Vol. 142: 55–73, 1996 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published October 24

Characterization of phytoplankton communities in the lower St. Lawrence Estuary using HPLC-detected pigments and cell microscopy

Suzanne Roy[1,2,*], Jean-Pierre Chanut[1,3], Michel Gosselin[1,3], Télesphore Sime-Ngando[4]

[1]GREC (Groupe de Recherche en Environnement Côtier), 310 Allée des Ursulines, Rimouski, Québec, Canada G5L 3A1
[2]INRS-Océanologie, 310 Allée des Ursulines, Rimouski, Québec, Canada G5L 3A1
[3]Département d'océanographie, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, Québec, Canada G5L 3A1
[4]CNRS URA-1944, Biologie Comparée des Protistes, Université Blaise Pascal, Clermont-Ferrand II, 24 Avenue des Landais, Les Cézeaux, F-63177 Aubière Cedex, France

ABSTRACT: The seasonal variation in the composition of algal communities in the lower St. Lawrence Estuary was examined using HPLC pigments and cell taxonomy, and the efficiency of these 2 techniques was compared. A major centric diatom bloom was observed in July 1992, preceded by an increase in pennate diatoms in June, likely caused by bottom resuspension due to spring runoff. Grazing in June and July was indicated by the presence of pyropheophorbide *a*, a copepod grazing product tracer, and chlorophyll degradation pigments, likely associated with sloppy feeding and with the presence of cells (diatoms) with high chlorophyllase activity and acidic cell sap. Various pheopigments and degradation products of chl *a* were found in these 2 months. This is consistent with observations of maximum abundances of major copepod species and of herbivorous ciliates in June preceding the summer diatom bloom. May was characterized by nanoflagellates from Chrysophyceae, Cryptophyceae and Chlorophyceae, lower values of algal biomass and production and higher light harvesting efficiency. Mixing prevented the establishment of vertical fluorescence patterns in May and September and probably lowered the effective daily light exposure of algae, which translated into lower light acclimation than in summer and higher ratios of photosynthetic pigments to chl *a*. Low-light acclimation was also observed in the deep (>20 m) June and July populations, affecting marker pigment coefficients used to calculate relative algal contributions. Increases in relative amounts of chlorophyllide *a* and in the allomer of chl *a* in September were interpreted as signs of algal senescence. The September populations were composed of a number of chlorophyte and chromophyte (fucoxanthin-containing) algae. Low pigment concentrations and low numbers of observations complicated the identification task for that month. Pigment and microscopic approaches were compared on the basis of (1) clustering, using each separately, (2) correlations between pigments and cell groups, and (3) transformation of pigment data into algal group contributions to chl *a*. The 2 approaches generally gave similar results even though they showed different characteristics: the presence of small cells was often a problem for microscopic identifications, while the lack of specificity of some markers (e.g. fucoxanthin) reduced taxonomic precision from the pigment approach. Combining both was certainly advantageous, in that cell-pigment correlations helped in the assignment of a number of pigment markers. Pigments also helped in ascribing taxonomic identities for unidentified flagellates, which were numerous in June and September. Thus, the choice between using the methods singly or together will depend partly on the degree of taxonomic detail needed.

KEY WORDS: HPLC · Pigments · Cell microscopy · Phytoplankton communities · Cluster analysis · St. Lawrence Estuary

INTRODUCTION

Phytoplankton from the lower St. Lawrence Estuary (LSLE) is generally characterized by its small size, which is attributed to the frequency of destabilization events (Levasseur et al. 1984, Gratton et al. 1988). Previous studies have emphasized the importance of physical (stratification, irradiance and temperature) and chemical (nutrients) control of these populations (Levasseur et al. 1984, Vézina 1994), particularly with

