INTER-ANNUAL VARIATIONS IN PHYTOPLANKTON ASSEMBLAGES IN THE MACROTIDAL ESTUARY SOUTHAMPTON WATER, ESTIMATED FROM MICROSCOPIC COUNTS AND HPLC PIGMENT CHEMOTAXONOMY

Elham M. Ali[1,*], Duncan A. Purdie[2] and Arantza Iriarte[3]
[1]Department of Biological and Geological Sciences, Faculty of Education (El-Arish), Suez Canal University, Egypt, [2]School of Ocean and Earth Sciences, University of Southampton, National Oceanography Centre, Southampton, UK, [3]Department of Plant Biology and Ecology, University of the Basque Country in Bilbao, Spain

ABSTRACT

Phytoplankton biomass and community structure in Southampton Water Estuary (on the south coast of the UK) have been investigated. Inter-annual changes in chlorophyll a coupled with changes in total carbon biomass have been analysed in 2004 & 2005 (during the productive period of the estuary). HPLC method has been also used to measure chlorophyll a and other accessory pigments that help, as quantitative biomarkers, to provide information on changing phytoplankton dynamics in such a highly dynamic estuary. Microscopy and chemotaxonomy give a high level of agreement phytoplankton characterization along the estuary; however, some limitations are present in both techniques. HPLC derived chlorophyll a showed a good correlation with the total phytoplankton biomass during the sampling period although it was underestimated in some samples. Fucoxanthin showed a strong correlation with total diatom biomass however high chlorophyll a concentrations during bloom time affected this relationship. Similar finding was obtained for peridinin and dinoflagellates. Although, *Cryptomonas* sp. was recorded in some samples, no correlation was detected between its biomass and alloxanthin concentration due to microscopic confusion with small flagellates that were numerically abundant at the same time. Peaks in alloxanthin were, however,

coincided with peaks in the biomass of the autotrophic ciliate *Mesodinium rubrum*. By relating the biomass of specific phytoplankton groups to their corresponding biomarker pigment, the dominance of diatoms (fucoxanthin & chlorophyll *c1+c2*) in spring and dinoflagellates (peridinin) in summer was established. Dinoflagellates as well as ciliates were found to grow better in the intermediate sites along the estuary. Combining the pigments together to give diagnostic indices shows a very similar pattern to that of Chl *a* as well as carbon biomass microscopically detected. Diagnostic pigment indices (represented as microplankton, picoplankton and nanoplankton) confirmed the previous finding that phytoplankton species succession in Southampton Water started with diatoms and followed by flagellates/ciliates and then dinoflagellates.

INTRODUCTION

In estuarine and coastal waters, phytoplankton is exposed to rapidly changing environmental conditions that may have pronounced effects on their dynamics and community structure. Phytoplankton blooms in Southampton Water, as a macrotidal estuary, are known to be short lived due to 1) rapidly changing conditions of irradiance (i.e. solar irradiance and water column turbidity) and 2) intensity of tidal mixing due to the spring-neap tidal cycle **[Holley & Hydes (2002) and Iriarte & Purdie (2004)]**. Previous analysis of phytoplankton in Southampton Water estuary revealed a sequence of different species that became numerically dominant for short periods (i.e. less than 7 days) during the productive period of the estuary **[Ali *et al.*, (2000)]** with a mixed diatom community being dominant during spring followed by a large increase in numbers of euglenoid flagellates. Dinoflagellate species grow preferably in summer coincided with high daily irradiance levels. Similar phytoplankton species succession is previously recorded along the estuary. The study of phytoplankton dynamics in such variable environments (like Southampton Water) requires sustained and frequent sampling as well as analysis methodologies that need short time and give reproducible **[Schluter *et al.*, (2000) and Garibotti *et al.*, (2003)]**. Relevant temporal and spatial scales are also a demand for accurate and reliable determination of the composition of natural phytoplankton communities **[Cloern (1996)]**.

Microscopic analysis is the most reliable technique to enumerate individual species in a mixed natural sample, but it is a time consuming

and tedious technique [**Millie** *et al.,* **(1993)**] if many samples are analyzed. For accurate identification of phytoplankton species a high level of expertise is also required [**Breton** *et al.,* **(2000)**]. Moreover, cell counting and identification often provide limited information on the small delicate phytoplankton groups that are difficult to identify [**Wong & Crawford (2006)**] or can not survive sample preservation [**Reid (1983)**]. The high performance liquid chromatography (HPLC) technique is now recognized as a powerful method in oceanography [**Mantoura & Llewellyn (1983); Barlow** *et al.,* **(1993); Jeffrey** *et al.,* **(1997); Schluter** *et al.,* **(2000) and DiTullio** *et al.,* **(2003)**] for analysis of phytoplankton pigments and their degradation products allowing the presence of dominant organisms in mixed assemblages to be evaluated. Phytoplankton accessory pigments can be used as quantitative biomarkers of some classes as well as provide information on changing phytoplankton dynamics, natural community structure and estimation of decomposition and grazing processes [**Quiblier-Lloberas** *et al.,* **(1994); Barlow** *et al.,* **(1997-1998); Breton** *et al.,* **(2000) and Pinckney** *et al.,* **(2001)**]. HPLC also allows the quantification of small phytoplankton cells (<5 $\square$m) which may be underestimated in microscopic counts [**Rodriguez** *et al.,* **(2002)**], and cells with membranes of low visibility, for example, small picoplanktonic green algae [**Breton** *et al.,* **(2000)**]. However, the interpretation of pigments using HPLC is not always clear and should be carefully compared with and validated against microscopic observations.

At a qualitative level, all previous studies of HPLC pigment analysis in oceans [**Trees** *et al.,* **(2000); DiTullio** *et al.,* **(2003) and Claustre** *et al.,* **(2004)**], lakes [**Descy** *et al.,* **(2000) and Trees** *et al.,* **(2000)**], estuaries and coastal waters [**Pinckney** *et al.,* **(1998), Brunet** *et al.,* **(1996); Breton** *et al.,* **(2000); Trees** *et al.,* **(2000) and Ansotegui** *et al.,* **(2001)**] as well as Antarctic environments [**Rodriguez** *et al.,* **(2002) and Garibotti** *et al.,* **(2003)**] and sub-arctic areas [**Obayashr** *et al.,* **(2001) and Suzuki** *et al.,* **(2002)**] verify the validity of phytoplankton pigments as reliable biomarkers using fucoxanthin as the indicative for brown algae, chl *b* for green algae, peridinin for Dinophycea and alloxanthin for Crypotophyceae. Chemotaxonomic sample analysis requires only a short time and the results are reproducible [**Schluter** *et al.,* **(2000) and Garibotti** *et al.,* **(2003)**]; however, its major limitation that cell pigment ratios change with growth conditions and species composition. This could introduce uncertainty in biomass quantification.

