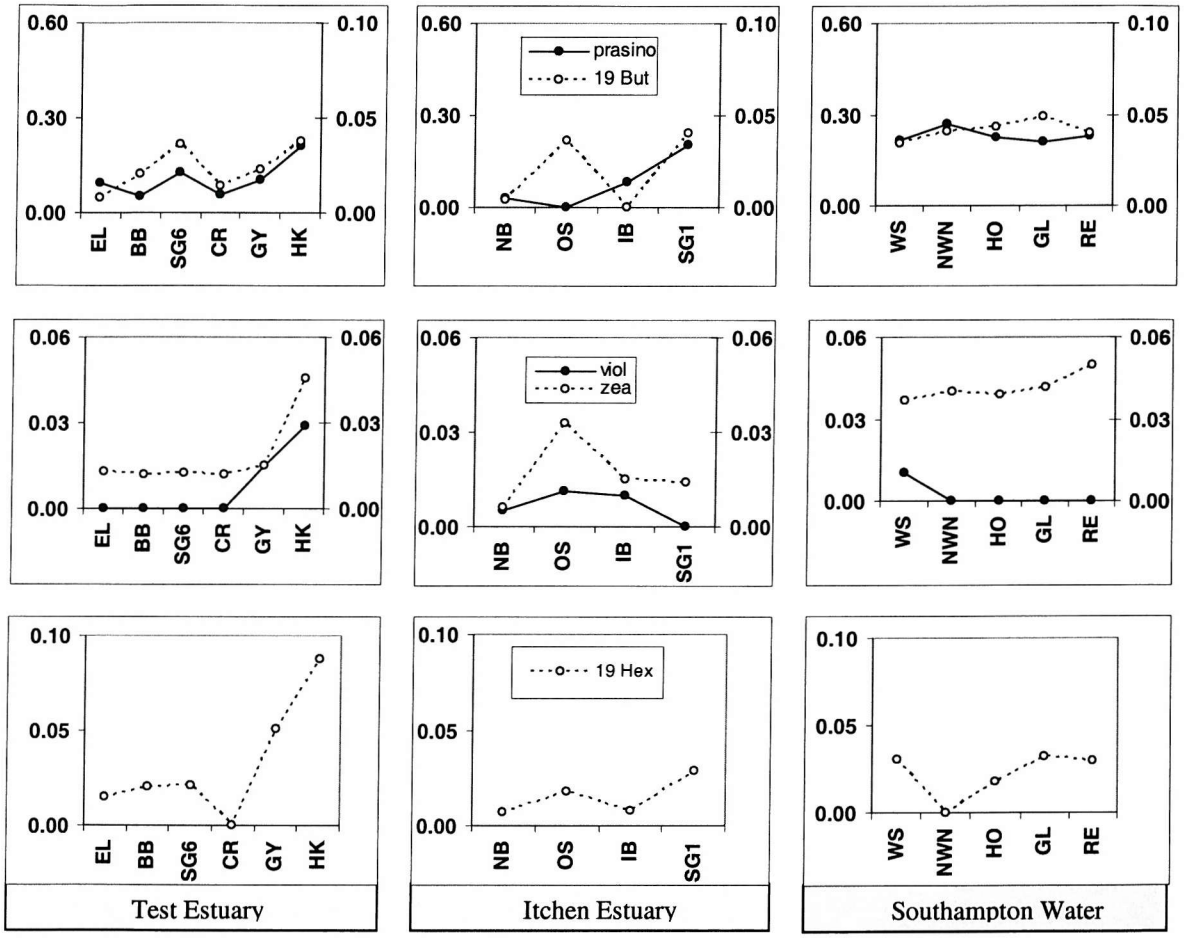


Figure 7.31. Spatial distribution of 3 minor pigments in surface water samples collected throughout estuary on 15th August 2000.

Using the HPLC pigment chemotaxonomy of water samples collected from bottom waters, phytoplankton community composition could be predicted, in part, form the pigment profile distribution (figure 7.32), however no microscopic analysis done for bottom samples. For example, figure 7.32 showed that Fuc/ Chl *a* ratio was higher in bottom waters due to sinking tendency. Chl *c1+c2*/ Chl *a* showed the same pattern as of Fuc/Chl-a with a much higher ratio (0.5) in bottom waters at SG6.

In contrast, Peri/Chl-a was much lower in bottom waters, indicating that dinoflagellates grew better in surface waters. The Diad/ Chl *a* ranged between 0.05 and 0.12 with higher values in the upper sites. Allo/ Chl *a* ratio was clearly high in bottom waters (figure 7.32) compared to that in surface waters, this could be explained by the aggregation of senescent cells, particularly flagellate (e.g. *Cryptomonas* sp.) that contain this pigment.

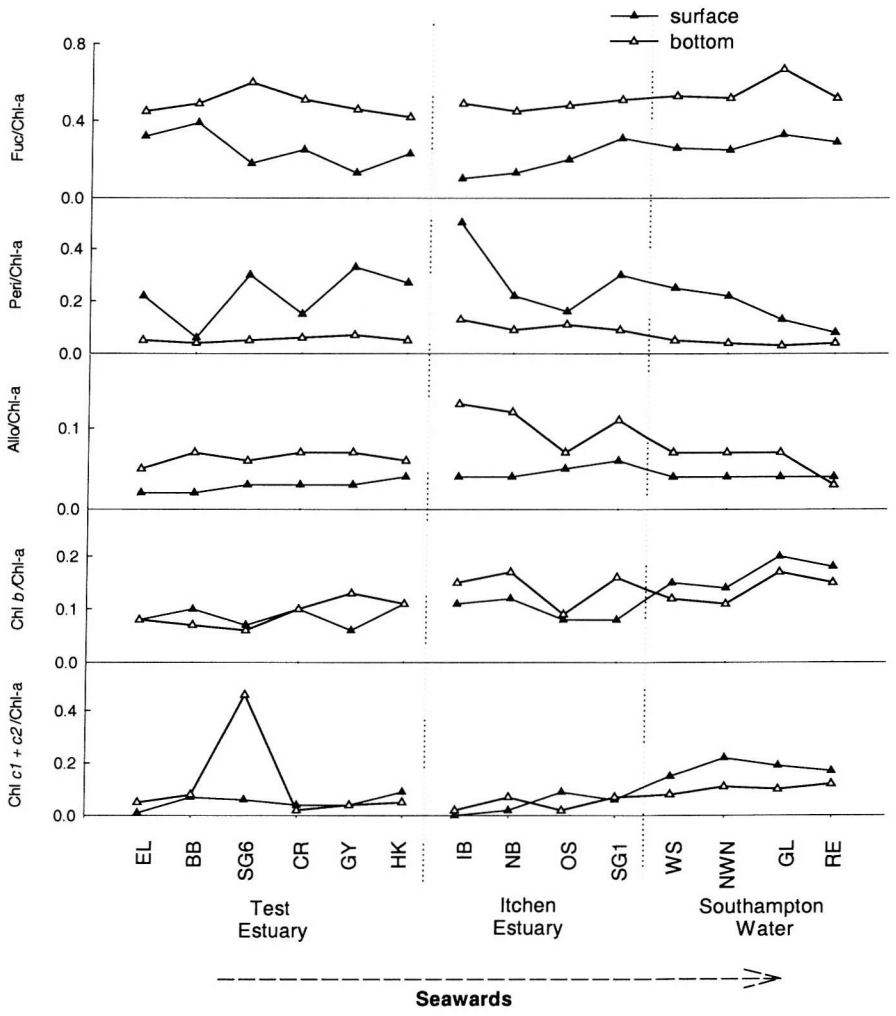


Figure 7.32. Spatial distribution of ratio of 5 biomarker pigments/Chl-a as estimated in surface (black symbols) and deep water (hollow symbols) samples collected along Southampton Water on 15th August 2000.

7.4 DISCUSSION

7.4.1 TOTAL PHYTOPLANKTON BIOMASS AND CHL A

HPLC measured Chl *a*, as a universal indicator of phytoplankton biomass, showed a clear correspondence with total phytoplankton biomass derived from microscopic observations (see chapter 2) with a mean value of correlation coefficient r = 0.72 during the temporal and spatial study conducted in Southampton Water in 1999 and 2000 (figure 7.33a). Although both variables were significantly correlated ($p > 0.01$, table 7.12), carbon biomass was, in some water samples, overestimated. This may occur, for example, during the bloom of the autotrophic ciliates *Mesodinium rubrum* in July 2000 (figure 7.8), particularly in the mid estuary. Similar findings were obtained when the large-sized diatoms (e.g. *Odontella sinensis* & *G. flaccida*, figure 7.15 & 7.19) in June and July 1999. Biomass measurements by HPLC analysis were sometimes overestimated (Breton et al., 2000) as recorded in coastal waters when diatom species of smaller cells (e.g. *Skeletonema costatum* and *Thalassiosira rotula*, 30-40mm) were apparently abundant (see figure 7.19). In contrast, Chl *a*, did not always give a good estimation of the total phytoplankton biomass as concluded by Breton et al. (2000) during a 20 month study undertaken in the Eastern Channel shown to be due to the varying environmental conditions (e.g. nitrogen depletion, light stress and seasonal variations in phytoplankton community).

Results presented in the current study have shown that there is a significant correlation between HPLC measured Chl *a* and Chl *a* measured fluorometrically (figure 7.33b) with a mean correlation coefficient of a value of 0.74 ($p < 0.01$) (table 7.12). Chlorophyll was clearly overestimated by the fluorometer method (22%-41% higher) in most analyzed samples due to the interference of other pigments and chlorophylls according to the method applied (Trees et al., 1985). In some studies particularly, in the ocean (Poulton, 2002), fluorometric measurements of Chl *a* can be underestimated (18% - 22% less) in some samples and overestimated (14% - 33% higher) in others.

Table 7.12. Summary of linear regression of relationship between HPLC measured Chl *a* and fluorometrically measured Chl *a*, total accessory pigments (carotenoids) and total carbon biomass of photosynthetic species. Data from temporal and spatial surveys conducted in 1999 and 2000 are grouped for the regression analysis.