*E-mail: suzanne_roy@uqar.uquebec.ca

respect to the summer diatom bloom. Fewer studies have dealt with the seasonal variation in phytoplankton communities in this environment (Levasseur et al. 1984). The study described here was undertaken in 1992 to examine this aspect. Considering the frequently small size of cells, which renders microscopic identification difficult, it was thought appropriate to use, for the first time in this region, HPLC-based chlorophyll and carotenoid signatures as a taxonomic complement to cell identification. In addition to their taxonomic role, pigments can provide information on the physiological condition of algae (Hallegraeff & Jeffrey 1985), on light acclimation responses (Demers et al. 1991, Johnsen et al. 1992, Johnsen & Sakshaug 1993, Claustre et al. 1994), and on the fate of these cells (grazing, sedimentation; Welschmeyer & Lorenzen 1985, Bathman & Liebezeit 1986, Klein & Sournia 1987, Redden 1994, Barlow et al. 1995).

The signature role of pigments has been summarized in various papers (Liaaen-Jensen 1978, Jeffrey 1981, Rowan 1989, Gieskes 1991, Wright et al. 1991, Millie et al. 1993). The taxonomic role of some of them is unambiguous, e.g. alloxanthin is found only in Cryptophyceae, while Prochlorophyceae are the only algae with divinyl-chlorophyll derivatives, both the *a* and *b* types (Goericke & Repeta 1992). Frequently found carotenoids from cyanobacteria include myxoxanthophyll and echinenone (Hertzberg & Liaaen-Jensen 1969, Goodwin 1980, Rowan 1989). Peridinin is found in photosynthetic dinoflagellates, but not all dinoflagellates possess this pigment: a number of species from the genera *Gymnodinium*, *Gyrodinium* and *Peridinium* have fucoxanthin and fucoxanthin derivatives in place of peridinin (Jeffrey et al. 1975, Bjørnland & Tangen 1979, Tangen & Bjørnland 1981, Johnsen & Sakshaug 1993, Millie et al. 1995). Combinations of pigments are often used when a single tracer pigment is not available. For example, chlorophyte algae contain chl *b* and a number of carotenoids, some of which are characteristic of particular algal classes. Lutein, violaxanthin, zeaxanthin, neoxanthin, β,β-carotene and β,ε-carotene are typically found in higher plants and Chlorophyceae. Prasinophyceae have been separated into 4 groups based on pigmentation (Ricketts 1970): (1) those with the common green algal carotenoids just mentioned, (2) as the first group + siphonaxanthin, (3) as the first group – lutein and + siphonein + magnesium 2,4-divinylphaeoporphyrin a_5 monomethyl ester (MgDVP), a pigment closely-related to chl *c*, and (4) same as the third group + prasinoxanthin. Recent work by Egeland et al. (1995) shows that Prasinophyceae containing prasinoxanthin also possess characteristic carotenoids such as uriolide, micromonal and micromonol. Lutein is sometimes present in these algae, but only in high-light acclimated cells (Egeland et al. 1995). Chromophyceae algae have, instead of chl *b*, a number of chl *c* derivatives in addition to a different set of carotenoids. Dinoflagellates possessing peridinin also have chl c_2, while those with fucoxanthin or 19'-acylfucoxanthin derivatives (often bloom-forming species) generally have chl c_3 (Bidigare et al. 1990, Johnsen & Sakshaug 1993, Millie et al. 1995). Chl c_3 also co-occurs with 19'-butanoyloxyfucoxanthin-like (BFU) or 19'-hexanoyloxyfucoxanthin-like (HFU) in many Prymnesiophyceae, some Chrysophyceae and 1 diatom species (Stauber & Jeffrey 1988). BFU seems to be more typical of Chrysophyceae (Bjørnland et al. 1984, Vesk & Jeffrey 1987), but not exclusively (Barlow et al. 1993), while HFU commonly occurs in Prymnesiophyceae (Wright & Jeffrey 1987). Bidigare (1989) sub-divides Chrysophyceae into 3 pigment subgroups: Type 1, 'true' Chrysophyceae, with fucoxanthin, violaxanthin, antheraxanthin and zeaxanthin; Type 2, 'aberrant' Chrysophyceae, with fucoxanthin, diadinoxanthin and diatoxanthin; and Type 3, same as Type 2 + BFU + chl c_3 (Withers et al. 1981, Bidigare et al. 1990). Prymnesiophyceae are also categorized into sub-groups (Stauber & Jeffrey 1988, Jeffrey & Wright 1994): Type 1 possess fucoxanthin and chl c_1 and c_2, Type 2 fucoxanthin and chl c_2 and c_3, Type 3 chl c_2 and c_3, with HFU (dominant) and fucoxanthin (BFU < 5%), and Type 4 chl c_2 and c_3 and BFU (with variable quantities of fucoxanthin and HFU; Jeffrey & Wright 1994). Pigments common to all Prymnesiophyceae include diadinoxanthin and β,β-carotene. Diatoms usually contain chl c_1 and c_2, fucoxanthin, diadinoxanthin, diatoxanthin and β,β-carotene, but a few species, often tropical pennate ones, possess chl c_3 instead of chl c_1 (Stauber & Jeffrey 1988).

