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SPATIAL AND TEMPORAL VARIATIONS IN PHYTOPLANKTON ASSEMBLAGES IN SOUTHAMPTON WATER ESTUARY (SOUTHERN UK) ESTIMATED FROM MICROSCOPIC COUNTS AND HPLC PIGMENT CHEMOTAXONOMY

Elham M. Ali[1,*]; Duncan Purdie[2] and Arantha 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 and 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 and chlorophyll c_1+*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.

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* Corresponding author: Tel.: +0162239339; Fax: +683350065
E-mail address: elhamali05@yahoo.co.uk (Dr. Elham Mohamed Ali)

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 and Hydes, 2002**; **Iriarte and 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** and **Ali, 2009**) 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**). 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 and 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 (e.g. **Mantoura and 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. HPLC also allows the quantification of small phytoplankton cells (<5 μm) 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**).

At a qualitative level, all previous studies of HPLC pigment analysis in oceans (**Claustre *et al*., 2004**; **Llewellyn *et al*., 2008**), in estuaries and coastal waters (**Llewellyn *et al*., 2005**; **Ediger *et al*., 2006**; **Ali 2003 and 2009**), in Antarctic and sub-arctic environments (**Rodriguez *et al*., 2002**; **Ras *et al*., 2008**

and **Fujiki *et al*., 2009)** and other marine systems (**Llewellyn *et al*., 2005**; **Delizo *et al*., 2007**).

In this research 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 and 2005. Our main question is whether these methodologies are comparable and suitable to characterize phytoplankton assemblages in a highly dynamic estuarine system as Southampton Water 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).

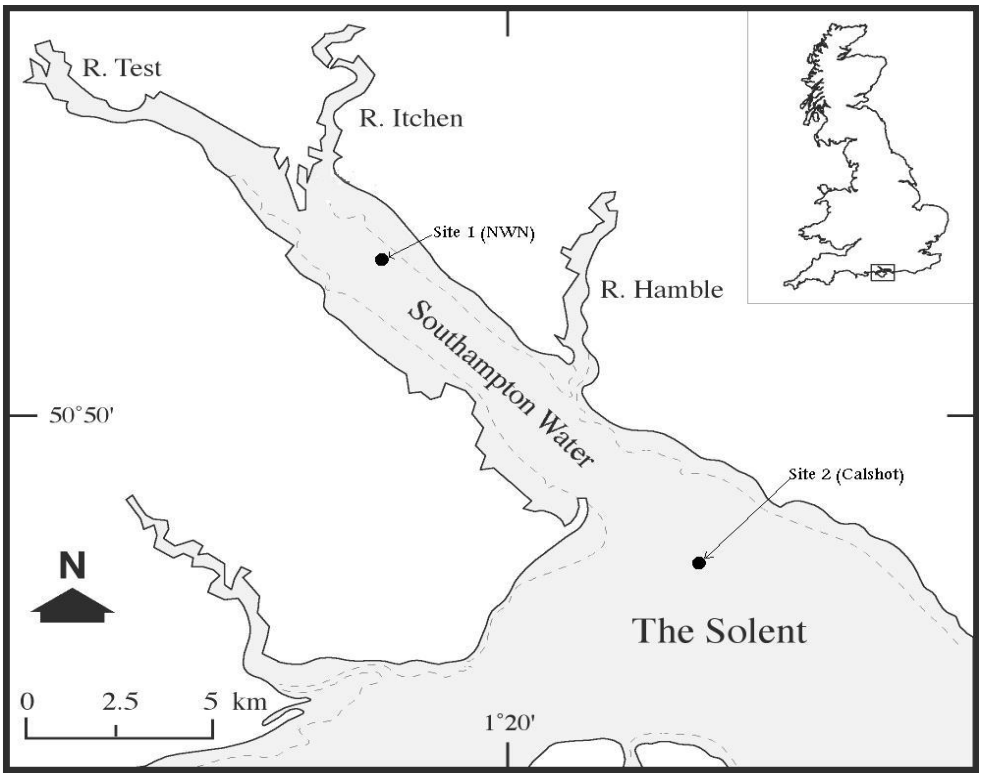

Figure (1): Map of the study area showing the position of the sampling sites (1 and 2)

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 (**Phillips, 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 and 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). These sampling sites were chosen to cover different environmental conditions along the main body of the estuary concerning the effect of rive-fresh water inflow. NWN, at the middle of the estuary (Figure 1), represented the medium environment between the fresh-water environment and coastal environment (i.e. medium salinity, medium nutrients). CA, at the mouth of the estuary (Figure 1), is however, represents the coastal high salinity site and less affected by river water (less nutrient). 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**).

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 and Larrance, 1966**). However, carbon values for some species were calculated according to a recent estimate of carbon per cell volume (**Menden-Deuer and Lessard, 2000**).