Independent	Dependent	r	n	*p*
Fluorometric Chl *a*	T Chl *a* (HPLC)	0.74	113	<0.01
T Chl *a* (HPLC)	T accessory pigments	0.79	113	<0.01
T Chl *a* (HPLC)	T photosynthetic carbon	0.72	93	<0.01

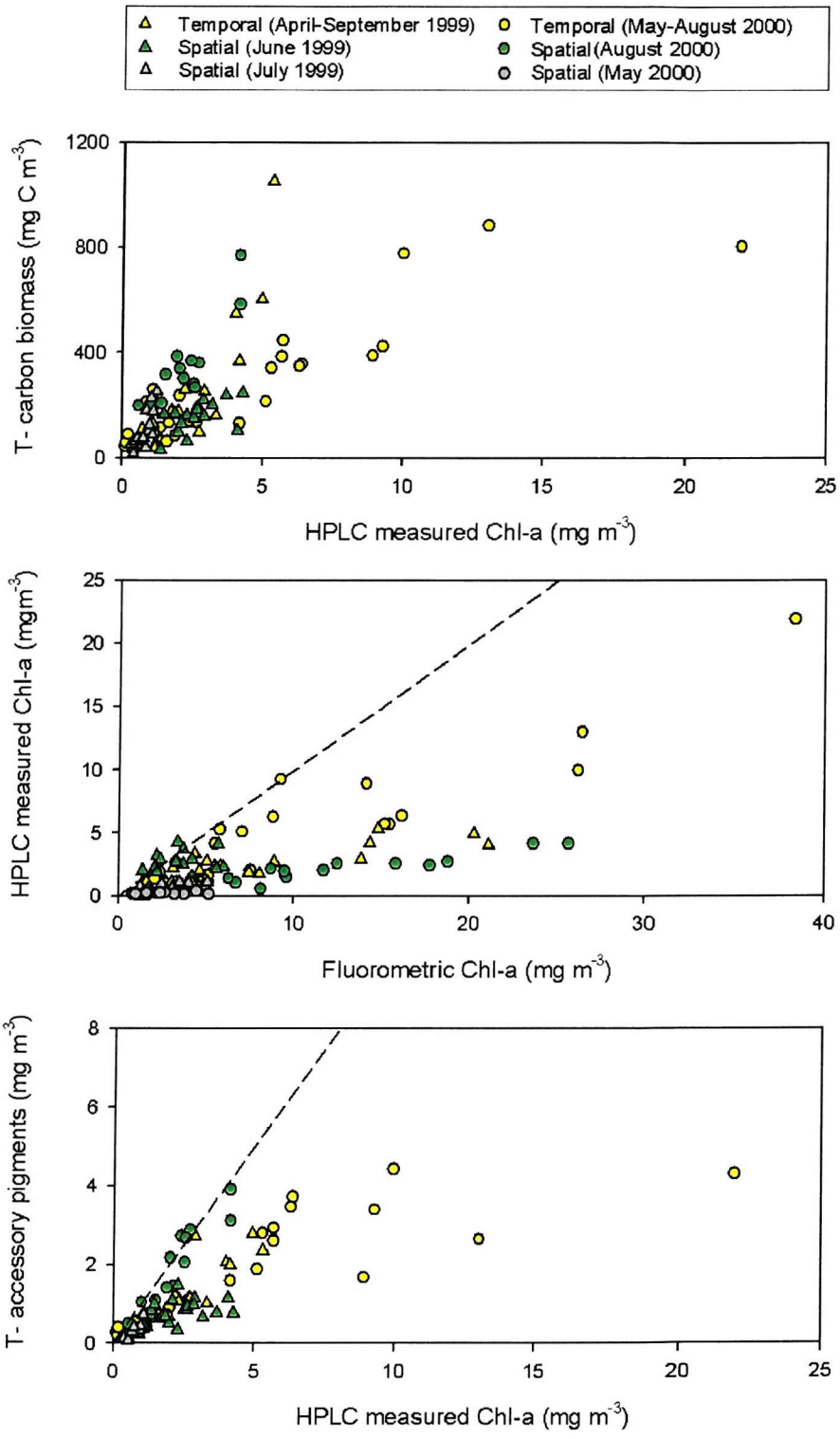


Figure 7.33. Comparison of T Chl *a* (measured by HPLC) and (a) T carbon biomass (estimated from cell counts), (b) fluorometric measurements of Chl *a* and (c) T- accessory pigments. Data from temporal and spatial surveys conducted in 1999 and 2000 are grouped for the regression analysis.

Total Chl *a*, concentration measured by HPLC significantly (p <0.01) correlated with total accessory pigment concentration (figure 7.33c) throughout the period of study with a mean value of correlation coefficient (r = 0.79), indicating that Chl *a*, concentration is related to the total concentration of accessory pigments as suggested by Trees et al. (2000) and can be used as an internal comparison of HPLC measurements of other pigments (Trees et al., 2000; Poulton, 2002). The gradient of the regression line (figure 7.3) ranged between 0.23-0.89 which was, in some samples, less than the value estimated by Trees et al., (2000). This could be explained by the fact that T Chl *a*, (the current study) did not include Chlorophyll a allomer, Chlorophyll a epimer and Chorophyllide a which were included in Trees et al. (2000) measurements. In addition, some accessory pigments (e.g. prasinoxanthin, diatoxanthin, dinoxanthin, violaxanthin, B carotene) were not quantified in some samples. In samples collected in 1999 and through the biweekly sampling conducted in 2000, Chl *c3* was also not fully quantified.

7.4.2 FUCOXANTHIN AND BACILLARIOPHYCEAE

A good relationship was estimated between fucoxanthin concentrations and total biomass of Bacillariophyceae in most water samples collected from Southampton Water (figure 7.34). Fucoxanthin is the taxonomic indicator pigment for diatoms (Vesk & Jeffrey, 1987; Jeffrey & Wright, 1994). However, it did not always give a good estimation of total diatom biomass. When the large-sized diatoms (e.g. *Odontella sinensis* and *Guinardia flaccida*) dominate the diatom community (table 7.4 and 7.5); total diatom biomass was overestimated (figure 7.15 & 7.21) by microscopic analysis (carbon biomass derived from cell counts). Thus, in these samples total diatom biomass showed a significant relationship with fucoxanthin (table 7.4 and 7.5), only when the biomass of these large diatoms was omitted (Breton et al., 2000). This could be due to the relatively low cellular pigment content of the cells of these large-sized diatoms (Stauber & Jeffrey, 1988) which could explain the discrepancies occurred between both measurements.

7.4.3 PERIDININ AND DINOPHYCEAE

Peridinin, the biomarker pigment for Dinophyceae (Jeffrey, 1974; Rodriguez et al., 2002) was shown to be a good quantitative marker of photosynthetic dinoflagellates in Southampton Water (table 7.13) during the period of this study (spring-summer 1999 & 2000). Peridinin concentrations were quantitatively related to the biomass of photosynthetic dinoflagellates (figure 7.34). In some water samples, the biomass of Dinophyceae (mg C m^{-3}) did not correlate strongly with concentration of peridinin (mg m^{-3}) possibly due to the microscopic confusion that could occur between photosynthetic and heterotrophic dinoflagellates, e.g. various species of *Gymnodinium* (see June 1999

data &, figure 7.15) were not identified to species level (Breton et al., 2000) and may contain endosymbionts as suggested by Anderson et al. (1996) and Jeffrey & Vesk (1997).

7.4.4 ALLOXANTHIN AND CRYPTOPHYCEAE

The relationship between Cryptophyceae and Alloxanthin as their respective biomarker is well documented (see Jeffrey & Vesk 1997; Jeffrey et al., 1999). During the current study, alloxanthin was a good biomarker for the *Cryptomonas* sp. (see table 7.1 & figure 7.7) that was numerically dominant in some samples. However, *Cryptomonas* sp. and alloxanthin concentration were not strongly correlated (table 7.7 & 7.13), for example, when the autotrophic ciliates *Mesodinium rubrum* was abundant. This ciliate is known to contain alloxanthin due to the presence of endosymbitic cryptophyte (Hibbred, 1977; Meyer-Harms & Pollehne, 1998) that may vary the pigment signature of the host (*Mesodinium rubrum*) (Ansotegui et al., 2001). Alloxanthin showed a similar temporal (May-September 2000; figure 7.22, table7.8) and spatial (along salinity gradient, on 15th August; figure7.27, table 7.11) distribution pattern to the biomass of *Mesodinium rubrum*, with a strong correlation ($r = 0.94$, $p < 0.01$) between both variables ($n = 93$, table 7.13) throughout the whole period of this study (see figure 7.34).

The microscopic confusion between *Cryptomonas* sp. and other small flagellates (2-3 μm) could also cause some discrepancy in the correlation between Alloxanthin concentration and biomass of *Cryptomonas* sp, particularly when these flagellates were numerically very dominant (section 7.3.1 II).

Table 7.13. Linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water. Data from temporal and spatial surveys conducted in 1999 and 2000 are grouped for the regression analysis. NS indicates insignificant correlations.

Pigment biomarker	Phytoplankton group	r	n	*p*
Fucoxanthin	Bacillariophyceae	0.74	93	*<0.01*
Chl *c1+c2*	Bacillariophyceae	0.54	93	*<0.01*
Peridinin	Dinophyceae	0.78	93	*<0.01*
Chl *b*	Green algae	0.67	93	*<0.01*
Alloxanthin	Cryptomonas sp.	0.1	93	NS
	Mesodinium rubrum	0.94	93	*<0.01*
Diadinoxanthin	Bacillariophyceae	0.66	93	*<0.05*
	Dinophyceae	0.59	93	*<0.01*
Zeaxanthin	Cyanophyceae	0.35	93	NS

7.4.5 CHL C3 AND "FLAGELLATES"

Chl *c3*, which is considered as a good biomarker pigment for the prymnesiophyte *Phaeocystis* sp. (Claustre et al., 1990; Breton et al., 2000) was not fully quantified in all samples. However, a clear increase in Chl *c3* peak area was noticed at the time when *Phaeocystis* sp. was numerically abundant in May 2000, particularly at the coastal site (see section 7.3.1). *Phaeocystis* sp. is known to be present in coastal waters (e.g. at Calshot) in Southampton Water around this time of year (Iriarte, 1991). Chl c3 concentration also correlated with the abundance of some small flagellates (~3μm) (not presented) with a moderate correlation ($r = 0.6$, $p < 0.05$, see table 7.11) on 15th August 2000 when abundance of *Phaeocystis* sp. was less.

In mid May, the Chl *c3*: Chl *a* ratio increased, particularly in coastal waters, during the growth of *Phaeocystis* sp. in May 2000. A higher increase in Chl *c3*: Chl a ratio, however, was found in late July and was coincident with the numerical increase of other small flagellates (2 – 3 μm) at the three sampling sites. Unidentified small flagellates with a similar cell size were shown to be significantly correlated to Chl *c3* concentration in a study conducted by Rodriguez, et al. (2002). *Phaeocystis* sp. may also contain 19-Hex (Jeffrey & Wright, 1994) however distribution of 19-Hex was not always correlated to the biomass of this flagellate and this could be due to variations in cellular content of 19-Hex in *Phaeocystis* sp. (Jeffrey & Wright, 1994). In addition, 19-Hex has shown to be a minor pigment of *Phaeocystis* sp. as previously found in the strains isolated from the north of Europe (Vaulot et al., 1994) and in the eastern part of the English Channel (Breton et al., 2000).