In addition, some classes infer different pigment signatures, e.g. Prymnesiophyceae showing four different pigments [Jeffrey & Wright (1994)] and some biomarker pigments are present in several classes [Breton *et al.,* (2000) and Rodriguez *et al.,* (2002)]. For example, chlorophyll *c3* (Chl *c3*) and 19'-hexanoyloxyfucoxanthin (19-Hex), which are pigment biomarkers of the Prymnesiophycean species *Phaeocystis* [Claustre *et al.,* (1990) and Jeffrey & Wright (1994)], are found in some 19-Hex containing-coccolithophors (e.g. *Emiliana huxleyi*) [Jeffrey & Wright (1994)]. In addition, Chl *c3*, the alternative biomarker of Prymnesiophyceae [Claustre *et al.,* (1990)] is, however, not found in all Prymnesiophyceans [Stauber & Jeffrey (1988)].

Here we monitor the inter-annual changes in phytoplankton community composition, bloom timing and bloom duration in Southampton Water Estuary using microscopic observations, with a view to examine the possibility of using HPLC chemotaxonomy in such a highly changing environment to present a descriptive study of the changes in phytoplankton pigments during the main productive period (Spring/Summer) of the estuary in 2004 & 2005. Our main question is whether these methodologies are comparable and suitable to characterize phytoplankton assemblages. Furthermore, three simple diagnostic indices are then used, by combining some pigments together, to provide purely taxonomic information of the phytoplankton community structure and species succession along the estuary.

MATERIALS AND METHODS

Study area

Southampton Water (Southern England) is a partially-mixed estuary, approximately 10 km long and 2 km wide being a north-westerly extension of the Solent (Figure 1). The estuarine system is macrotidal (mean tidal range >4 m) and water depth in the dredged deep-water channel is maintained at 10 m below chart datum to above Southampton Docks. The system receives most fresh water from the rivers Test and Itchen with a mean annual discharge of 8.8 and 3.3 m^3s^{-1}, respectively. The river Hamble also discharges into the system but makes a minor contribution compared to the other two rivers. Salinity structure along Southampton Water depends on the seasonal cycle of fresh water flow as well as the tidal-state [Phillip (1980)]. Surface salinity ranges between ~18 to > 32. However at the entrance to the system at Calshot, in the

open Solent, salinities throughout the water column generally exceeding 34 and the water column remains almost permanently well-mixed [Sylaios & Boxall (1998)].

Sample collection

Samples were collected from two sampling positions in the estuary (Figure 1), one located in the middle estuary adjacent to North-West Netley buoy (NWN), and the other near Calshot buoy (CA). Water samples were collected weekly from March to July in 2004 and from February to September in 2005. Surface water (1m depth) was collected using a 1.5-L Niskin bottle.

Phytoplankton counts

Aliquots of 100 ml were preserved in acid Lugol's iodine solution [Parsons *et al.*, (1984)] and kept in dark bottles until counted. Phytoplankton cells were counted in 10 mL sedimentation chambers using a Flouvert inverted microscope and where possible identified to species level, using [Tomas (1997)].

Fig. (1): Map of the study area showing the position of the sampling sites (1 & 2)
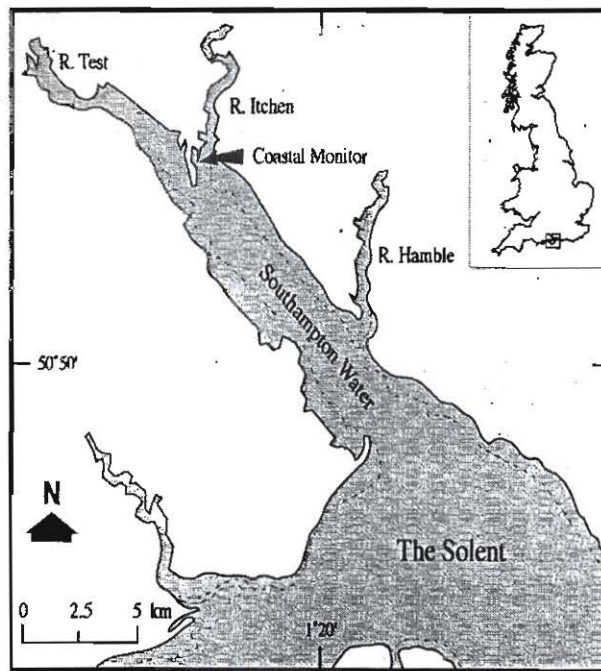
Biomass estimation

Samples for chlorophyll *a* analysis were filtered (50 ml) through 25-mm diameter GF/F filters and immediately frozen. Chlorophyll *a* was extracted in 8 mL of 90% acetone by sonication followed by centrifugation. Chlorophyll *a* was measured using a Turner AM10 fluormeter. Chlorophyll *a* concentration was determined using Parsons' equation [**Parsons *et al.*, (1984)**] and the fluormeter calibrated against a standard Chlorophyll *a* solution (Sigma Ltd.).

Total phytoplankton biomass (as mg C m^{-3}) was estimated from microscopic enumeration of cells by estimating cell volume of individually measured cells and converted to carbon using the cell volume/carbon relationship given by [**Eppley *et al.*, (1970)**] as described by [**Holligan *et al.*, (1984)**] using a standard spreadsheet algorithm provided by Derek Harbour [**Kovala & Larrance (1966)**]. However, carbon values for some species were calculated according to a recent estimate of carbon per cell volume [**Menden-Deuer & Lessard (2000)**].

HPLC Pigment Measurements
Method Outline

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 auto-sampler (AS3000), a UV detector (UV1000), a fluorometer (FL3000), integrator (SN4000) and integration software PC1000. Pigmet extracts were loaded into the auto-sampler which retained a temperature of 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

1L water samples were filtered through 47-mm GF/F filters and frozen immediately. The frozen samples were subsequently extracted in 90% HPLC-grade acetone by sonication followed by centrifugation. . The extracts were filtered through 0.2 μm Nylaflo filters and 100 μL injected into the HPLC system for pigments analysis. The ion-pairing reverse-phase HPLC technique of [Mantoura & Llewellyn (1983)] was used as described with modifications by [Barlow *et al.*, (1993)].

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 all pigments were identified by comparing their retention times with authentic standards in acetone obtained from Sigma Chemical Company or DHL, Denmark. Chlorophyll *a* standards were loaded every 6-8 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) and Dransfeld (1999)]. An inline photodiode array detector was used in this work for more accurate identification of accessory pigments. This method does not separate chlorophylls *c1* and *c2* and so these were reported together as chlorophyll *c1+c2* (Chl *c1+c2*). Table (1) gives the common accessory pigments used as biomarkers for particular groups of phytoplankton.