HPLC Pigment Measurements

Method Outline

Pigments were separated, in this study, by ion-pairing reverse-phase HPLC as described by **Mantoura and 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 ^{0}C. A 100 µl filtered sample (500 µl sample mixed with 500 µl 1M ammonium acetate) was injected into the column.

Extraction Procedure for HPLC pigment analysis

One litter 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 and Llewellyn** (**1983**) was used as described with modifications by **Barlow *et al.*** (**1993**).

Detection and Identification of Chlorophylls and Accessory Pigments

Carotenoids 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. An inline photodiode array detector was used in this work for more accurate identification of accessory pigments. This method does not separate chlorophylls c_1 *and* c_2 and so these were reported together as chlorophyll c_1+c_2 (Chl c_1+c_2). Table (1) gives the common accessory pigments used as biomarkers for particular groups of phytoplankton. 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 and Vesk, 1997; 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 mg m^{-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).

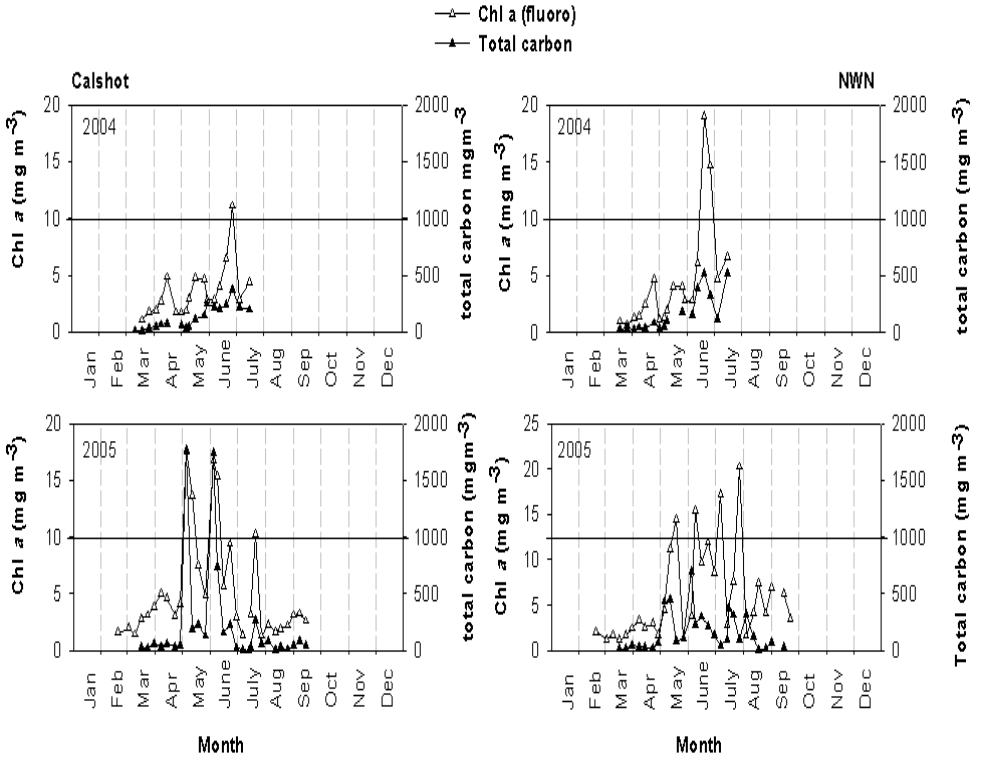

Figure (2): Distribution of fluorometrically estimated Chl *a* at Calshot (CA) and NW Netley (NWN) during 2004 and 2005

In 2004, the first and only major chlorophyll *a* peak recorded at Calshot delayed until end of June (~11 mg m^{-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 mg m^{-3}) measured at Calshot and NWN, respectively (Figure 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 and 4); however dinoflagellates (mainly, *Scrippsiella trochoidea*) dominated the late spring/early summer ones, particularly at NWN.