7.4.6 CHL B AND GREEN ALGAE

During the period of the current study green algae were represented mainly in Southampton Water by *Eutreptiella marina* and few *Scenedesmus* spp. Chl *b* distribution did not always show a good correlation with that of the biomass of green algae (table 7.5), however a clear correlation (mean r = 0.66, p, table 7.1, 7.4 and 7.7) was shown between the pigment concentration and the biomass of *E. marina* (figure 7.34). The lack of the of the correspondence between Chl *b* and the total biomass of green algae in some samples could be explained by the smaller size of the green algal cells (i.e. picoplankton) that could be missed during microscopic analysis as suggested by Breton et al. (2000) when recording high concentration of Chl *b* but no green algae were detected. This hypothesis is supported by Brunet (1994) when he found that green algae in the English Channel were dominant (using fractionation filtration) in the 0.5-0.7 μm, which could be easily missed by light microscopy.

7.4.7 ZEAXANTHIN AND CYANOPHYCEAE

Cyanophyceans are generally small in size and difficult to detect using light microscopy. Zeaxanthin was detected in most of the water samples collected from Southampton Water during the period of this study, however no (or few cells) Cyanophyceae were detected. Breton et al. (2000) clearly detected zeaxanthin in April-May in the English Channel; however they did not observe any Cyanophyceae by light microscopy. They recommended the use of Scanning electron microscopy and epifluorescence microscopy to identify smaller cells as well as to determine the distribution of cyanobacteria.

7.4.8 PIGMENT/CHL A RATIO

The ratio of biomarker pigments to Chl *a* could be used not only to indicate the presence but also the dominance of various classes and/or species of phytoplankton. It also helps detecting the minor pigments (Everitt et al., 1990; Letelier et al., 1993) and small-sized phytoplankton species (Rodriguez et al., 2002). In the spatial and temporal studies throughout Southampton Water in 1999 and 2000, a good agreement was found between the ratio of the biomarker pigment / Chl *a* and the total biomass of the relevant species and/or class. For example, ratios of Fuc and Chl *c1+c2* to Chl *a*, increased in spring and early summer during the bloom period then decreased over the summer (figure 7.7 & 7.10), indicating that the relative contribution of diatoms to the total phytoplankton biomass was highest during spring. The ratio of Fuc to Chl *a*, during the temporal study in 2000 showed, however, high values on 14 August (figure 7.12) at all 3 sites and this was coincident with the numerical increase in some small diatoms (*Thalassiosira rotula;* 20–30 μm and *Chaetoceros spp.*) with relatively higher ratios in the coastal water site compared to the other two estuarine sites (figure 7.12). Similar findings were obtained during the large scale one-day (15th August) survey (see figure 7.28 & 7.32) This indicates that the relative contribution of diatoms to the total biomass was higher in the coastal water at this time and the community had transferred from a dinoflagellate dominated community to a diatom dominated community.

During the temporal study in 1999 & 2000, the Peri to Chl *a*, ratio was low during spring and increased over summer with highest ratios on 1st July 1999 & 15th August 2000 (figure 7.6 & 7.12) at the time of summer dinoflagellate bloom (figure 7.11) and was much higher in estuarine waters. A higher ratio was estimated in the mid estuary (~0.3) indicating that dinoflagellates prefer to grow under intermediate conditions with respect to nutrient availability and tidal mixing (i.e. in calmer water). Lowest Peri/ Chl *a*, ratios were also estimated in coastal waters on 15th August 2000, indicating their reduced tendency to grow in more turbulent waters (White, 1976; Pollinger & Zemel, 1981).

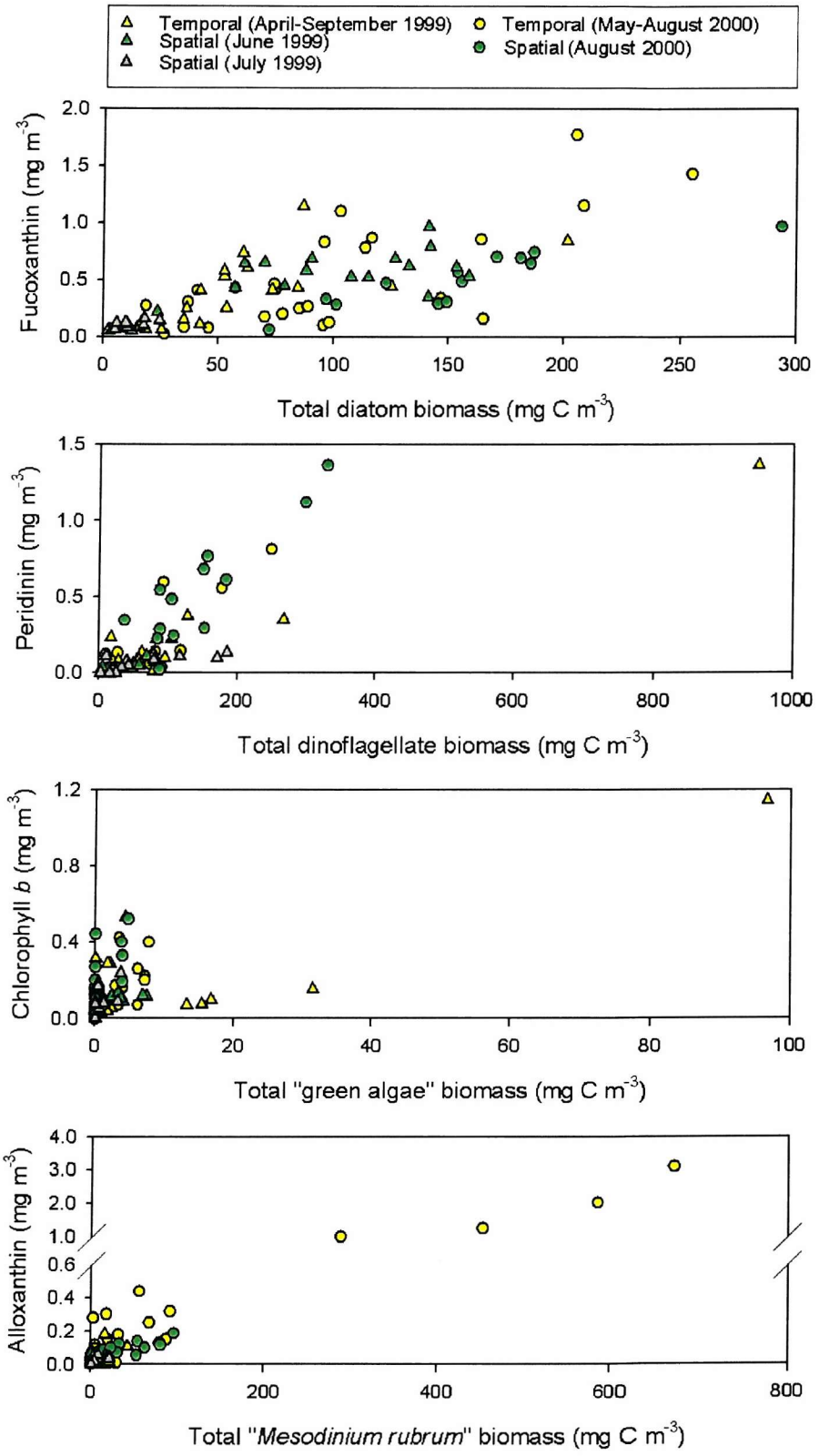


Figure 7.34. Plot of linear regression of specific pigment content (mg m^{-3}) versus the biomass of the corresponding algal group (mg C m^{-3}) encountered in Southampton Water. Data from temporal and spatial surveys conducted in 1999 and 2000 are grouped for the regression analysis. Total "green algae" presented in this figure was mainly of *E. marina*.

Recent laboratory studies with dinoflagellates (Thomas & Gibson, 1990; Berdalet, 1992; Berdalet & Estrada, 1993; Thomas et al., 1995) support this finding and have demonstrated a direct effect of turbulence on their cell growth, cell division and also their physiological behaviour.

Higher Allo: Chl *a*, ratios were exclusively found (at all 3 sites) during the temporal study undertaken in 2000, particularly in July at the time of the peak growth of the ciliate *Mesodinium rubrum*. A relatively high ratio of this carotenoid to Chl *a*, was found early in spring in the upper estuarine site coincident with an increase in the abundance of a small cryptomonad-like flagellate at this time of the sampling period (data not presented)

The agreement between algal biomass and the relevant biomarker pigment of certain groups of phytoplankton provides a prediction of the species present in water samples where cell counts were not made, e.g. bottom samples May and August 2000 (figure 7.25, 7.26, 7.27 & 7.32). Figure 7.32 showed that the Fuc/Chl-a ratios were higher in bottom waters (on 15th August) at all sites possibly due to diatoms accumulation in deep water by sinking. The ratio of Chl *c1+c2*/Chl-a showed the same pattern as of Fuc/Chl-a with a much higher ratio (0.5) noticed in bottom waters at SG6. In contrast, the Peri/Chl-a ratios on the same day were much lower in bottom waters, indicating that dinoflagellates favour near surface waters and also they are able to regulate their position in the water column (Lauria, 1998) for optimum light and nutrient availability.

The Diad/Chl *a* ratio ranged between 0.05 to 0.12 with higher values at the upper estuarine more turbid sites similar to that shown by Ansotegui et al. (2001). The higher Allo/Chl *a* ratio in bottom waters in comparison to that estimated in surface waters, could be due to patchy distribution of the ciliate *M. rubrum* as seen by Crawford & Lindholm et al. (1997).

CHAPTER EIGHT

CHAPTER EIGHT

8- GENERAL DISCUSSION AND CONCLUSIONS

The main aim of the research reported in this thesis was to investigate the effect of environmental factors on phytoplankton growth, abundance and bloom development throughout a macrotidal temperate estuary. A number of previous studies have investigated various aspects of phytoplankton ecology in Southampton Water. In this study an initial objective was to describe the temporal variations in phytoplankton population in relation to varying environmental conditions. Wright et al. (1997) described changes in chlorophyll at a position in the lower estuary from a continuously sampling fluorometer together with salinity and temperature data but samples were not collected at regular intervals adjacent to the sensor for phytoplankton identification. Results presented in chapter 3 of this thesis have linked changes in chlorophyll from a calibrated continuously monitoring fluorometer (at a fixed position in the upper estuary) with phytoplankton population changes and changes in a number of environmental variables. This combination of data has revealed a sequence of phytoplankton blooms during the monitored spring/summer period and showed that a succession of dominant diatoms is followed by small flagellates and then dinoflagellates. The phytoplankton species composition was broadly similar to those previously reported from local estuarine studies (e.g. Kifle, 1992; Howard et al., 1995; Lauria, 1998) and other similar estuarine environments, for example, in Peconic Bay (Bruno et al., 1980), the lower Westerschlde (Tripos, 1991) and inner Oosterscheld (Bakker et al., 1994).