Thus, assigning taxonomic identities from pigment signatures is not always a straightforward task. The sole use of pigment signatures without concurrent microscopic verification can sometimes be misleading: high chl *b* concentrations in the open ocean led researchers to believe that green algae were much more important than previously thought. Further work, however, suggested that most of this chl *b* belonged to Prochlorophyceae (Chisholm et al. 1988, Gieskes et al. 1988, Gieskes 1991). On the other hand, microscopic studies are often plagued by problems such as poor fixation or small size, making identification difficult. Thus, a combination of both approaches has been recommended; indeed, a number of earlier studies used both pigments and phytoplankton species when examining algal communities (e.g. Hallegraeff 1981, Jeffrey & Hallegraeff 1987a). However, recent field studies have tended to rely mostly on pigment chemo-taxonomy because of shorter analysis time (e.g. Everitt et al. 1990, Barlow et al. 1993, Letelier et al. 1993)

The objectives of this study were 2-fold: (1) to examine the seasonal changes in the population composition of phytoplankton from the LSLE, making use of both species and pigment information and (2) to compare results from both approaches in order to estimate whether pigment chemo-taxonomy could substitute for cell identification and enumeration in future studies in this area.

METHODS

Study sites. Six stations within a 900 km^2 area were selected in the middle portion of the LSLE (Fig. 1); the same area has been referred to as zone III in previous studies (Therriault & Levasseur 1985). This region was chosen on the basis of its importance in terms of primary production in the area and its relative homogeneity in physical and chemical characteristics. The stations were sampled once a month, during 2 d cruises, on the following dates: May 30 to 31, June 28 to 30, July 29 to 30 and September 29 to 30, 1992. The presence of ice prevented regular ship work prior to May. Monthly sampling gives the general seasonal phytoplankton pattern in this environment (Therriault & Levasseur 1985).

Physical and chemical characteristics. Vertical profiles of temperature and salinity were obtained at each station with a Guildline digital CTD attached to a sampling rosette of 5 l Niskin bottles. Irradiance was measured at 1 m intervals, from the surface to 22 m, using a 2π submarine photometer (Kahl Scientific Instrument). Incident irradiance was also measured every 2 to 3 h during daytime with a 2π meter. Nutrients are generally not limiting in this environment (Levasseur et al. 1984). Ammonium was the only nutrient measured, as a general index. It was determined on filtered (Whatman GF/F) seawater by the alternative method described in Parsons et al. (1984).

Phytoplankton. Vertical profiles of *in situ* fluorescence were taken between the surface and 30 m by pumping water through a Turner-Designs model 10-005R fluorometer. The standard fluorometric method of Yentsch & Menzel (1963; modified by Holm-Hansen et al. 1965) was used (as in previous studies in this area) to determine chl *a* concentrations from a series of depths generally corresponding to 50, 10 and 1% (sometimes 0.1%) of incident light, and also from 50 and 70 m. This method was chosen because it has shorter analysis times than does chromatography, allowing us to make 2 size fractions for both chlorophyll biomass and algal productivity.