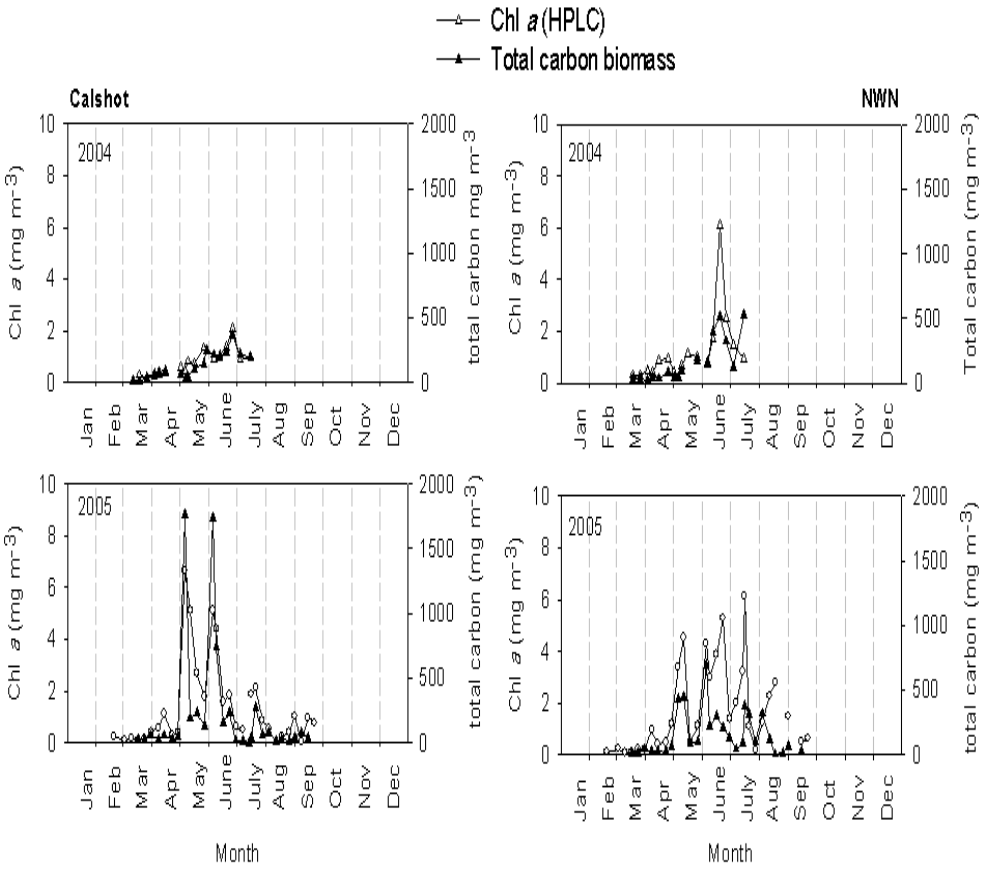

Figure (3): Distribution of HPLC estimated Chl *a* at Calshot and NWN in relation to variations in total phytoplankton biomass during 2004 and 2005

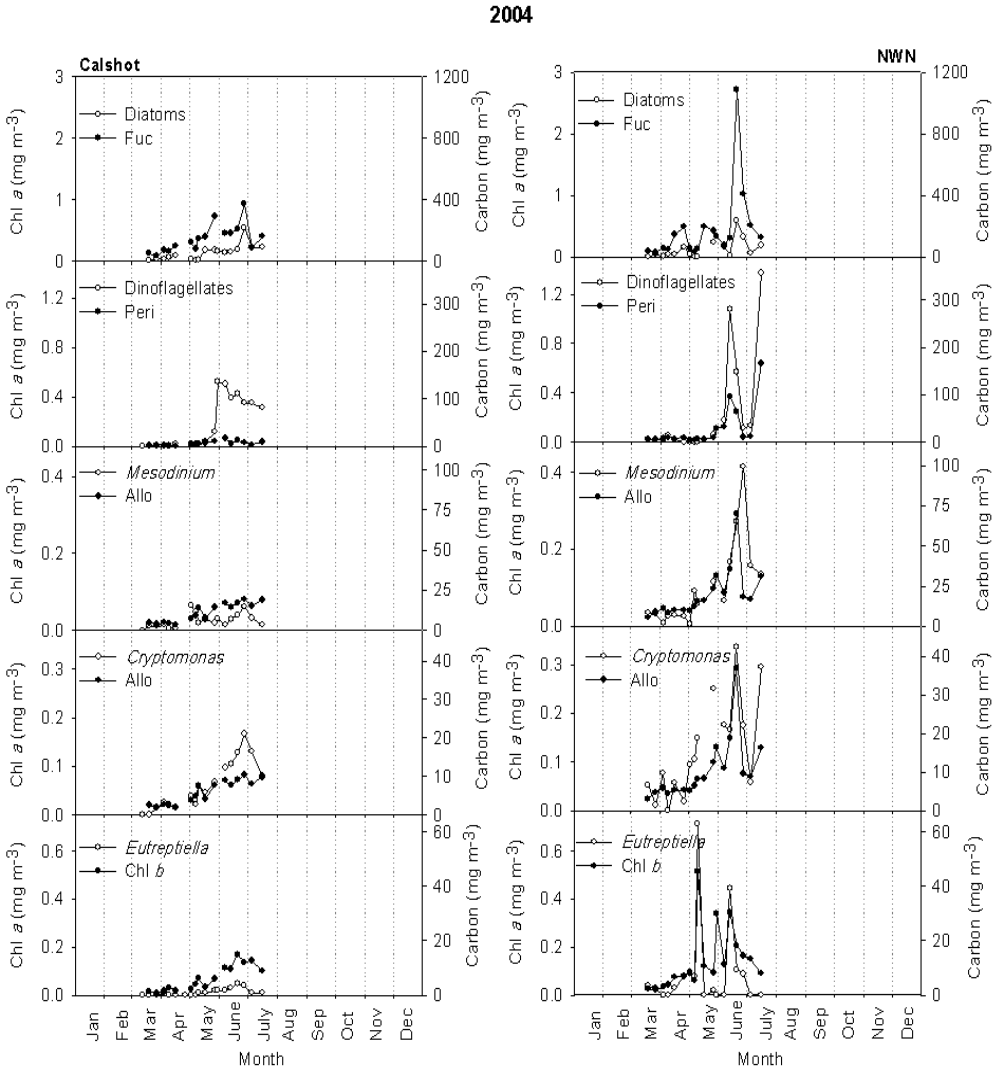

Figure (4): Distribution of 4 specific biomarker pigments (fucoxanthin (Fuc), Peridinin (Peri), alloxanthin (Allo) and, Chl *b*) at Calshot and NWN in relation to variations in carbon biomass of the relevant group and/or species identified during 2004