The data set has revealed for the first time that diatom blooms can develop in the estuary over a spring tide in contrast to previous findings (e.g. Hydes & Wright 1999). In addition attempts were made to correlate environmental conditions to the development of the bloom. A mean water column irradiance level of over 100 W h m^{-2} day^{-1} appeared to be required for the onset of this diatom bloom (dominated by *Guinardia delicatula*) together with a water temperature of ~14°C and some degree of salinity stratification. This agreed well with a threshold of 100 W h m^{-2} day^{-1} previously suggested by Jahnke (1989), Peperzak (1993) and Peperzak et al. (1993) for phytoplankton growth to occur in Spring, in coastal waters of the North Sea, although Riley (1957) had earlier reported that a level of only 50 W h m^{-2} day^{-1}, was required for temperate diatom bloom initiation.

Following the collapse of the bloom of this relatively large-celled diatom (40-80 μm), a transition period occurred before the dinoflagellates began to dominate the phytoplankton assemblage with a period of a few weeks dominated by flagellates (mainly *E. marina*) and the photosynthetic ciliate *M. rubrum*. These non-Si requiring organisms were generally abundant when nutrient concentrations were depleted, particularly Si, immediately after the main spring bloom. Dinoflagellate blooms were shown to develop in summer when water column irradiance levels were higher (up to 700 W h m^{-2} day^{-1}) and cell abundance increased particularly between neap and spring tides with temperature stratification being increased during neap tide periods. In addition, river flow rates are minimal at this time of year causing reduced flushing of phytoplankton populations from the estuary. Previously Kifle (1992) described phytoplankton species and chlorophyll changes in Southampton water in 1988 from frequent water samples collected at 2 sites in the estuary (NW Netley and Calshot). A similar species succession was reported during this research in 1999 at a site in the lower Itchen estuary approximately 1km from North West Netley Buoy although in this study phytoplankton biomass was also calculated from cell biovolume measurements.

The second aim of the research was to investigate the spatial distribution of phytoplankton species throughout Southampton Water in relation to various environmental parameters. The original aim was to undertake these large surveys during bloom periods to contrast the effect of diatom and dinoflagellate blooms on nutrient distribution in the estuary. This proved difficult to achieve although one survey was conducted in August 2000 during a widespread dinoflagellate bloom. The main finding from these surveys was that certain species of phytoplankton are not distributed equally throughout the estuary and their distribution may be influenced by a combination of factors such as salinity, physical structure of the water column and nutrient availability. For example, the diatom *Guinardia delicatula* was more abundant in high salinity waters towards the mouth of the estuary whereas *Odontella* and *Nitzschia* species were more numerous in surface waters towards the head of the estuary. In addition, cell counts and HPLC

pigment data showed that diatoms were more abundant in deeper waters in May and June and flagellates and dinoflagellates were more abundant in surface waters in June, July and August in agreement with observations previously made by Kifle (1992) and Lauria (1998).

A further observation of the spatial distribution of phytoplankton species along the salinity gradient indicated that the autotrophic ciliate, *M. rubrum* was dominant in the middle part of the estuary (mainly at NW Netley). This could be attributable to the mid estuarine conditions, with respect to salinity, nutrient concentrations and mixing being optimum for growth of this highly motile photosynthetic ciliate in this part of the estuary. Similar findings were previously reported for this organism by Crawford, (1992) and Crawford et al (1998) in Southampton Water.

In 2000, a sampling programme was designed to investigate the changes in phytoplankton community composition in three different regions of the estuary over the spring/summer period. These results were then compared to changes in the phytoplankton assemblage in seawater samples collected from each of the three sites and incubated for up to two weeks in the laboratory under non-limiting light conditions. All incubated water samples showed an increase in chlorophyll and carbon biomass with concurrent reduction in nutrients. Phytoplankton biomass (as Chl *a*) initially collected from the upper estuary, mid estuary and coastal waters increased from the *in situ* values of 1-16, 1-24 and 2-6 mg m^{-3}, respectively to experimental peak values of 146-205, 34-82 and 27-70 mg m^{-3} during the four experiments (May, June, July & August 2000) when incubated under optimum light conditions. These peak levels of phytoplankton biomass that developed in laboratory incubated samples were considerably greater than the maximum values measured during the same period in the estuary, thus confirming that the estuarine system is light rather than nutrient limited. Nutrients are not considered to be a limiting factor for phytoplankton growth in Southampton water and concentrations of nitrate and phosphate are generally high enough to support phytoplankton growth within the estuary throughout the whole year as suggested Hydes & Wright (1999) based on results from the SONUS (Southern Nutrient Study) programme. The results from incubation experiments conducted during this study showed conclusively that nutrients (N, P and Si) were removed by the developing phytoplankton populations at close to Redfield ratios during an initial nutrient unlimited growth phase. Following the peak in chlorophyll biomass in the incubation containers however nutrient uptake ratio changed indicating either P-limitation particularly in the outer estuary site in June and July and N+P-limitation in August. Silicate limitation was indicated in the mid estuary in June immediately following the spring diatom bloom period when initial silicate levels were considerably depleted in the incubation samples. When light was non-limiting in laboratory incubated samples, diatoms generally out-competed other groups e.g. flagellates and dinoflagellates during the incubation period. These results also showed that dinoflagellates are

generally weak competitors for nutrients compared to diatoms under optimum light conditions. Similar findings were also reported by Chang and McClean, (1997) and Smayda (1997). Results from the culture experiments using the diatom *T. rotula* and the dinoflagellate *P. micas* (both shown to be abundant species occurring in Southampton Water) indicated that *P. micans* had a lower growth rate (in all cultures) compared to T. *rotula.* Most Dinophyceae are known to have substantially lower growth rates compared to diatoms as reported several workers (e.g. Chan, 1980; Brand & Guillard, 1981; Langdon, 1993). This in part explains the succession of phytoplankton species seen in Southampton Water and other similar macrotidal temperate estuaries where diatoms dominate in spring as they can divide rapidly and increase to bloom levels despite the high mixing (wind and tidally driven) and rapid water exchange in the estuary (i.e. during peak spring tides and high river run off), while slower growing dinoflagellates dominate in summer under more thermally stratified conditions with reduced estuarine flushing.

Results from the seawater incubation experiments also showed that small-sized diatom cells were more abundant in phosphate depleted cultures as previously recorded by Labry et al (2002) when species of smaller sized-cells (3-20 μm) were shown to dominate the spring diatom bloom in the Bay of Biscay as a consequence of early P-limitation recorded (Labry, et al. 2002). In this respect the species diversity of the incubated samples did not reflect that occurring in the estuary, particularly in summer months, due to the different physical conditions in incubated samples.

Although, Southampton Water is not generally a nutrient-limited system, nutrient loadings and ratios (particularly, N:P) may also play an important role in influencing the growth of phytoplankton and bloom development. The effect of different initial nutrient supply ratios on the growth rate and biomass yield was tested during the culture experiments conducted with *T. rotula* & *P. micans*. Results showed that both species attained a higher growth rate and cell yield in cultures with a Redfield N:P ratio (16:1). A similar cell yield was attained in phosphate replete cultures (for both species when compared to 16N:1P) with some small variation in growth rate whereas phosphate limited cultures attained a much lower cell yield and reduced growth rate in comparison to Redfield ratio cultures.

In situ maximum cell concentration of *T. rotula* was attained in Southampton water (current study) during spring/summer 1999 and 2000 when nutrient ratios of N:P, N:Si and P:Si were close to the Redfield ratio with values of 16:1 (or 25:1), 1:1 respectively as previously suggested for phytoplankton, in general (Redfield et al., 1963; Broecker & Henderson, 1998) and for diatoms (16:1, 2.3:1 and 0.14:1), in particular (Peeters & Peperzak, 1990). In addition, the value of the N:P ratio was 16.2 before the bloom of *G. delicatula* and 21.6 before the bloom of *T. rotula* at the mooring site in 1999. A value of N:P close to the Redfield ratio was sometimes

recorded in Southampton Water, however the relative ambient concentrations of nitrate – N and phosphate – P was generally higher than 16μM:1μM.

One of the main objectives of this research was to investigate changes in phytoplankton populations and dominance with respect to space and time using HPLC pigment analysis as a chemotaxonomic indicator of different phytoplankton groups. Parallel microscopic observations were also conducted to investigate how powerful the HPLC technique is in such complex estuarine systems. HPLC results showed mostly a good relationship with cell count data. It produced a quick indication of the presence of dominant groups compared to microscopic analysis, and clearly shows seasonal and spatial patterns of distribution of phytoplankton groups. The HPLC method identifies the main abundant classes (e.g. Bacillariophyceae, Dinophyceae and Cryptophyceae), and sometimes indicates the presence of certain species (e.g. *Phaeocystis*). However, some discrepancy was recorded in the relationship between pigment and carbon measurements derived from cell counts which was mainly attributable to microscopic errors either when small sized-cells (green algae and small flagellates) were abundant and not accurately counted or when microscopic confusion existed between autotrophic and heterotrophic species or when large rare diatoms biased the carbon estimates. These results clearly demonstrated that microscopic enumeration is invaluable, and should be used in parallel to HPLC pigment analysis as recommended by Ansotegui et al. (2001) and Breton et al. (2000). Breton et al. (2000) also recommended the use of scanning electron microscopy and epifluorescence microscopy for small cells.

Phytoplankton biomass estimates were made for the first time on samples collected from the Southampton Water estuary and these provided a further insight into the changes in phytoplankton populations from the C:Chl *a* ratio. Results showed that the this ratio varied markedly according to the species community structure with higher values (40-70) estimated when large celled-diatoms (e.g. *R. styliformis*, *O. sinensis*) and/or carbon rich dinoflagellate species (e.g. S. *trochoidea*) were present. However lower C: Chl *a* (20-30) ratios were recorded when smaller-sized cells (e.g. *S. costatum*) were more abundant.

CONCLUSIONS

1- Continuous monitoring data was shown to provide a more reliable indication of short-time scale variations in phytoplankton growth and bloom development in estuaries and allowing the detection of several episodic blooms which lasts only for a short period (i.e. less than 7 days).

2- Spring diatom blooms were coincident with increased irradiance and reduced water column turbidity and their development was independently of the spring-neap tidal cycle, whereas summer dinoflagellate blooms coincided with both high daily irradiation and reduced mixing (i.e. neap tides).

3- Flagellates species, particularly dinoflagellates, tended to dominate in more stratified regions of the estuary with diatoms mainly found in more turbulent waters

4- The autotrophic ciliate, M. rubrum was more abundant in the mid estuary (mainly at NW Netley) where a combination of conditions favored its growth including, moderate nutrient concentrations and reduced mixing..

5- Phytoplankton in Southampton water were shown to have a succession of Spring diatoms followed by a transition period dominated by flagellates and/or ciliates (non-Si required organisms) and then dinoflagellates, which dominated summer blooms

6- The dinoflagellate P. micans which was numerous in the estuary during summer showed a slower growth rate than the diatom T. rotula at all three nutrient ratios explaining, in part, the seasonal succession in Southampton Water estuary. Both species grew optimally under "balanced" N:P cultures (16:1)

7- Phytoplankton populations throughout Southampton water were shown to be generally light rather than nutrient-limited although at times, particularly following the Spring bloom, nutrient ratios (N:P) indicated short periods of silicate and phosphate limitation..