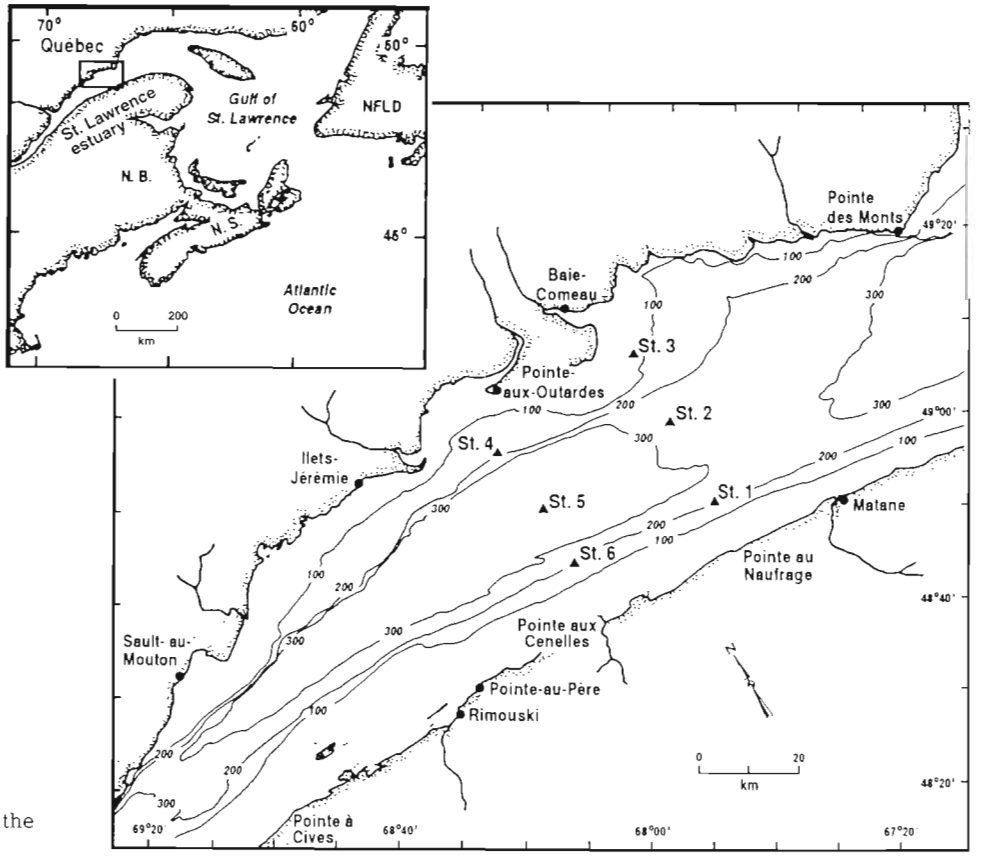

Fig. 1 Location of stations in the lower St. Lawrence Estuary

Poretics polycarbonate filters, of pore sizes 0.4 and 0.5 µm, were used for biomass and for the determination of photosynthetic activity by the ^{14}C method (Parsons et al. 1984). Subsamples (250 ml) from the optical depths (50, 10 and 1 or 0.1% of surface light levels) were inoculated with 5 µCi (0.19 MBq) of $NaH^{14}CO_3$. Two transparent and 1 opaque 70 ml Corning tissue culture flasks were filled with the inoculated water. The total amount of radioisotope in each bottle was determined by immediate pipetting of 50 µl into Aquasol-II scintillation cocktail containing 50 µl of 6 N NaOH. Inoculated bottles were placed in temperature-controlled (subsurface water flow-through) Plexiglas deck incubators for 3 to 5 h. At the end of the incubation period, bottle contents were split into two 35 ml aliquots and filtered. The filters were then put into scintillation vials and acidified with 0.1 ml of HCl (0.5 N; Lean & Burnison 1979). Following evaporation of the acid, Aquasol-II scintillation cocktail was added (10 ml per vial). Radioactivity was counted using a Beckman LS 5801 liquid scintillation counter. Daily production rate was obtained by dividing the total C uptake by the fraction of daily irradiance received during the incubation. In May, photosynthetic activity was measured at only 2 stations because of weather problems.