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 mg m^{-3}) were selected as primary taxonomic markers of the dominant phytoplankton groups; fucoxanthin (Fuc) for diatoms together with Diadinoxanthin (Diad) and

chlorophyll c_1+c_2 (Chl c_1+c_2), Peridinin (Peri) for dinoflagellates, alloxanthin (Allo) for cryptophytes, chlorophyll *b* (Chl *b*) for chlorophytes and chlorophyll c_3 (Chl c_3) for prymnesiophytes. Some other pigments (e.g. 19' hexanoyloxyfucoxanthin, 19'butanoyloxyfucoxanthin, violaxanthin, zeaxanthin and prasinoxanthin) were recorded in small traces (~ <0.1 mg m^{-3}) in some samples of which, 19' hexanoyloxyfucoxanthin (19`Hex) was included in the next analysis as an important pigment for prymnesiophyta. Ranges of the selected pigment concentrations are given in Table (2) .The breakdown products (phaeophorbides *a*1 and *a*2 and phaeophytin *a*1 and *a*2) 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.

Table (2): Range of concentrations (minimum and maximum) of the specific pigments (mg m^3) detected from Southampton water estuary in 2004 and 2005. Values in brackets are for Calshot (CA).

	2004		2005	
	Minimum	**Maximum**	**Minimum**	**Maximum**
Chl *a*	0.19 ± **0.19**	2.12 ± **6.10**	0.04 ± **0.07**	6.65 ± **0.14**
Chl c_1+c_2	-	0.18 ± **0.45**	-	0.20 ± **0.39**
Chl c_3	-	0.11 ± **0.15**	-	0.80 ± **3.10**
Chl b	0.01 ± **0.02**	0.17 ± **0.41**	-	0.48 ± **0.54**
Fuc	0.09 ± **0.08**	0.94 ± **2.72**	0.06 ± **0.03**	3.43 ± **3.76**
Peri	-	0.06 ± **0.06**	-	0.13 ± **1.18**
19 Hex	0.01 ± **0.01**	0.08 ± **0.08**	-	0.55 ± **0.31**
19 But	-	0.02 ± **0.03**	-	0.14 ± **0.11**
Allo	0.02 ± **0.02**	0.08 ± **0.29**	-	0.36 ± **0.26**
Zea	-	0.01 ± **0.03**	-	0.45 ± **0.22**
Diad	0.01 ± **0.01**	0.09 ± **0.42**	-	0.33 ± **0.48**

N.B. Chl *a* (chlorophyll *a*), Chl c_1+c_2 (chlorophyll c_1+c_2), Chl c_3 (chlorophyll c_3), Chl *b* (chlorophyll *b*), Fuc (fucoxanthin), 19 Hex (19'-hexanoyloxyfucoxanthin), 19 But (19'-Butanoyloxyfucoxanthin,), Allo (alloxanthin), Diad (diadinoxanthin), Zea (zeathanthin), - Undetected or zero.

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 mg m^{-3} < 20 mg m^{-3}) of chlorophyll *a* was measured in July and a smaller peak (<10 mg m^{-3}) in August at NWN, however, no peaks in carbon biomass were microscopically detected at the same time.

Microscopic analysis of water samples during 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 and 5) and chlorophyll c_1+c_2 (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.