Simple diagnostic pigment indices [Barlow *et al.*, (2004) and Wong & Crawford (2006)] were used to give a broad indication of community structure from the raw pigment data. Three major phytoplankton groups were characterised in this work, namely microplankton, nanoplankton and picoplankton. Microplankton pigments are defined as fucoxanthin+peridinin. Nanoplankton pigments were defined as 19-hex+19-but+alloxanthin. Picoplankton pigments were defined as chlorophyll *b* + zeaxanthin.

Phytoplankton community biomass concentration estimated as C and as Chl *a* (or diagnostic pigment) for each date both sites (microscopic and HPLC results, respectively) were compared by simple linear correlations. Level of significance was set as $p < 0.01$ for all statistical analysis.

Table (1): Distribution of major accessory pigments for some phytoplankton taxa as given by [**Barlow *et al.*, (1993); Jeffrey & Vesk (1997) and Jeffrey *et al.*, (1997)**].

Algal 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, zeathanthin (Zea)
Dinoflagellates	peridinin (Peri), diadinoxanthin (Diad), fucoxanthin (Fuc)

RESULTS

Chlorophyll a dynamics

Chlorophyll *a* (Chl *a*) is a universal indicator of phytoplankton and showed wide variations over the sampling years at both sites. The first chlorophyll *a* (flurometrically measured) bloom (>10 mgm^{-3}) was mostly measured during Spring/summer (Figure 2) with higher chlorophyll *a* values at mid estuary (NWN) compared with values measured at the mouth of the estuary (Calshot). In 2004, the first and only major chlorophyll *a* peak recorded at Calshot delayed until end of June (~11 mgm^{-3}) and lasted only for short period (less than a week). This peak was dominated by diatoms (mainly *Guinardia delicatula, Rhizosolenia setigera* and *Cerataulina pelagica*). While the delayed major chlorophyll *a* peak at NWN lasted for a longer period and was dominated by a mixture of both diatoms (mainly *Rhizosolenia setigera*) and dinoflagellates (*Scrippsiella trochoidea*). In 2005, Many peaks of chlorophyll *a* were recorded at both sites compared to that in 2004 with 5 and 7 major blooms (>10 mgm^{-3}) measured at Calshot and NWN, respectively (Fig. 2).

The early phytoplankton peak events recorded over the sampling period were, in general, dominated by diatoms (with *Guinardia delicatula* and

Thalassiosira rotula the most abundant) at both sites (see Figures 3 & 4); however dinoflagellates (mainly, *Scrippsiella trochoidea*) dominated the late spring/early summer ones, particularly at NWN.

Fig. (2): Distribution of flurometrically estimated Chl *a* at Calshot (CA) and NW Netley (NWN) during 2004 and 2005.
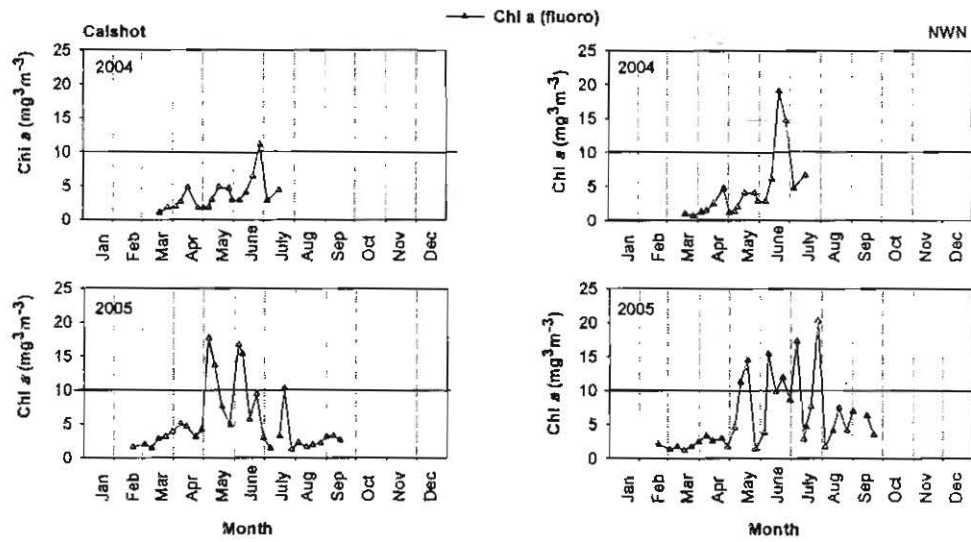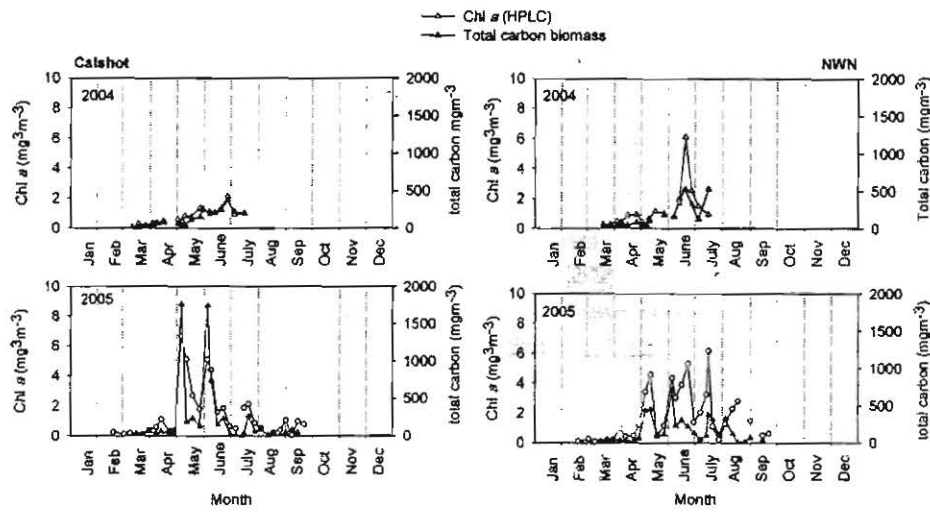
Fig. (3): Distribution of HPLC estimated Chl *a* at Calshot and NWN in relation to variations in total phytoplankton biomass during 2004 and 2005

Phytoplankton biomarker pigments and Community composition

About 20 pigments were recorded from the HPLC absorbance chromatogram, among which (in addition to Chlorophyll *a* as an indicator of the total phytoplankton biomass) 7 important pigments (> 0.1 mgm^{-3}) were selected as primary taxonomic markers of the dominant phytoplankton groups; fucoxanthin (Fuc) for diatoms together with Diadinoxanthin (Diad) and chlorophyll *c1+c2* (Chl c1+c2), Peridinin (Peri) for dinoflagellates, alloxanthin (Allo) for cryptophytes, chlorophyll *b* (Chl *b*) for chlorophytes and chlorophyll *c3* (Chl *c3*) for prymnesiophytes. Some other pigments (e.g. 19' hexanoyloxyfucoxanthin, 19'butanoyloxyfucoxanthin, violaxanthin, zeaxanthin and prasinoxanthin) were recorded in small traces (~ <0.1 mgm^{-3}) in some samples of which, 19' hexanoyloxyfucoxanthin (19'Hex) was included in the next analysis as an important pigment for prymnesiophytes. Ranges of the selected pigment concentrations are given in Table (2) .The breakdown products (phaeophorbides *a1* & *a2* and phaeophytin *a1* & *a2*) are not included in the following data analysis as the indications beyond these products are not of this work interest; however, they were detected in the HPLC chromatogram.