8- Smaller species of diatoms were noted to be more abundant in P-depleted waters particularly in the outer estuary.

9- The HPLC pigment analysis was shown to be a powerful technique for indicating the presence of particular dominant groups of phytoplankton but was more useful when used in combination with microscopic cell enumeration.

10- Higher C: Chl a ratios (40-70) were recorded when large celled-diatoms (e.g. R. styliformis, O. sinensis) and/or dinoflagellate species (e.g. S. trochoidea) were present with lower C: Chl a ratios (20-30) recorded when smaller-sized cells (e.g. S. costatum) were much abundant.

CHAPTER NINE

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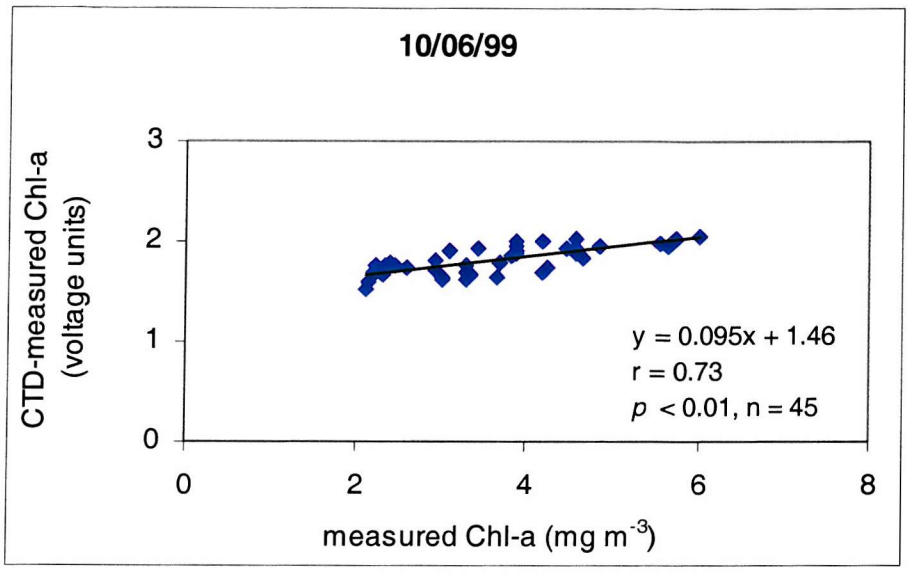
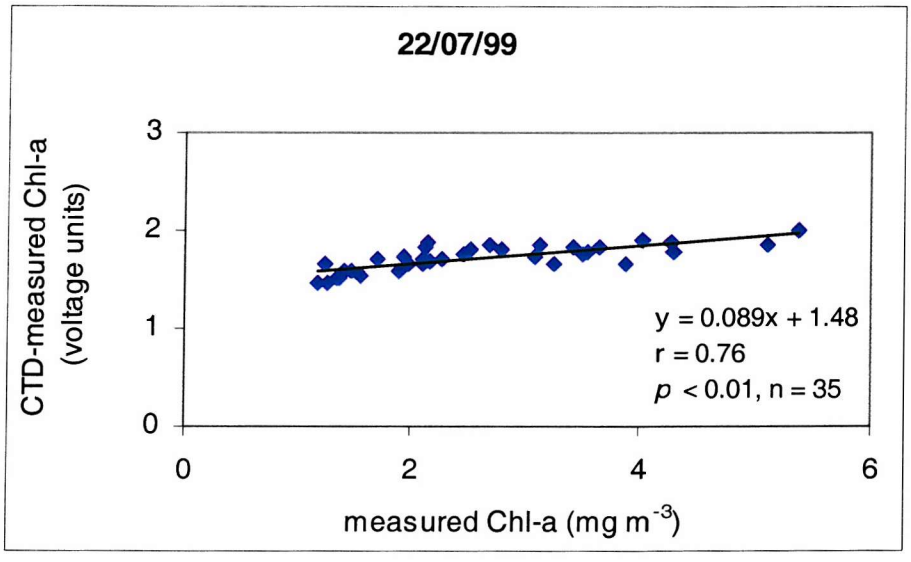
CHAPTER TEN

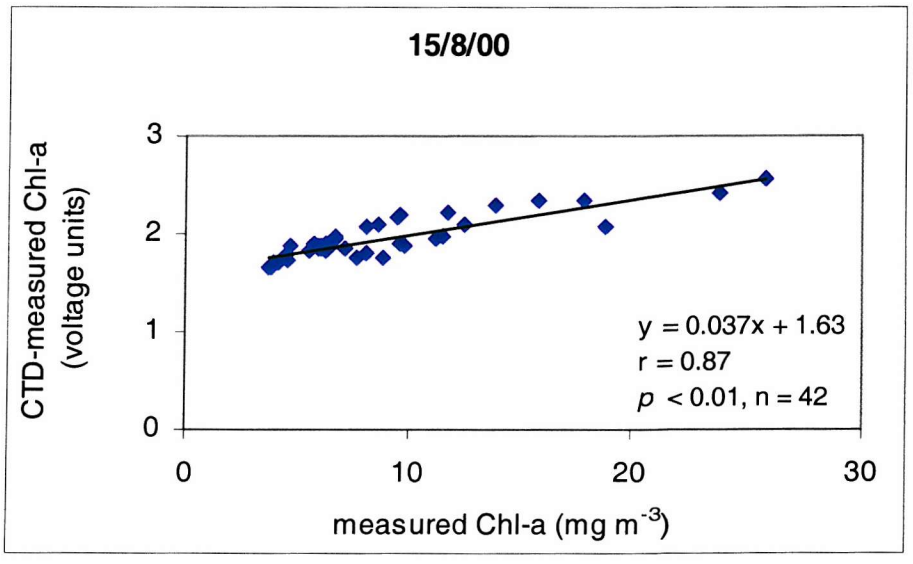
"APPENICES"

APPENDIX I

FLOUROMETRIC VALUES OF CHLOROPHYLL A AGAINST THE CTD VOLTAGE VALUES


10/06/99
CTD-measured Chl-a (voltage units)
3
2
1
0
0
2
4
6
8
y = 0.095x + 1.46
r = 0.73
p < 0.01, n = 45
measured Chl-a (mg m-3)

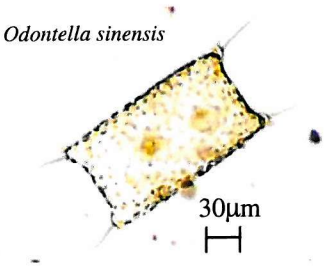

22/07/99
CTD-measured Chl-a (voltage units)
3
2
1
0
0
2
4
6
y = 0.089x + 1.48
r = 0.76
p < 0.01, n = 35
measured Chl-a (mg m-3)

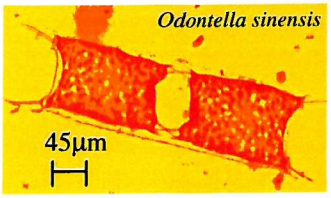

15/8/00
CTD-measured Chl-a (voltage units)
3
2
1
0
0
10
20
30
y = 0.037x + 1.63
r = 0.87
p < 0.01, n = 42
measured Chl-a (mg m-3)

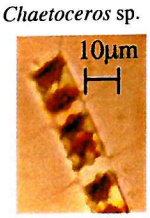
APPENDIX II

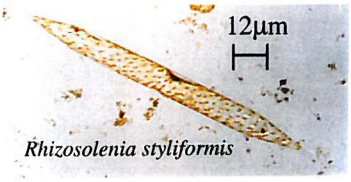
LIGHT-MICROSCOPIC IMAGES OF SOME DOMINANT PHYTOPLANKTON SPECIES IDENTIFIED IN WATER SAMPLES COLLECTED FROM SOUTHAMPTON WATER DURING 1999 & 2000

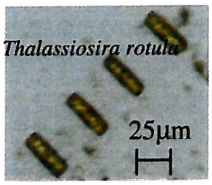
I- Dominant diatom species

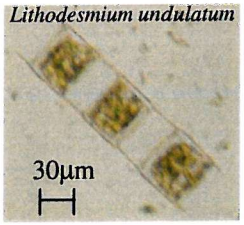


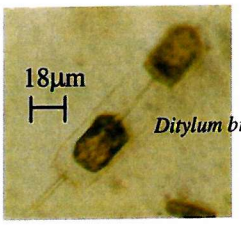




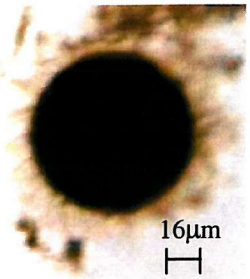


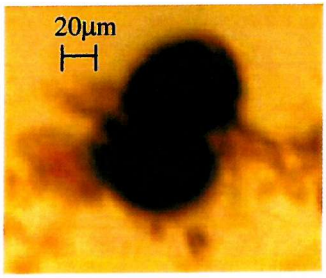


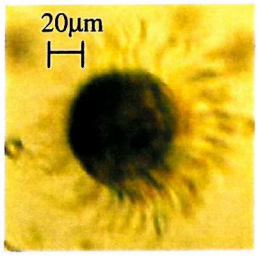




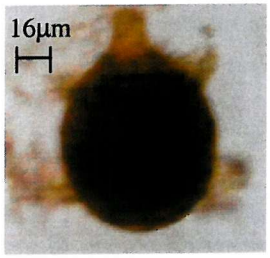
II- Dominant ciliate species



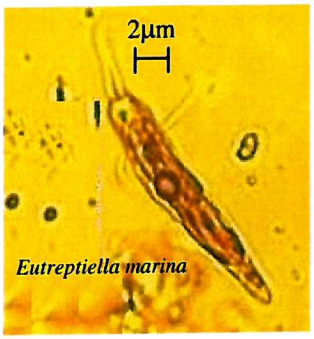


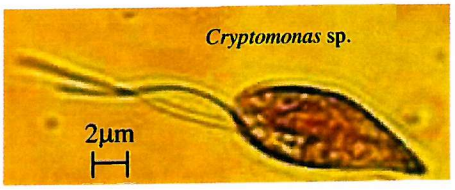


Mesodinium rubrum

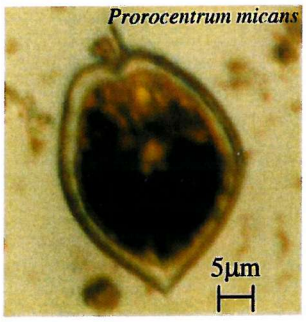


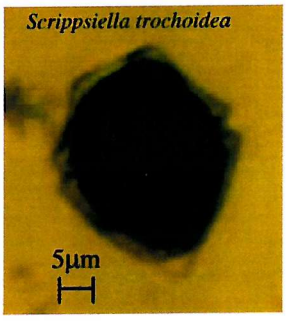
III- Dominant flagellates species

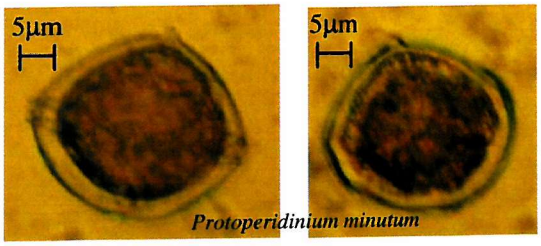




III- Dominant dinoflagellate species







APPENDIX III

SEM PICTURES OF SOME DOMINANT PHYTOPLANKTON SPECIES IDENTIFIED IN WATER SAMPLES COLLECTED FROM SOUTHAMPTON WATER DURING 1999 & 2000