Cell identification and enumeration were done on acid Lugol-preserved water samples collected with 5 l Niskin bottles at the same depths reported above. Volumes of 10, 25, 50 or 100 ml were sedimented for 24 to 48 h in sedimentation chambers and observed with a Wild inverted microscope at a magnification of 200 or 400 (Utermöhl's technique; Lund et al. 1958).

Identity and concentration of pigments were determined from chromatographic analysis of filtered volumes of sea water (4 l, Whatman GF/F filters). Sampling depths were the same as above, except that deep samples from May and September gave undetectable values for all pigments except chl *a*. Filters were immediately frozen in liquid nitrogen on board ship and analyzed back in the laboratory using reversed-phase HPLC (Wright et al. 1991). Extraction was done in 5 ml of 98% methanol:2% ammonium acetate. The HPLC system consisted of a Shimadzu LC-600 pump, a Spherisorb-ODS2 column (150 × 4.6 mm, 5 µm particle size) and 2 detectors in series: a photodiode array spectrophotometric detector (Waters 991), for screening of absorbances between 400 and 700 nm, and a fluorometric detector (ABI Spectroflow 980) for detection of the fluorescent chlorophylls and their breakdown products. Calibration was done with external standards obtained commercially (chl *a* and *b* and β,β-carotene), graciously donated by Hoffman-Laroche, Switzerland (zeaxanthin), or prepared locally (TLC) from algal cultures (chl c_2, fucoxanthin, peridinin). Pyropheophorbide *a* was also prepared locally, following Pennington et al. (1964). The method used did not distinguish between chl c_1 and chl c_2; hence, this peak is hereby referred to as chl $c_{1,2}$. Concentrations of these standards were determined spectrophotometrically using extinction coefficients found in Jensen (1966), Jeffrey & Haxo (1968), Jeffrey & Humphrey (1975) and Davies (1976).

Statistical analyses. Cell abundances and pigment data were used separately to calculate an association matrix between samples. The cell data were summed into major taxonomic groups and pigment concentrations were expressed as fmol l^{-1} and rounded off to integer values for subsequent calculation of similarity index. The Bray & Curtis (1957) semi-metric distance index was used, as recommended in Legendre & Legendre (1984) for this type of data. The choice of this index has 2 advantages: calculations are performed on raw data and they are not affected by double absence of the same species in 2 samples. Flexible clustering was then applied on each matrix (pigments and cells) using the NTSYS (Numerical Taxonomy and Multivariate Analysis System, Exeter Software, Setauket, New York) program. A number of sample groups was identified and shown graphically on a 3-dimensional reduced space using non-metric multidimensional scaling (MDS) available in the NTSYS program. Spearman rank order correlations (r_S) were also calculated (using SigmaStat) between cell and pigment data.

RESULTS

Algal fluorescence, biomass and production

In situ fluorescence showed little vertical structure in May and September, highest values at the surface (2 to 12 m) in June, and deeper maxima (10 to 22 m) in July coinciding with the bottom of the mixed layer. Extracted fluorometrically-measured chl *a* concentrations varied between 0.1 and 10 mg m^{-3}. Highest values were observed in July around 10 m and the lowest in September (<1 mg m^{-3}), at all stations. Daily carbon fixation rates varied between 0.1 and 879.3 mg C m^{-3} d^{-1}, highest values being found in July, lower values in June and the lowest values in May and September. Small algal cells (<5 µm) constituted, on average, 37 and 26% of chl *a* in May and June, respectively, in the surface layer (0 to 20 m) and 47% in September, while they represented only 23% in July, both in terms of biomass and primary production (Table 1). At depths between 20 and 35 m (deepest sampling depth for primary production), small cells contributed >60% of total average primary production. Their contribution to chlorophyll biomass was smaller and more variable (13 to 30%). Small cells clearly dominated algal production in September at all depths.