The pattern of change in total phytoplankton biomass (expressed as total carbon) was similar to that of HPLC measured chlorophyll *a* (see Figure 3), although some variations were detected on some dates (e.g. at NWN in 2005). For example, two major peaks (>10 mgm^{-3}< 20 mgm^{-3}) of chlorophyll *a* was measured in July and a smaller peak (<10 mgm^{-3}) in August at NWN, however, no peaks in carbon biomass were microscopically detected at the same time.

Microscopic analysis of water samples during both sampling years revealed that phytoplankton community composition was mainly dominated by diatoms and dinoflagellates, while smaller-sized species (e.g. flagellates) were numerically abundant in some water samples. HPLC data showed that over the sampling period fucoxanthin (see Figures 4 & 5) and chlorophyll *c1+c2* (not shown) were the most abundant taxonomic pigment in spring/early summer at both sites, indicating that diatoms dominated the phytoplankton assemblages at this time of the year. Fucoxanthin (Fuc) generally showed temporal variations during the sampling period with highest peaks recorded during May at the time of the diatom bloom of *Guinardia delicatula*, whereas, in 2004 the biomass peak delayed until end of June at both sites when a diatom mixture of the genera *Guinardia delicatula* and *Cerataulina pelagica* was dominant. Other relatively high peaks of fucoxanthin were measured late in July at NWN in all years. These peaks mostly coincided with the bloom of *Chaetoceros spp.* and small pennate diatoms (e.g. *Nitzschia closterium, Nitzschia serriata*). Although, high Fucoxanthin peaks were recorded at NWN (figure 5) in 2005 (July and August), these peaks could not be identified microscopically, this could be due to the dominance of smaller pinnate diatoms (<2µm) that difficult to recognised by light microscopy. A strong correlation (p <0.001) between Fucoxanthin concentrations and Chlorophyll *a* was recorded in both years with r values ranged between 0.88-0.96, for both years, indicating the dominance of diatoms in Southampton Water Estuary. Highest concentrations (maximum 0.45 mg m^{-3} in 2004 and 0.39 mg m^{-3} in 2005) of Chlorophyll *c1+c2* (included in most diatoms) were measured during spring bloom (May/June) of the diatom *Guinardia delicatula* (at Calshot) and *Thalassiosira & Nitzschia* (at NWN). A good correlation of Chl *c1+c2* however less than that of fucoxanthin, to Chllorophyll *a* was found (r = 0.73) with a more scattered plot between both variables especially when large-celled chlorophyll-rich diatoms (e.g. *Guinardia delicatula & Rhizosolenia setigera*) were mostly abundant. Variations in

cell pigment/chlorophyll *a* ratio among species could be an explanation of the discrepancy of the scatter plot.

Fig. (4): Distribution of 4 specific biomarker pigments (Fuc, Peri, Allo and, Chl *b*) at Calshot and NWN in relation to variations in carbon biomass of the relevant group and/or species identified during 2004.
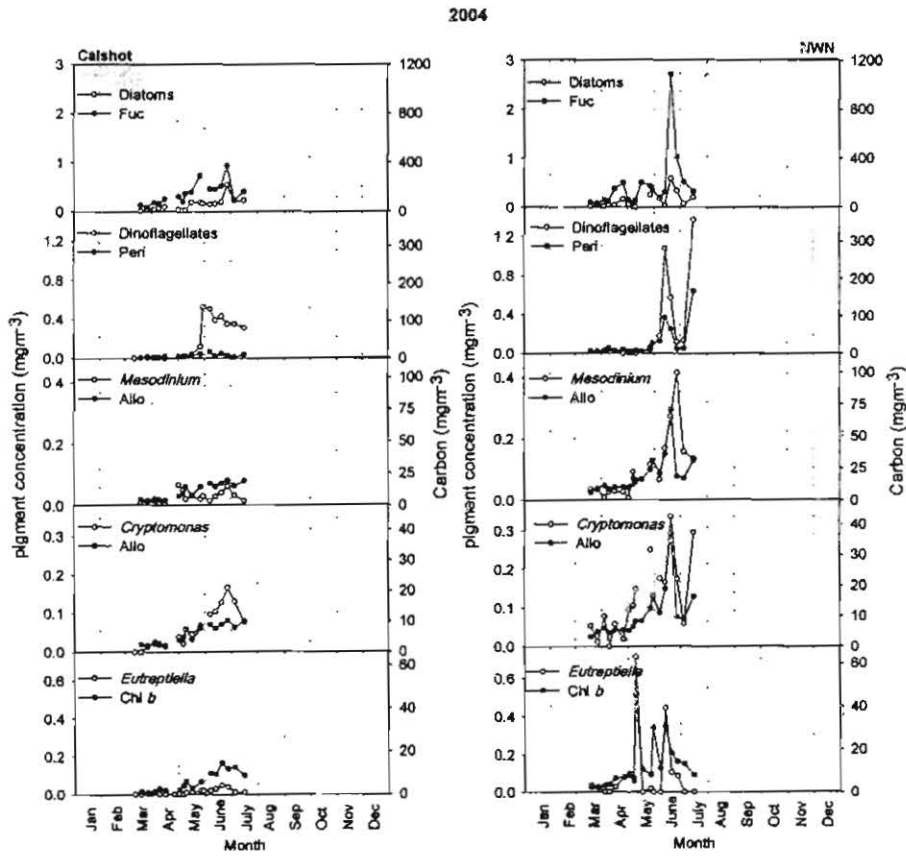
Fig. (5): Distribution of 4 specific biomarker pigments (Fuc, Peri, Allo and, Chl
b) at Calshot and NWN in relation to variations in carbon biomass of
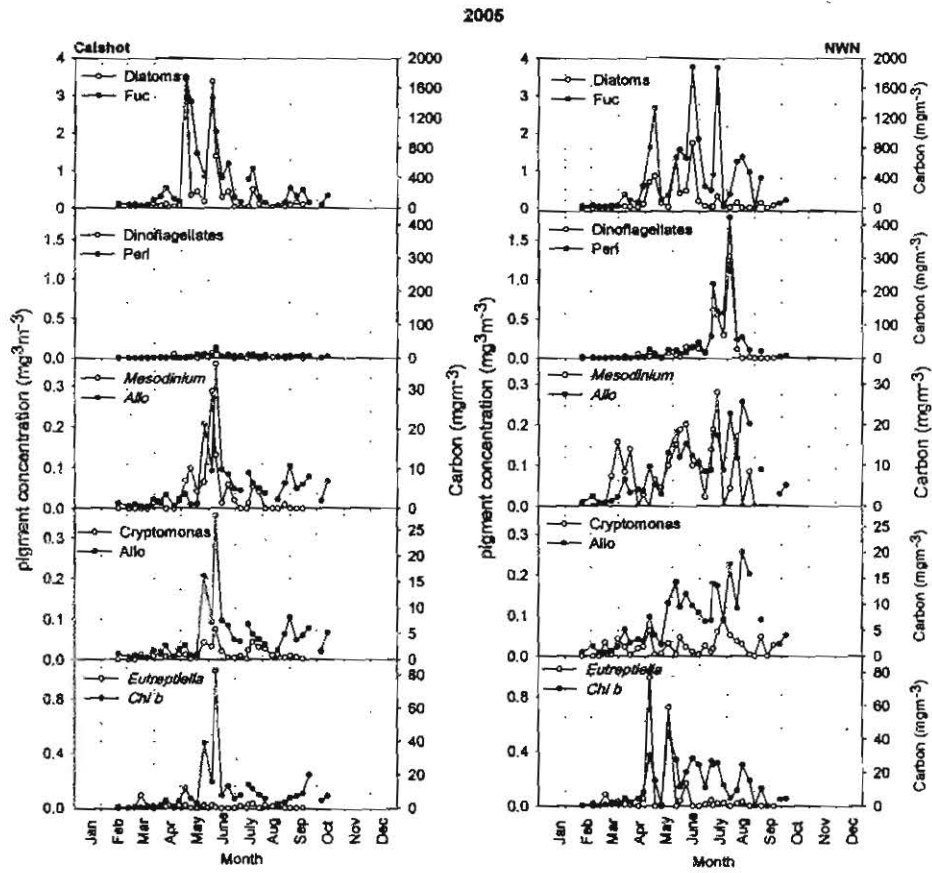the relevant group and/or species identified during 2005.