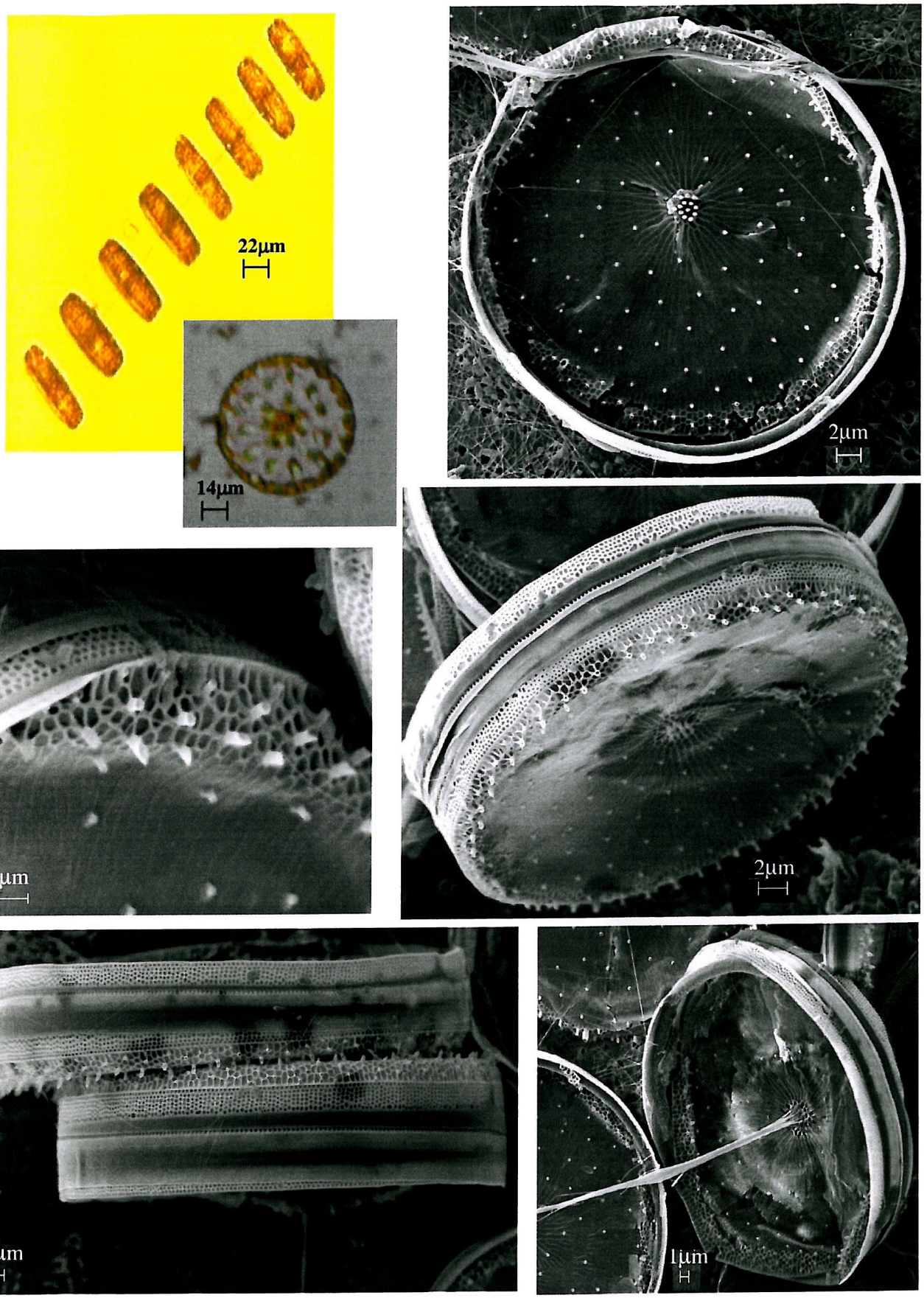
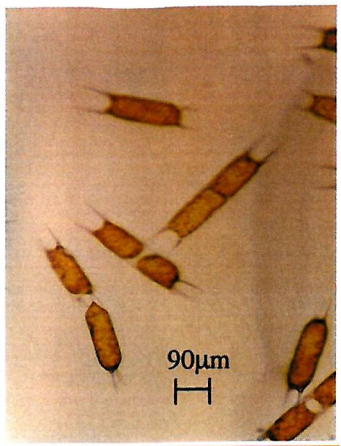
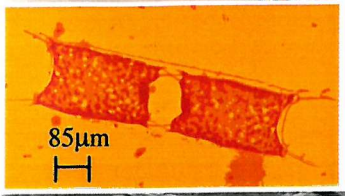
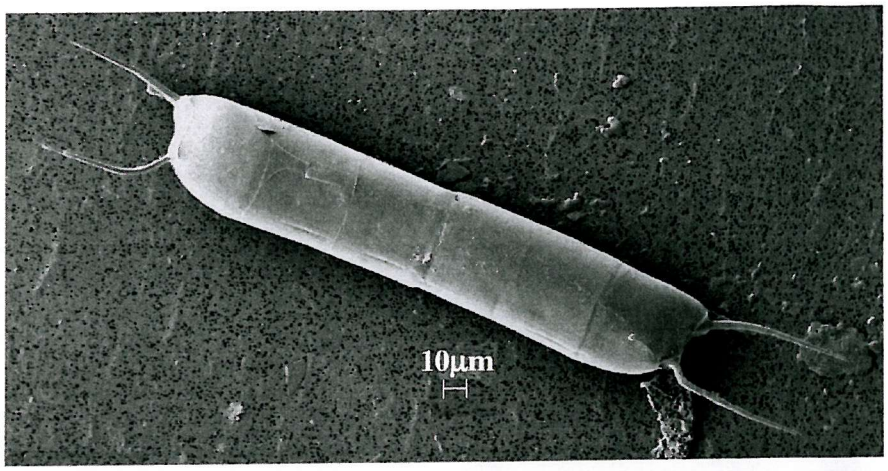


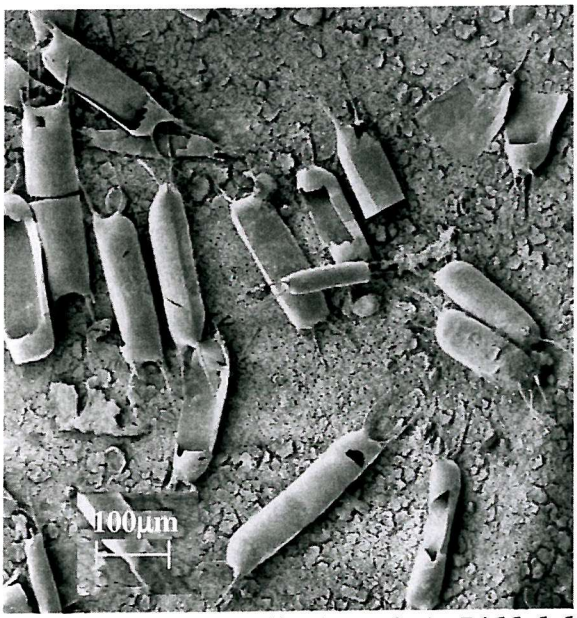
Plate 1. *Thalassiosira rotula*

Plate 2. ***Ditylum brightwellii***









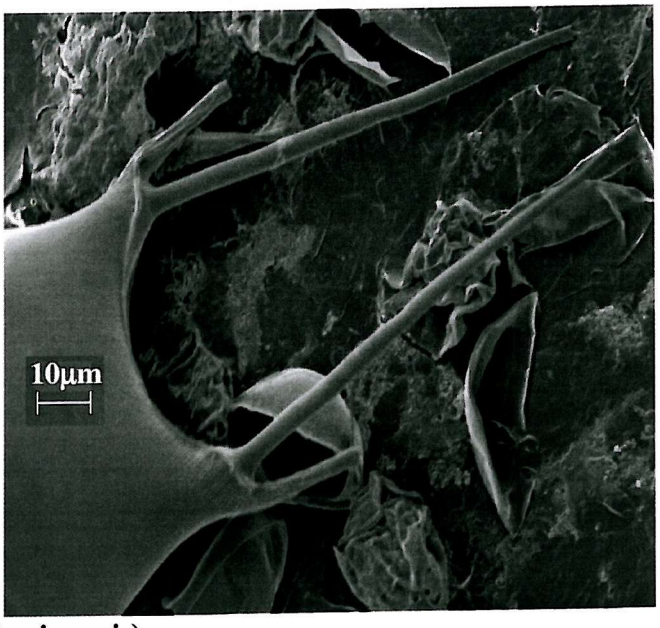
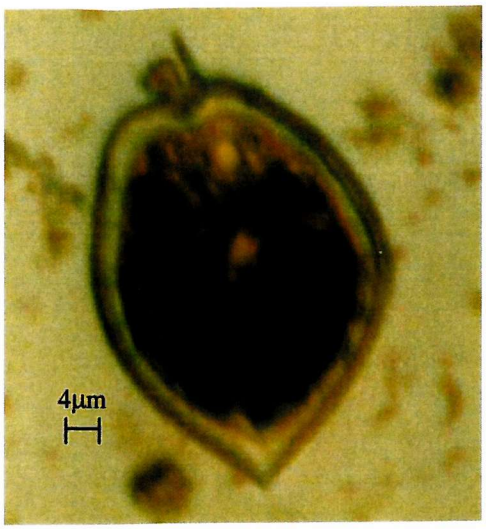
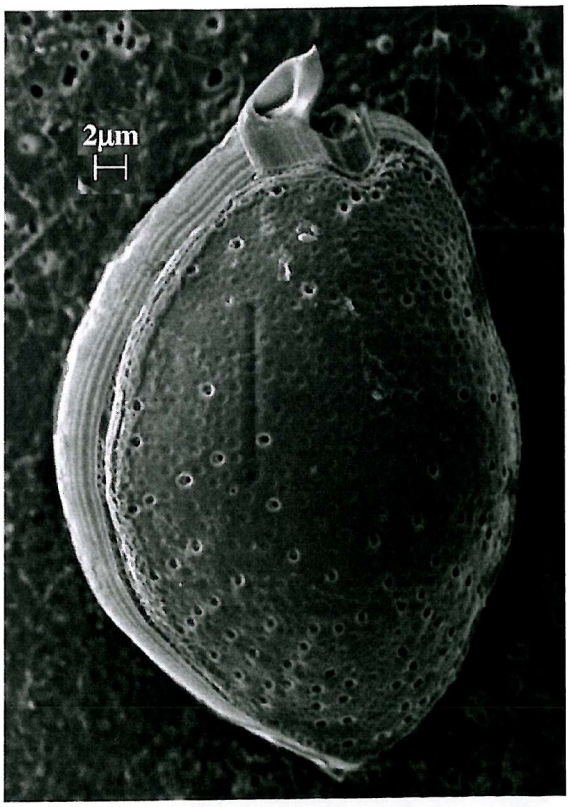
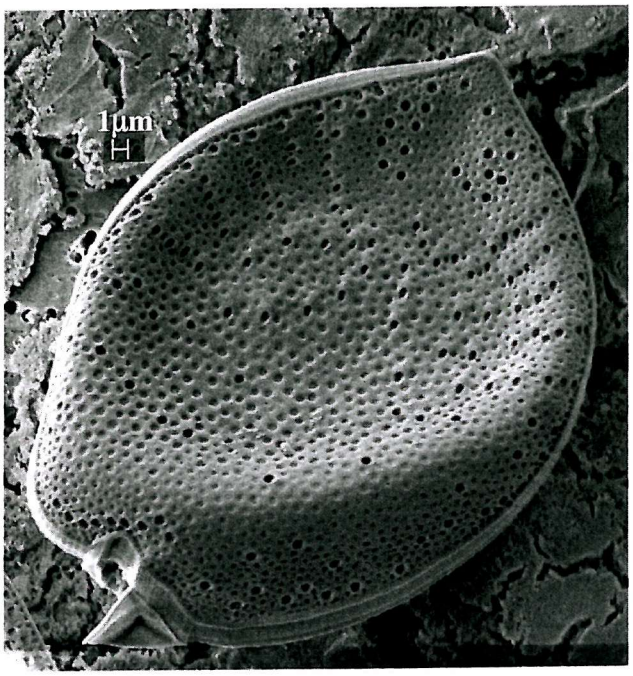
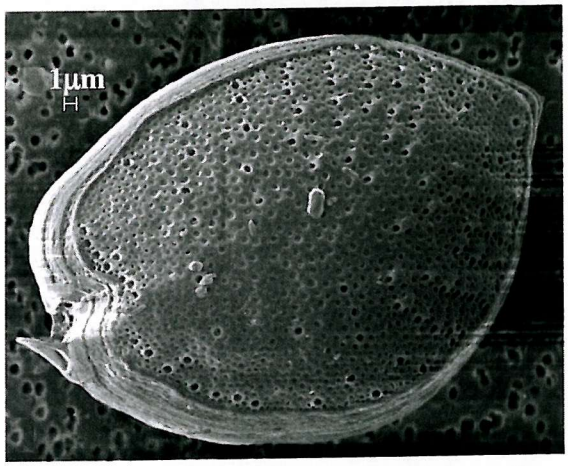