Table 1. Average daily primary production (mg C m^{-3} d^{-1}; SD in parentheses) and fluorometrically-determined chl *a* concentration (mg m^{-3}) in the surface (0 to 20 m) and deeper (21 to 35 m) layers for the 4 monthly samplings. Total production and biomass (filtration at 0.4 µm) and small-size (<5 µm) fractions, relative to total, are shown. Only surface values are available for May as primary production was measured at only 2 stations and only 1 deep value was recorded

Month, depth	Daily primary production, 0.4 µm (mg C m^{-3} d^{-1})	% primary production <5 µm	Chl *a*, 0.4 µm (mg m^{-3})	% chl *a* <5 µm
May, surface	19.95 (21.88)	55.7 (16.9)	1.36 (0.90)	36.5 (21.9)
June, surface	115.90 (118.52)	22.4 (17.6)	4.75 (1.74)	26.1 (15.9)
July, surface	261.93 (269.15)	23.3 (16.8)	5.72 (3.25)	22.7 (20.1)
September, surface	3.72 (3.55)	80.2 (22.9)	0.61 (0.29)	46.8 (25.3)
June, deep	1.89 (1.00)	77.5 (25.9)	1.15 (1.00)	29.6 (21.3)
July, deep	3.48 (4.45)	61.7 (38.4)	0.82 (0.29)	16.0 (19.8)
September, deep	1.22 (0.42)	78.9 (14.7)	0.13 (0.11)	13.0 (7.0)

Table 2. Average abundances (10^3 cells l^{-1}) of major species in various algal groups in the surface (0 to 20 m) and deeper (>20 m) layers

	May		June		July		September	
	Surface	Deep	Surface	Deep	Surface	Deep	Surface	Deep
Diatoms—centric								
Chaetoceros decipiens	6	0	1	82	73	10	7	9
Chaetoceros convolutus	1	1	27	184	17	21	2	2
Chaetoceros debilis	5	0	3	133	9	15	1	1
Chaetoceros difficilis	1	0	18	24	27	3	3	6
Chaetoceros gracilis	6	2	0	18	3	2	0	4
Chaetoceros sp.	1	3	29	242	61	28	4	8
Leptocylindrus danicus	0	0	9	63	25	8	2	4
Skeletonema costatum	4	11	26	199	4706	753	369	532
Thalassiosira sp.	47	75	11	51	921	61	72	105
Thalassiosira gravida	9	32	13	33	220	19	21	27
Diatoms—pennate								
Navicula sp.	3	1	0	3	4	1	3	5
Nitzschia closterium	0	1	3	8	63	3	3	9
Nitzschia seriata	2	0	817	2492	263	284	22	31
Nitzschia sp.	0	1	4	0	3	0	0	0
Dinoflagellates								
Gyrodinium sp.	14	12	29	18	20	20	9	15
Heterocapsa triquetra	78	20	27	9	17	3	24	3
Protoperidinium sp.	9	6	38	19	18	7	12	8
Pronoctiluca sp.	0	1	2	0	4	2	1	1
Chlorophyceae								
Chlamydomonas sp.	52	40	27	35	258	64	79	1
Prasinophyceae								
Pyramimonas sp.	36	3	209	4	110	1	33	0
Cryptophyceae								
Chroomonas sp.	4	1	37	0	40	0	35	0
Rhodomonas sp.	245	38	180	22	313	25	55	10
Chrysophyceae								
Chromulina sp.	63	19	209	9	96	4	30	0
Prymnesiophyceae								
Chrysochromulina sp.	78	25	751	57	62	2	120	0

Structure of phytoplankton communities

Diatoms and flagellates dominated the phytoplankton communities. Pennate diatoms (*Nitzschia* sp., *Navicula* sp.; Table 2) were more abundant in June, generally at 1 to 5 m, while centric diatoms made up the bulk of the samples in July (Fig. 2), especially at the surface and the depth of the chl *a* maximum (10 to 20 m). These reached maximum abundances of 10^7 cells l^{-1} for the dominant species, which included *Skeletonema costatum*, *Thalas-*