Diadinoxanthin (Diad), which is a dark-induced pigment included in most diatoms, was found with variable concentrations ranging between 0.01–0.48 mg m^{-3} (Table 2) during both sampling years with highest values of 0.42 and 0.48 mg m^{-3} at NWN in 2004 and 2005, respectively. The temporal and spatial variations in diadinoxanthin concentration (data not represented) and correlation with Chlorophyll *a* were similar to that of fucoxanthin, however, a scattered correlation was found between Diadinoxanthin and chlorophyll *a* in some occasions for the reason mentioned above.

Low concentrations of peridinin (Peri), the major carotenoid for autotrophic Dinophyceae, were mostly measured in spring at both sites during the sampling period (see figures 4 & 5) but increased towards the end of the sampling period with highest concentrations in summer (July/August). Maximum concentration in Peri of 1.78 mgm^{-3} was detected at NWN in 2005. This was during the summer dinoflagellate bloom, which was mainly composed of *Scrippsiella trochoidea* (with *Prorocentrum micans* & *Gymnodinium sp.* in some samples). insignificant correlations between peridinin and Chlorophyll *a* (r = 0.3) was generally recorded over the sampling period, indicating that dinoflagellates, with the exception of August bloom, contributed less to the total phytoplankton biomass along the estuary over the sampling period. This could be also related to the exclusive abundance of the relatively large-sized diatoms (e.g. the dominance of *Guinardia delicatula* & *Rhizosolenia spp.* during spring/summer in both years). Dinoflagellates were, however much contributed to the total phytoplankton biomass 2004 compared to 2005. It is worth mentioning that peridinin concentration was much higher at NWN (the middle part of the estuary) compared to that in coastal waters. This indicated that diatom species replaced dinoflagellates with increasing water turbulence. Dinoflagellates are known to live preferably at NWN where high daily irradiance and calm water state compared (Ali, *pers. Comm.*).

Alloxanthin (Allo), the major biomarker of Cryptophyceae was detected with different concentrations during sampling period with a range of 0.01 – 0.29 mg m^{-3} in 2004 and 0.01 – 0.33 mg m^{-3} in 2005 Table (2). Although variations in alloxanthin and biomass of the cryptophycean species, *Cryptomonas* showed no similarities in most dates (see Fig. 4 & 5), smaller peaks of alloxanthin were coincided with peaks of the biomass of *Cryptomonas* during 2004 (Fig. 4) and 2005

(Figure 5) when this species was numerically abundant and achieving high population (> 250 cell/ml). Modest to strong correlations (r = 0.6 – 0.76) were found between the pigment and chlorophyll *a* in all years particularly at NWN indicating that smaller flagellates (< 5µm) were significantly contributed to phytoplankton population at this site and was difficult to recognize by light microscopy. Some peaks of alloxanthin were coincided with peaks of the biomass of ciliate *Mesodinium rubrum* in 2004 (Fig. 4) and 2005 (Fig. 5), particularly at NWN when this species was achieving higher population. Concentrations of chlorophyll *b*, the carotenoid pigment of green algae widely varied over the sampling period (Figs. 4 , 5) but occurred in lower concentrations (0.00-0.54 mg m^{-3}) compared to other major pigments at both sites (higher at NWN) indicating that green algae (i.e. Chlorophyta) were relatively less contributing to the total phytoplankton community in Southampton Water (compared with Bacillariophyceae and Dinophyceae). Highest levels in Chl *b* was recorded during year 2005 (0.48 & 0.54 mg m^{-3}), particularly at NWN. Despite the fact that peaks in the biomass of the flagellate *Eutreptiella marina* in 2004 and 2005 (during May - June) coincided with peaks in Chl *b* (Fig. 4, 5) and the fact that Chl *b* correlated significantly to chlorophyll *a* in both years (r = 0.77 in 2004 and r = 0.79, in 2005), poor correlation (r < 0.3) was found between this carotenoid and the biomass of *Eutreptiella marina*. This finding might indicate that this flagellate is not the only chlorophyceans in Southampton Water.

Table (2): Range of Concentrations (minimum and maximum) of the specific pigments detected from Southampton water Estuary in 2004 and 2005. Values in brackets are for 2005.