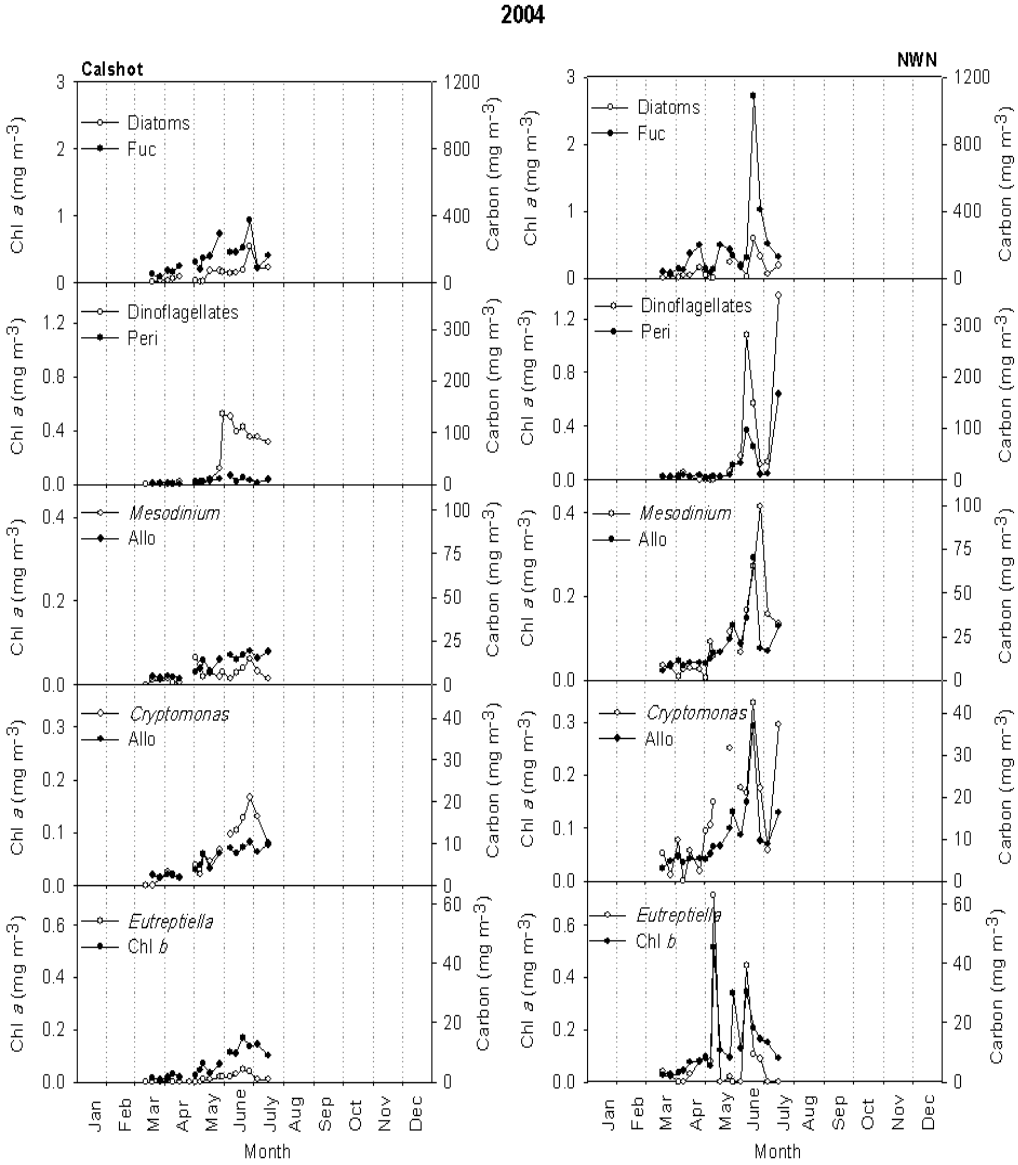

Figure (4): Distribution of 4 specific biomarker pigments (fucoxanthin (Fuc), Peridinin (Peri), alloxanthin (Allo) and Chl *b*) at Calshot and NWN in relation to variations in carbon biomass of the relevant group and/or species identified during 2004