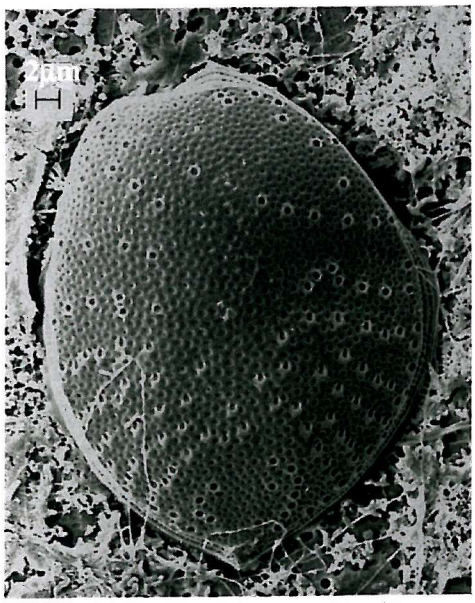
Plate 3. ***Odontella sinensis*** **(=** ***Biddulphia sinensis*****)**











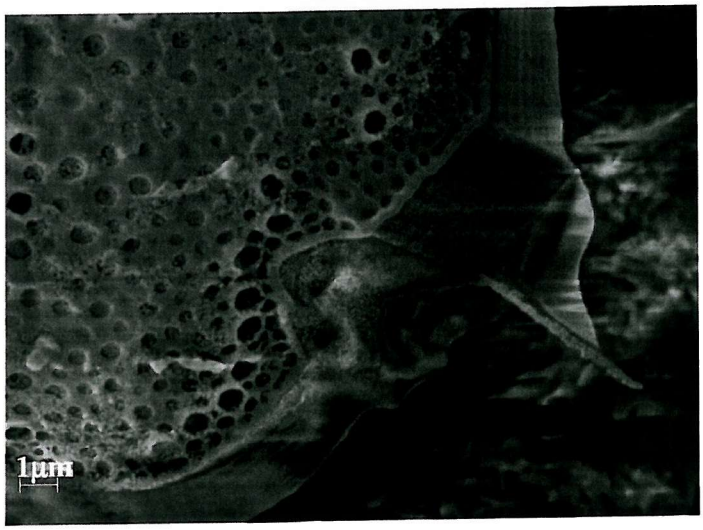


Plate 4. ***Prorocentrum micans***

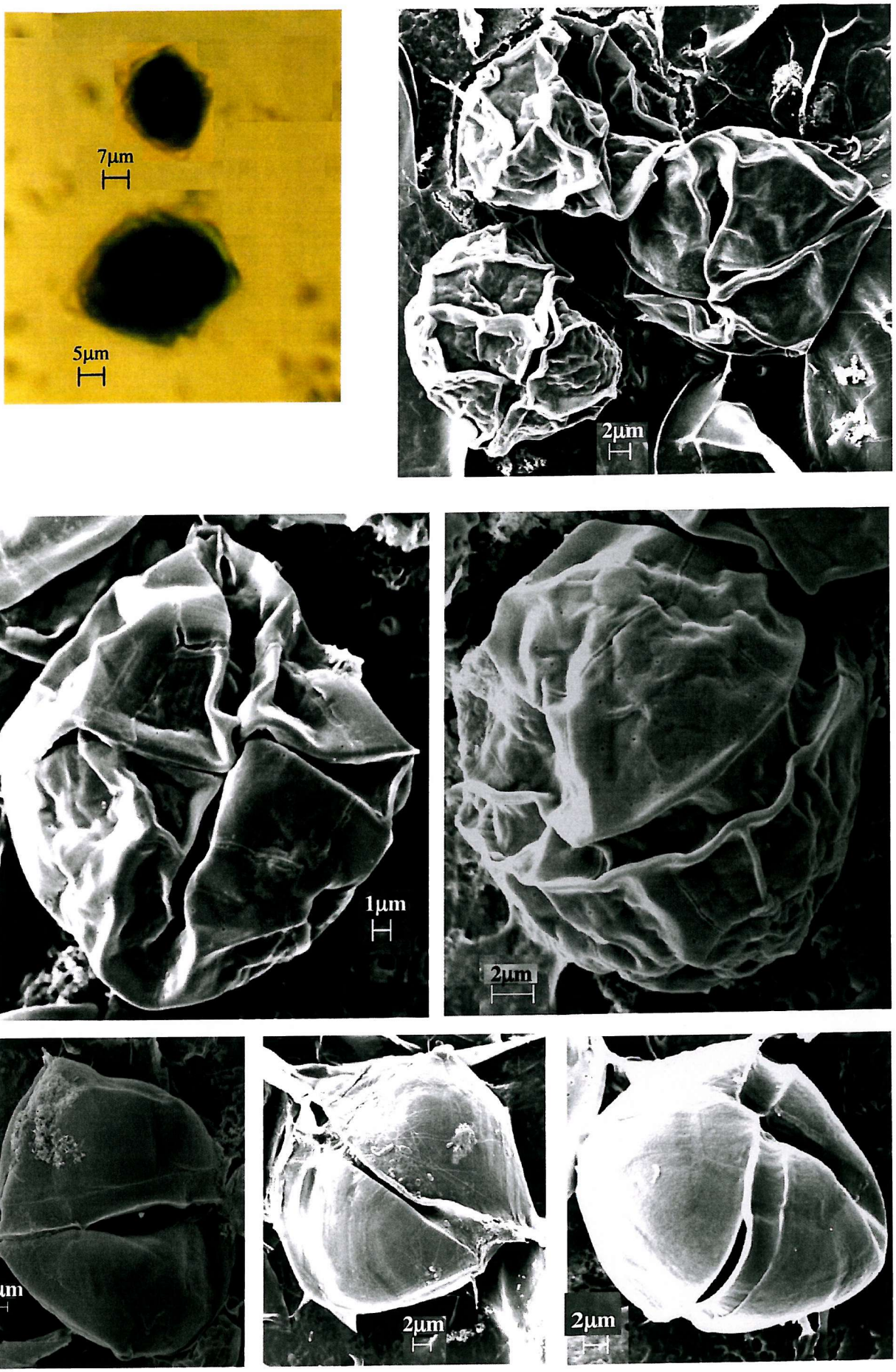
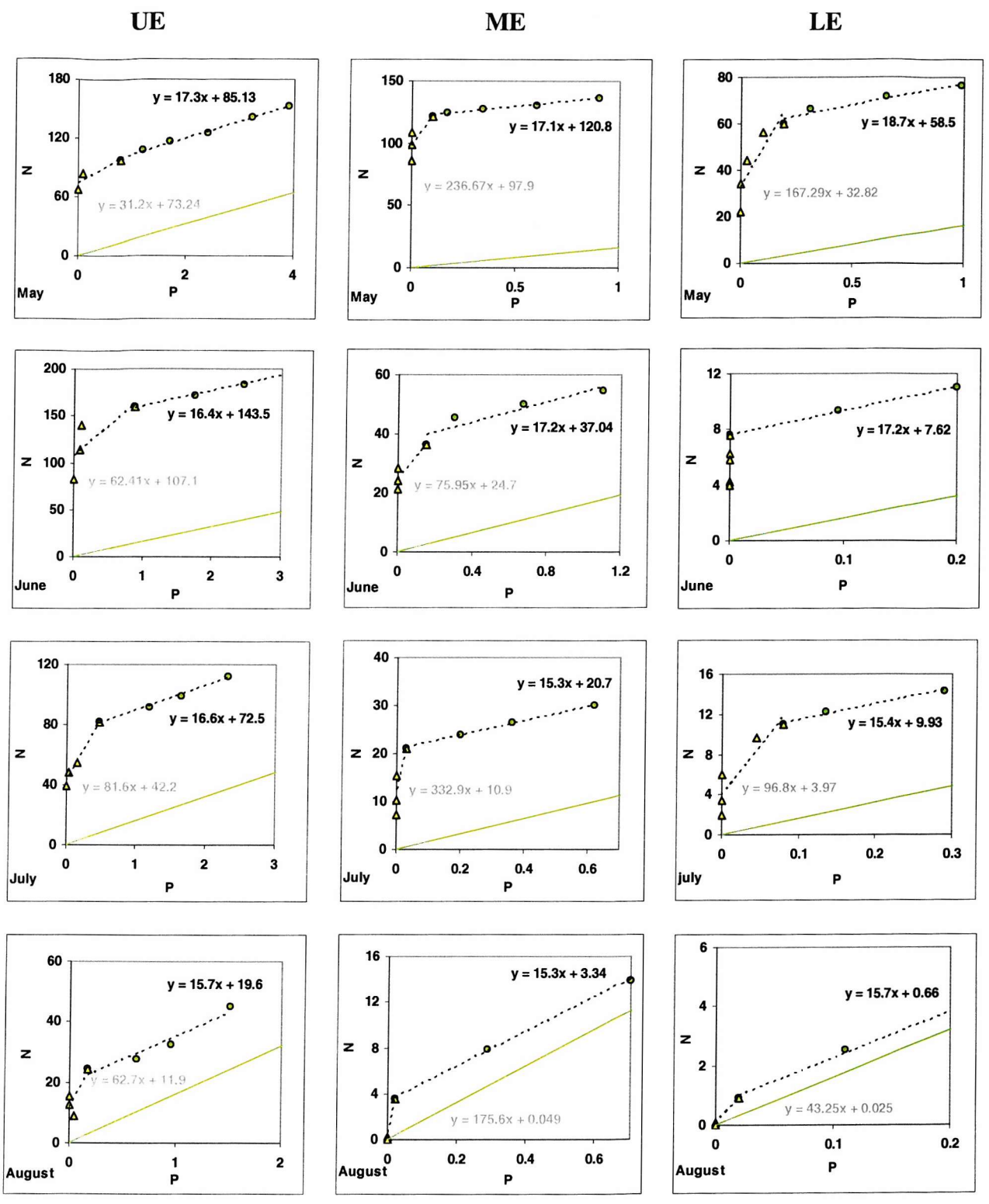