		Chl a	Chl c1+c2	Chl c3	Chl b	Fuc	Peri
2004	minimum	0.19 (0.19)	0.00 (0.00)	0.00 (0.00)	0.01 (0.02)	0.09 (0.08)	0.00 (0.00)
	maximum	2.12 (6.10)	0.18 (0.45)	0.11 (0.15)	0.17 (0.41)	0.94 (2.72)	0.06 (0.06)
2005	minimum	0 04 (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.06 (0.03)	0.00 (0.00)
	maximum	6.65 (0.14)	0.20 (0.39)	0.80 (3.10)	0.48 (0.54)	3.43 (3.76)	0.13 (1.18)

19 Hex	19 But	Allo	Zea	Diad
0.01 (0.01)	0.00 (0.00)	0.02 (0.02)	0 00 (0.00)	0.01 (0 01)
0.08 (0.08)	0.02 (0.03)	0.08 (0.29)	0.01 (0.03)	0.09 (0.42)
0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.55 (0.31)	0.14 (0.11)	0.36 (0.26)	0.45 (0.22)	0.33 (0 48)

Chlorophyll *c3* (Chl *c3*) was mostly found in low concentration (0.0 – 0.15 mg m^{-3}) at both sites apart from three high peaks recorded in 2005 particularly at NWN during summer (see Fig. 6). These peaks of chlorophyll *c3* might indicated the presence of Prymnesiophytes or/and Chrysophytes (both include Chlorophyll *c3*), of which most species are small in size (< 2µm) and are difficult to recognize microscopically. This

finding might explain the last 3 un-identified fucoxanthin peaks (see Figure 5) during July and August at this site (both Prymnesiophytes and Chrysophytes include fucoxanthin as well as Chl *c3*). Lowest concentrations in Chl *c3* were generally measured in year 2004 (Fig. 6). Microscopic analysis of phytoplankton confirmed that no prymnesiophycean species were noticed at this year. In contrast, *Phaeocystis* was numerically dominated the mid May phytoplankton community at Calshot; however no noticeable peak in chlorophyll *c3* was measured at this time.

Fig. (6): Variations in Chl c3 and 19 Hex at Calshot and NWN during 2004 and 2005.
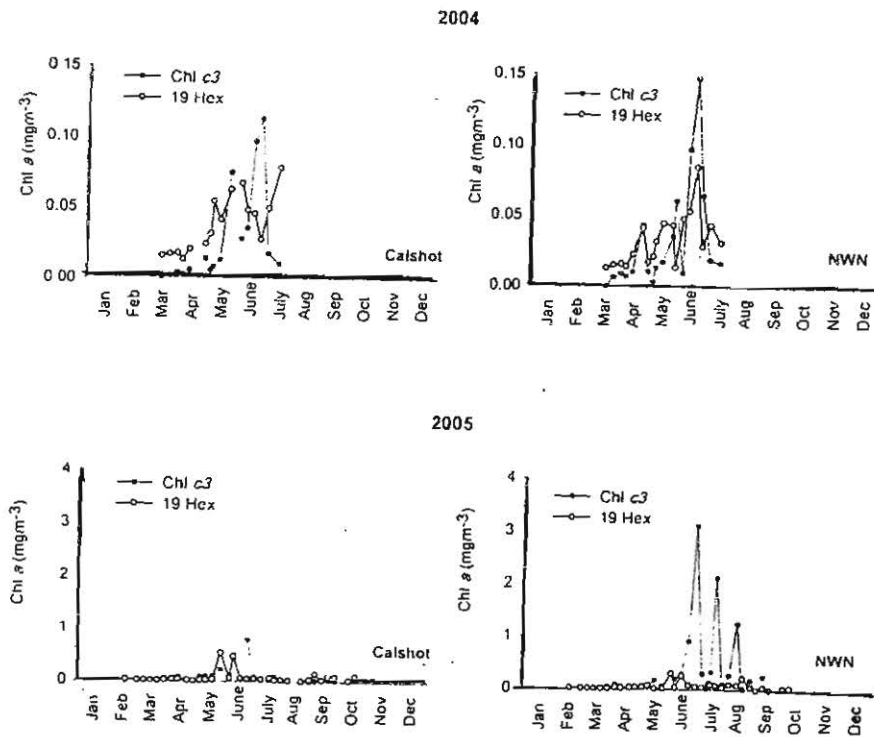
Combining the pigments together to give diagnostic pigment indices (DP), it revealed a sequence of different phytoplankton species that became dominant for short period at both sampling site. DP indices (fig. 7) showed a very similar pattern to that of carbon biomass (Fig. 4 & 5) and confirmed the species succession detected microscopically. DP indices (Fig. 7) showed that the early spring (May/June) phytoplankton community was mainly dominated by microplankton, with diatoms being most numerous, followed by a noticeable increase in picoplankton and/or nanoplankton in June/July. Microplankton peaked again in early summer (July August) at the time when dinoflagellates were over dominant.

Fig. (7): Evaluation of diagnostic pigment indices at Calshot and NWN during 2004 and 2005. These indices sum the relevant pigments in 3 broad fractions of the phytoplankton community to give an idea of the pigment content of microplankton (micro), nanoplankton (nano) and picoplankton (pico). See methods for details.
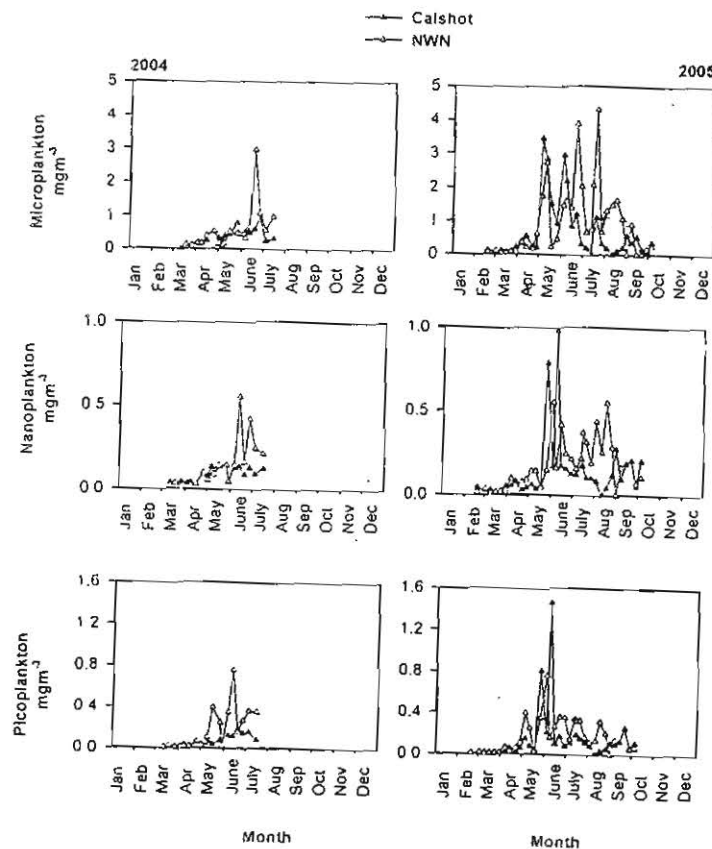
DISCUSSION AND CONCLUSION