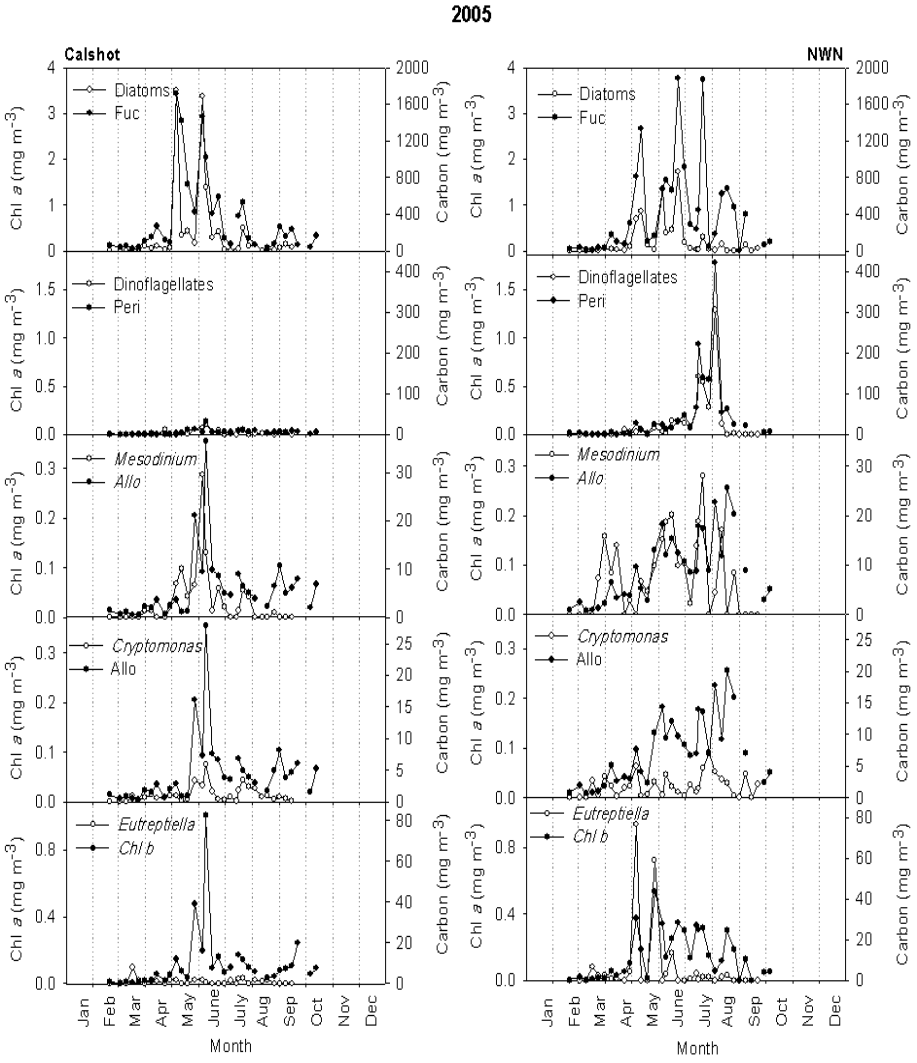

Figure (5): Distribution of 4 specific biomarker pigments (fucoxanthin (Fuc), Peridinin (Peri), alloxanthin (Allo) and, Chl *b*) at Calshot and NWN in relation to variations in carbon biomass of the relevant group and/or species identified during 2005

Fucoxanthin 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* were 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 c_1+c_2 (included in most diatoms) were measured during spring bloom (May/June) of the diatom *Guinardia delicatula* (at Calshot) and *Thalassiosira* and *Nitzschia* (at NWN). A good correlation of Chl c_1+c_2 however less than that of fucoxanthin, to chlorophyll *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* and *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.

Diadinoxanthin, 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, the major carotenoid for autotrophic Dinophyceae, were mostly measured in spring at both sites during the sampling period (see Figures 4 and 5) but increased towards the end of the sampling period with highest concentrations in summer (July/August). Maximum concentration in Peri of 1.78 mg m^{-3} was detected at NWN in 2005. This was during the summer dinoflagellate bloom, which was mainly composed of *Scrippsiella trochoidea* with *Prorocentrum micans* and *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* and *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 *et al*., 2000** and **Ali, 2009**).

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 Figures 4 and 5), smaller peaks of alloxanthin were coincided with peaks of the biomass of *Cryptomonas* during 2004 (Figure 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 (Figure 4) and 2005 (Figure 5), particularly at NWN when this species was achieving higher population. Concentrations of chlorophyll *b*, the carotenoids pigment of green algae widely varied over the sampling period (Figures 4 and 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 and 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* (Figures 4 and 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 carotenoids and the biomass of *Eutreptiella marina*. This finding might indicate that this flagellate is not the only chlorophyceans in Southampton Water.

Chlorophyll c_3 (Chl. c_3) 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 Figure 6). These peaks of chlorophyll c_3 might indicated the presence of Prymnesiophytes or/and Chrysophytes (both include Chlorophyll c_3), 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 (Figure 5) during July and August at this site (both Prymnesiophytes and Chrysophytes include fucoxanthin as well as Chl c_3). Lowest concentrations in Chl c_3 were generally measured in year 2004 (Figure 6).