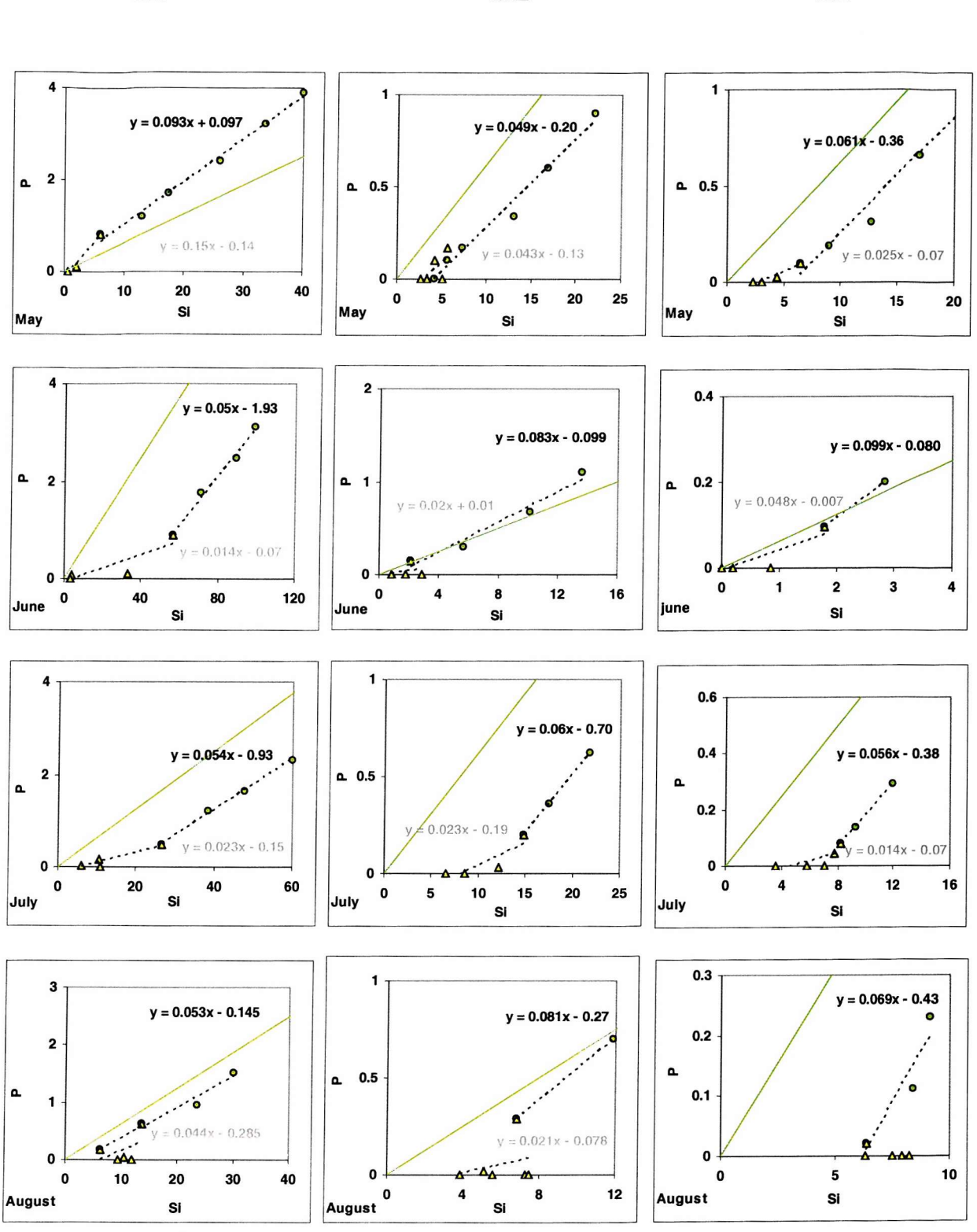
Plate 5. ***Scrippsiella trochoidea***

APPENDIX IV

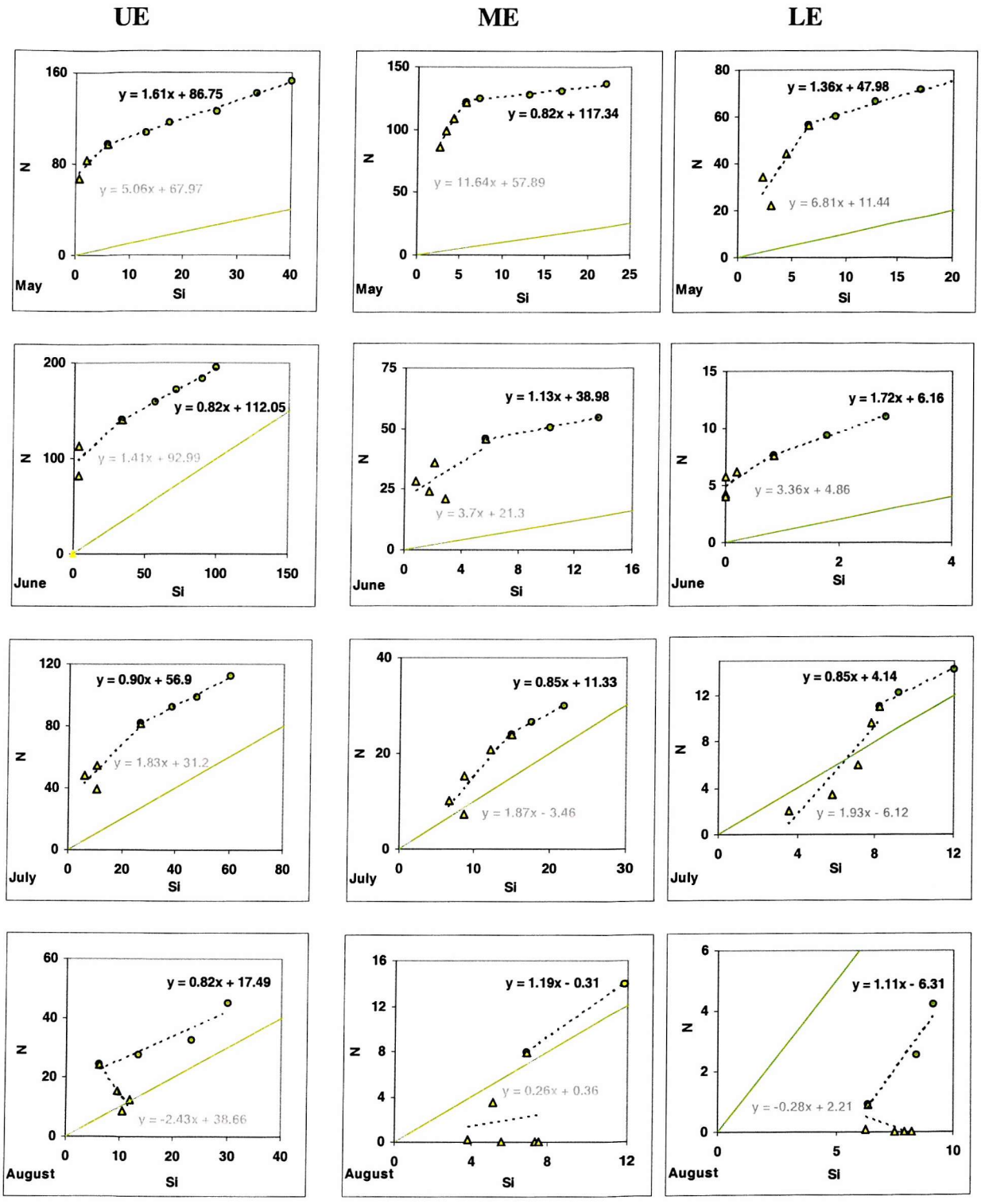
NUTRIENT RATIOS (N:P, P:Si, N:Si) BEFORE AND AFTER PHYTOPLANKTON PEAK BIOMASS IN THE INCUBATED CULTURES UNDER NON-LIMITED LIGHT CONDITIONS (See Chapter 5)



Appendix IVa. Changes in N:P ratio occurring during the 4 incubation experiments (May, June, July and August 2000) for the 3 cultures, UE (upper estuary), ME (mid estuary) and LE (coastal waters). Green circles and yellow triangles indicate uptake of N and Pi before and after the peak day (i.e. day of maximum Chl *a* concentration), respectively.



Appendix IVb. Changes in P:Si ratio occurring during the 4 incubation experiments (May, June, July and August 2000) for the 3 cultures, UE (upper estuary), ME (mid estuary) and LE (coastal waters). Green circles and yellow triangles indicate uptake of P and Si before and after the peak day (i.e. day of maximum Chl *a* concentration), respectively.



Appendix IVc. Changes in N:Si ratio occurring during the 4 incubation experiments (May, June, July and August 2000) for the 3 cultures, UE (upper estuary), ME (mid estuary) and LE (coastal waters). Green circles and yellow triangles indicate uptake of N and Si before and after the peak day (i.e. day of maximum Chl concentration), respectively.