Pigment concentrations and internal relationship

Total chlorophyll *a* concentration from HPLC and acetone extraction (Fluorometric chlorophyll *a*) showed a good significance (r = 0.94, *p* < 0.001) between both methods (Figure 8a) with relatively similar variation patterns (see figure 2 & 3) although the later was always overestimated by a mean value of 0.2% - 38%. This could be due to the interference of other pigment and chlorophylls according to the method used (Barlow, *pers. comm.*)

Figure. 8b showed that HPLC measurements of Chlorophyll *a* significantly (*p*> 0.001) correlated with that of total accessory pigments (carotenoids, Chlorophyll *c* and Chlorophyll *b*) with a mean correlation coefficient (r) range of about 0.91 close to the value (r = 0.89) previously suggested by **Trees** (italic) (2000). Such relationship indicates that Chlorophyll *a* can be used as internal comparison of HPLC measurements of other pigments This also indicates that Chlorophyll *a* concentrations are related to the concentration of accessory pigments despite variations in cell physiology and community structure which, might led to a more scattered relationship between both variables (lower correlation coefficient) and increase the discrepancy of the scatter plot. HPLC measured chlorophyll *a* showed a very similar spatial and temporal variation to that of the total phytoplankton biomass over the sampling period (Fig. 3), with a significant correlation (r = 0.75, Fig. 8c). HPLC measured Chlorophyll *a* was, however, not always give a good estimation [**Breton** *et al.*, (2000)] of the total phytoplankton biomass. In this work, some variability between both variables was, however, detected at some dates (e.g. at NWN in 2004 (July) and in 2005 (August)) this could be related to the differences in carbon/chlorophyll ratio among phytoplankton species. This might occurred when species with high chlorophyll content species are over dominated. Difficulty to distinguish heterotrophic phytoplankton species during identification and counting might lead to incorrect biomass evaluations causing. [**Breton** *et al.*, (2000)] recommended using Chlorophyll *a* as a biomass indicator should be undertaken with caution according to the environmental conditions (e.g. nitrogen depletion, light stress and seasonal variations). Highest concentrations of both variables was detected during June 2004 at both sites (maximum at NWN) during the diatom bloom (mainly *Guinardia* sp.), however, in 2005, more than one peak of both

phytoplankton biomass indicators (Chl *a* and carbon) were detected during the period from May to early August. Phytoplankton community structure widely changed during that time at both sites from a diatom-dominating community in May/June; *Guinardia delicatula* during May and *Thalassiosira rotula* during June. Both diatom species are common in Southampton Water and known to achieve high abundance [**Kifle & Purdie (1993)**]. Dinoflagellate species (mainly, *Scrippsiella trochoidea*) was then replaced diatoms (during July) until diatoms flourished again at the end of July/August with a different species composition (*Chaetoceros spp.* and *Cosinodiscus* were dominated). Flagellates (e.g. *Cryptomonas*) were numerically over dominating in June, when and after *Thalassiosira* sp was abundant. A similar succession of phytoplankton species was previously reported for Southampton Water [**kifle & Purdie (1993); Crawford & Purdie (1993) and Ali *et al.*, (2000)**]

Good agreement was found between the concentration of the biomarker pigments and the biomass of their respective class and/or species at both sites (see figure 4 & 5). Diatoms (r = 0.75) and dinoflagellates (r = 0.82) showed the best correlation with their respective pigment marker fucoxanthin and peridinin [**Jeffrey & Wright (1994)**], respectively. It was clearly identified, from the HPLC pigment analysis that dinoflagellates tend to grow favourably during summer, with increasing irradiance level and reducing rainfall and the flow rate. It was also noticeable that they grow better in intermediate sites in relatively calm water environment than in highly turbulent waters (coastal waters). Although, higher diatom biomass recorded in mid estuary, they grow also well in coastal waters, however, the diatom community composition may vary. Microscopic analysis of phytoplankton showed that the growth peak of the diatom *Guinardia delicatula* occurred in May/early June at both sites with a maximum level at coastal waters. This diatom was previously reported to in Southampton Water forming blooms during May [**Kifle & Purdie (1993)**].

Alloxanthin is the pigment biomarker for Cryptophycea (Jeffrey et al., 1999). The quality of the relationship between both variables is well documented [**Jeffrey & Vesk (1997)**]. Alloxanthin is however showed no correspondence with Cryptophyceae (only *Cryptomonas* sp was reported in this work). This was due to the confusion occurred during microscopic count when other small flagellates (2 µm) were numerically very abundant (>1400 cells ml^{-1}). Peaks in alloxanthin were,

however, coincided with peaks of the autotrophic ciliate, *M. rubrum* on some dates. This seemed to be due to endosymbiont [**Jeffrey & Vesk (1997)**] as described by [**Gieskes & Kraay (1983)**]. Alloxanthin, is detected in the ciliate *Mesodinium rubrum* [**Hibberd (1977)**], which could contain cryptophytes as endosymbionts as described by [**Gieskes & Kraay (1983)**]. Presence of some specific pigments in several species and/or groups could give false or inaccurate indications [**Breton *et al.*, (2000) and Rodriguez *et al.*, (2002)**], which is one of the limitation in the chemotaxonomic methodology.

Fig. (8): Plot and regression results (r value and significance level, p) of a) fluorometric Chl-a concentration (mgm^{-3}) versus the HPLC measured Chl a (mgm^{-3}), b) HPLC measured Chl a versus total accessory pigments and c) HPLC measured Chl a versus total phytoplankton biomass. All measurements during 2004 & 2005 of both sites were grouped for the regression line.
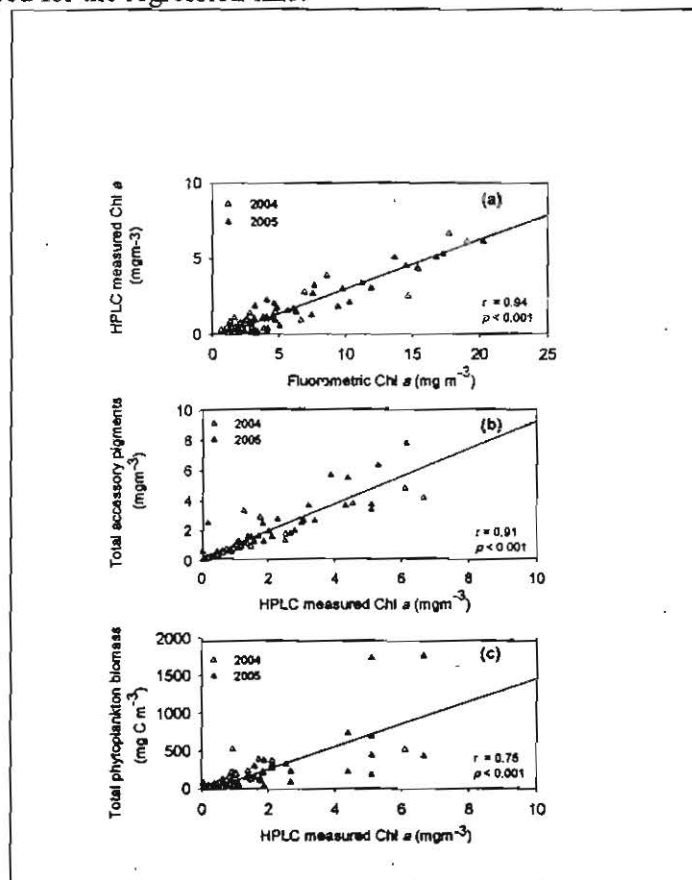
A modest to poor correspondence (r = 0.44) was estimated between Chlorophyll *b* and the total biomass of green algae (mainly *Eutreptiella marina*) in most samples analysed from Southampton water. The reason for not obtaining a stronger relationship between both variables could be explained by the small cells of green algae that could be missed in microscopic counting. A study conducted in the eastern English Channel [**Breton *et al.*, (2000)**] supported this finding. The fact that small flagellates may not survive sample fixation [**Reid (1983) and Booth *et al.*, (1993)**] could be another explanation for the poor relationship between both variables.