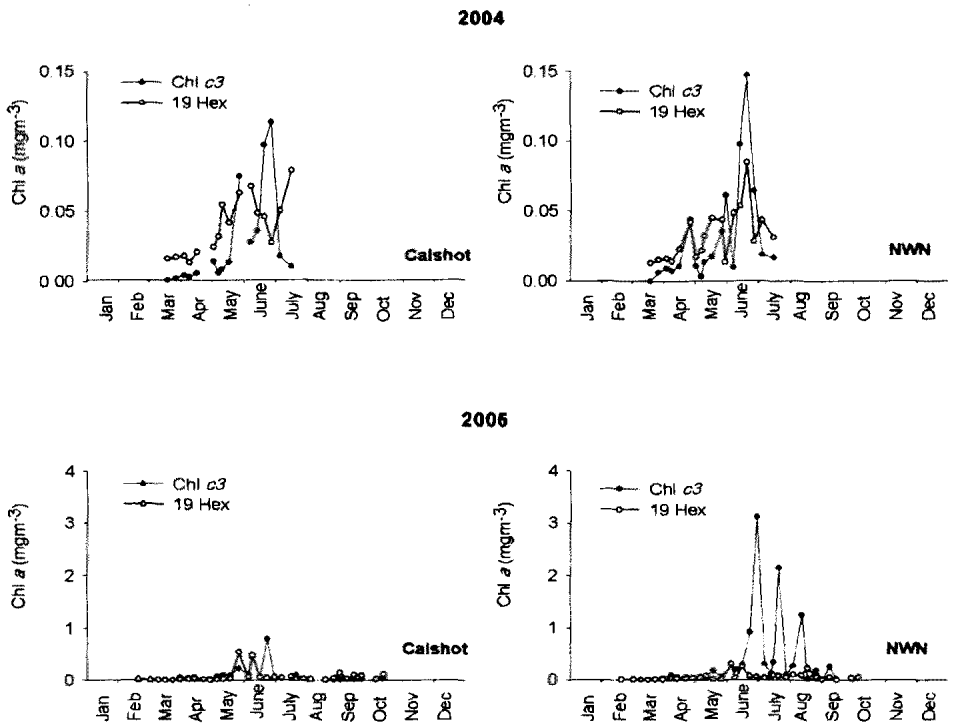

Figure (6): Variations in Chl c_3 and 19 Hex at Calshot and NWN during 2004 and 2005

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 c_3 was measured at this time.

Discussion

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 7a) with relatively similar variation patterns (Figure 2 and 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 7b 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 = 0.91) close to the value (r = 0.89) previously suggested by **Trees *et al***. (**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.

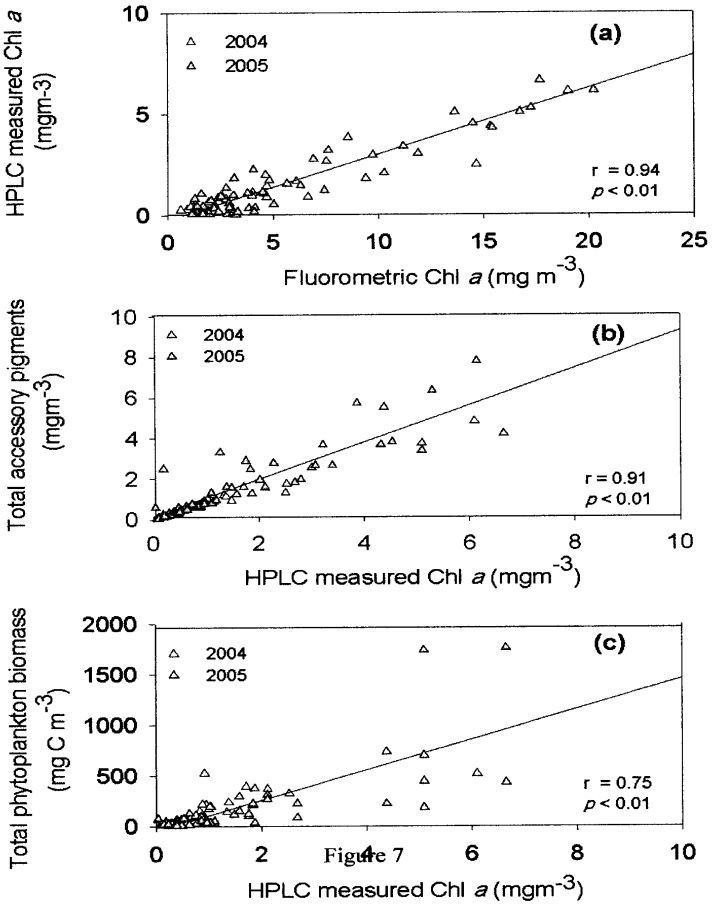

Figure 7

Figure (7): Plot and regression results (r value and significance level, p) of a)fluorometric Chl *a* concentration versus the HPLC measured Chl *a* , b) HPLC measured Chl *a* versus total accessory pigments. c) HPLC measured Chl *a* versus total phytoplankton biomass

****All measurements during 2004 and 2005 of both sites were grouped for the regression line.**

HPLC measured chlorophyll *a* showed a very similar spatial and temporal variation to that of the total phytoplankton biomass over the sampling period (Figure 3), with a significant correlation (r = 0.75, Figure 7c).

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 (e.g. **Kifle and 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 (e.g. **kifle and Purdie, 1993**; **Crawford and Purdie, 1993**; **Ali, 2003 and 2009**).

Good agreement was found between the concentration of the biomarker pigments and the biomass of their respective class and/or species at both sites (Figure 4 and 5). Diatoms (r = 0.72) and dinoflagellates (r = 0.74) showed the best correlation with their respective pigment marker fucoxanthin and peridinin (**Jeffrey and Wright, 1994**; **Delizo *et al*., 2007**), respectively (Table 3). 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 (e.g. **Kifle and Purdie, 1993**).

Table (3): Results of the linear regression analysis of some HPLC derived data versus other relevant data. Table includes the Pearson's moment correlation coefficients r, significance level s and confidence percentage (%).

	R value (r)	Significance level (s)	% Confidence
HPLC measured Chl *a* *Vs.* Fluorometeric Chl *a*	**0.94**	**<0.001**	**98%**
HPLC measured Chl *a* *Vs.* T. accessory pigments	**0.91**	**<0.001**	**98%**
HPLC measured Chl *a* *Vs.* T. phytoplankton biomass	**0.75**	**<0.001**	**98%**
Fuc *Vs.* Total diatom biomass	**0.72**	**<0.001**	**98%**
Peri *Vs.*Total dinoflagellates biomass	**0.74**	**<0.001**	**95%**
Allo *Vs.* biomass of Cryptophyceans	**0.33**	**<0.005**	**95%**
Chl *b* *Vs.* Total biomass of green algae (Only *Eutreptiella* in this research	**0.44**	**<0.005**	**95%**
Chl c_3 *Vs.*19'Hex	**0.27**	**<0.05**	**>95%**

Alloxanthin is the pigment biomarker for Cryptophycea (**Jeffrey *et al.*, 1999**). The quality of the relationship between both variables is well documented by **Jeffrey and Vesk,** (**1997**). Alloxanthin is however showed no correspondence (r= 0.33, Table 3) with Cryptophyceae (only *Cryptomonas* sp 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 and Vesk, 1997**) as described by **Gieskes and Kraay** (**1983**). Alloxanthin, is detected in the ciliate *Mesodinium rubrum* (**Hibberd, 1977**), which could contain cryptophytes as endosymbionts as described by **Gieskes and Kraay** (**1983**). Presence of some specific pigments in several species and/or groups could give false or inaccurate indications (**Breton *et al.*, 2000**; **Rodriguez *et al.*, 2002**), which is one of the limitation in the chemotaxonomic methodology.

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 (Table 3) 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.

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 **Breton *et al*.** (**2000**) (**2001**) and **Ansotegui *et al*.**. 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) بإستخدام طريقتي التعريف والعد الميكروسكوبي مقارنة بالتصنيف الصبغي-الكيميائي HPLC

إلهام محمود علي[1] – دانكان بيردي[2] – أرنثا إريارتي[3]

1- قسم العلوم البيولوجية والجيولوجية ، كلية التربية ، جامعة قناة السويس ، مصر.

2- مدرسة علوم البحار و الأرض ، جامعة ساوثاهمبتون ، المركز الوطني لعلوم البحار والمحيطات، ساوهثمبتون ،المملكة المتحدة.

3- قسم علوم البيولوجية النباتية والبيئة ، جامعة الباسك - بيلباو ، إسبانيا

تم فحص ودراسة الكتلة الحيوية ومكونات مجتمع الهائمات النباتية في مصب مياه ساوثهامبتون على الساحل الجنوبى للمملكة المتحدة. وقد تم تحليل التغيرات السنوية في محتوى الكلوروفيل بالاقتران مع التغيرات في إجمالي الكتلة الحيوية (مقدرة بالمحتوي الكربوني) في عامى 2004 ,2005 . تم استخدام جهاز ال HPLC لقياس الكلوروفيل و باقي الأصباغ الأخرى. وبمقارنة النتاج الخاصة بنتائج HPLC (للتصنيف الكيميائى) مع مثيلتها من الفحص الميكروسكوبى. أظهرت النتائج مستوى عال من توصيف العوالق على طول المصب بإستخدام تقنية ال HPLC (رغم وجود بعض القيود في هذه التقنية) كما أظهرت علاقة جيدة بين اجمالى الكتلة الأحيائية للهائمات النباتية وإجمالي الكلوروفيل كدليل أساسي خلال فترة الدراسة بالرغم من قلتها في بعض العينات.

أظهرت النتائج أيضاً علاقة طردية معنوية بين تركيز بعض الأصباغ والتي تساعد (بوصفها كدلائل لبعض الأجناس من عائلات الهائمات النباتية) على تقدير مدي سيادة بعض الأجناس من مجموعات دون غيرها مما يساعد علي تقديم معلومات عن التغيير الديناميكى للعوالق في مثل هذه المصبات ذات الديناميكية العالية دون اللجوء للتحليل الميكروسكوبي الذي رغم دقته إلا أنه يحتاج إلي الكثير من الوقت والجهد بالإضافة إلي الخبرة العالية.علي سبيل المثال: أظهرت النتائج ترابط قوي بين صبغ fucoxanthin و إجمالي الكتلة الأحيائية للدياتومات Diatoms بالرغم من التركيزات المرتفعة للكلوروفيل أثناء وقت الازدهار (النمو الكثيف) 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 غير واضحة بسبب تواجد بعض السوطيات (flagellates) الصغيرة والتي قد تكون تسببت في الخلط أو عدم الدقة في العريف الميكروسكوبي كما أنه تزامن التركيز العالي في alloxanthin الذروة في الكتلة الحيوية في *Mesodinium rubrum* أوضحت النتائج أيضاً سيادة الدياتومات في الربيع و أزدهار dinoflagellates في فصل الصيف. دلت علي أن ال dinoflagellates وكذلك ال ciliates تنمو بشكل أفضل في المواقع الوسيطة على طول المصب.