Diagnostic pigment indices confirmed that the phytoplankton community was dominated by microplankton, changed to a picoplankton and/or nanoplankton. This finding confirms the general phytoplankton species succession; microplankton (diatoms) – picoplankton/nanoplankton (flagellates & ciliates) – microplankton (dinoflagelates), previously known [**Kifle & Purdie (1993); Ali *et al.*, (2000) and Iriarte & Purdie (1994 & 2004)**] for Southampton Water Estuary.

To conclude, the comparison of the microscopic and HPLC pigment techniques allowed us to evaluate several methodological issues for monitoring phytoplankton distribution and species diversity. The high agreement between microscopy and chemotaxonomy found in this study suggests that both methodologies can be used efficiently for the characterization of phytoplankton community of estuaries and coastal waters. Both techniques revealed changes in phytoplankton species composition and biomass along the estuary and succeeded to identify and quantify the dominant group of different phytoplankton assemblages and in such highly variable system, but so me limitations are present in both techniques.

The use of specific biomarker pigments analysed by HPLC method of water samples collected from Southampton water provided considerable insight into the seasonal variability of phytoplankton community composition and species succession throughout the estuary. Microscopic observations is, however, still needed to identify the taxa contributing to these specific accessory pigments as recommended also by [**Ansotegui *et al.*, (2001) and Breton *et al.*, (2000)**]. It is also

recommended the use of scanning electron microscopy and epifluorescence microscopy for small cells. Finally, a further research is needed to assess the correct application of the chemotaxonomy to ecological studies of natural phytoplankton assemblages.

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التغيرات السنوية في مجتمع الهائمات النباتية في مصب ساوث هامبتون (الساحل الجنوبي-
المملكة المتحدة) باستخدام طريقتي التعريف/العد الميكروسكوبي و تصنيف
الصبغي/الكيميائي HPLC

إلهام محمود علي – دانكان بيردي – أرنثا إريارتي

تم فحص ودراسة الكتلة الحيوية ومكونات مجتمع الهائمات النباتية في مصب مياه
ساوثهامبتون على الساحل الجنوبي للمملكة المتحدة. وقد تم تحليل التغيرات السنوية في
محتوى الكلوروفيل بالاقتران مع التغيرات في إجمالي الكتلة الحيوية (مقدرة بالمحتوي
الكربوني) في عامى ٢٠٠٤- ٢٠٠٥. تم استخدام جهاز ال HPLC لقياس الكلوروفيل وباقي
الأصباغ الأخرى. وبمقارنة النتاج الخاصة ب HPLC (الكيموتكسونومى) مع مثيلتها من
التحليل الميكروسكوبى أظهرت النتائج مستوى عال من توصيف العوالق على طول المصب
باستخدام تقنية ال HPLC (رغم وجود بعض القيود في هذه التقنية) كما أظهرت ترابط جيد
بين اجمالى الكتلة الأحيائية للهائمات النباتية وإجمالي الكلوروفيل كدليل أساسي خلال فترة
أخذ العينات بالرغم من قلتها في بعض العينات.

أظهرت النتائج أيضاً علاقة طردية جيدة بين تركيز بعض الأصباغ والتي تساعد
(بوصفها كدلائل لبعض الأجناس/العائلات الهائمات النباتية) على تقدير مدي تواجد/سيادة
بعض الأجناس/العائلات دون غيرها مما يساعد علي تقديم معلومات عن التغيير الديناميكى
للعوالق في مثل هذه المصبات ذات الديناميكية العالية دون اللجوء للتحليل الميكروسكوبى
الذي رغم دقته، إلا أنه يحتاج إلي الكثير من الوقت،الجهد بالإضافة إلي الخبرة العالية.على
سبيل المثال: أظهرت النتائج ترابط قوي بين صبغ fucoxanthin وإجمالي الكتلة الأحيائية
للدياتومات Diatoms بالرغم من التركيزات المرتفعة للكلوروفيل أثناء وقت البلوم (زهرة
النمو) والذى اثر على هذه العلاقة. وتم الحصول على نتيجة مماثلة بين ال peridinin و
Dinoflagellates.

وبالنسبة لجنس Cryptomonas فبالرغم من أنه تم تسجيله في بعض عينات إلا أن العلاقة بين كتلته الحيوية وبين تركيز alloxanthin بسبب تواجـد بعــض ال flagellates الصغيرة والتي قد تكون تسببت في الخلط أو عدم الدقة في التعريف الميكروسكوبي كما أنــه تزامن التركيز العــالي فــي alloxanthin الـذروة فــي الكتلـة الحيويــة فــي rubrum Mesodinium أوضــحت النتــائج أيــضاً هيمنـة ال diatoms فــي الربيــع أمــا ال dinoflagellates فقـد أندهـرت فــي فـصل الـصيف. دلـت النتــائج علـي أن ال dinoflagellates وكذلك ال ciliates تنمو بشكل أفضل في المواقع الوسيطة علـى طـول المصب.

تم ضم بعض الأصباغ معا لإعطاء مؤشرات دالة علي تواجد/إذدهار الهائمات النباتية. أظهرت هذة الدوال البيولوجية نمط مماثل جدا الى النمط المعروف لتتابع النمو الموسمي للهائمات النباتية عند مصب مياه ساوث هامبتون والمعروف بأن يبدأ بالدياتومات ثم السوطيات/الهدبيات تليها الدينوفلاجيلات.