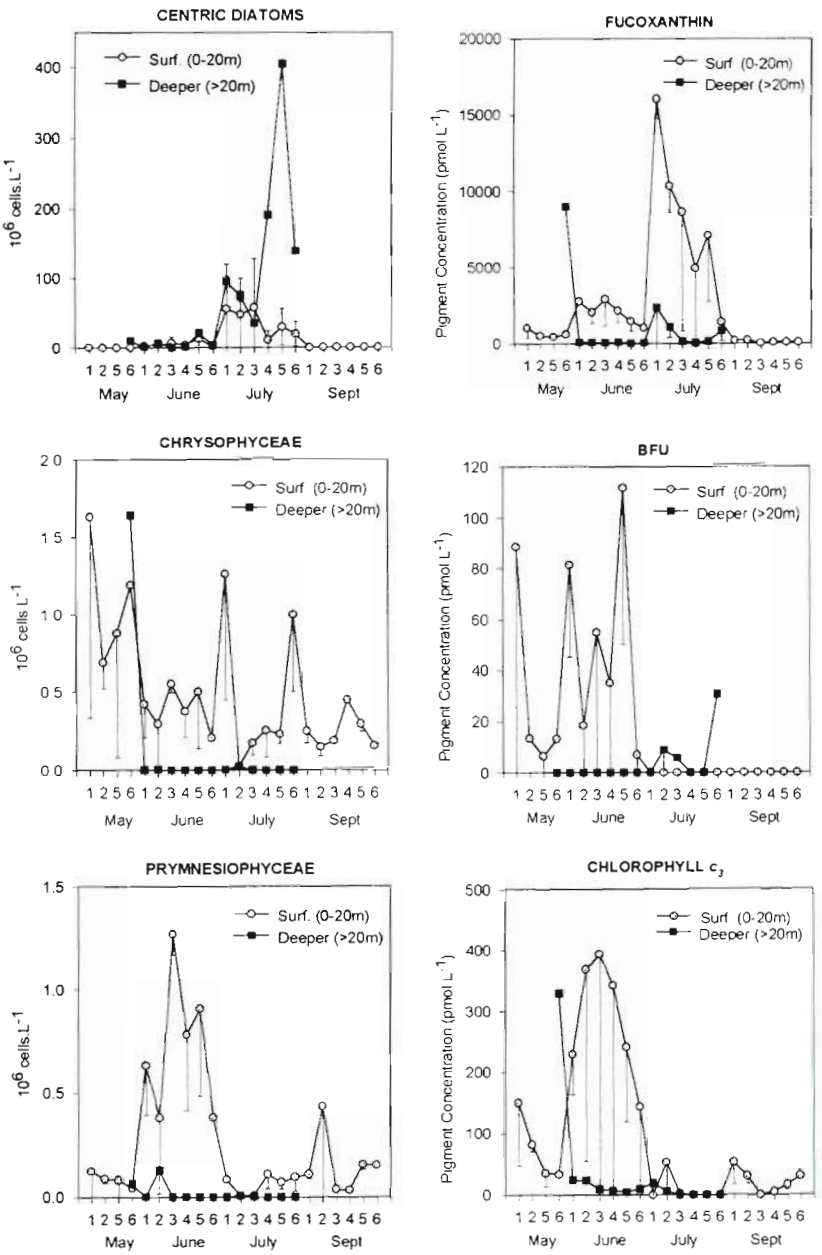

Fig. 2. Average cell counts (10^6 cells l^{-1}) for centric diatoms, Chrysophyceae and Prymnesiophyceae and pigment concentrations (pmol l^{-1}) for fucoxanthin, BFU and chl c_3 in the surface (0 to 20 m) and deeper (>20 m) layers. Error bars = SD

siosira gravida and *Chaetoceros decipiens* (Table 2). A group of unidentified flagellates as numerous as pennate diatoms was also prominent in June at the surface. Prymnesiophyceae, represented by *Chrysochromulina* sp., were most abundant in June at 1 to 5 m and also showed up in September at the surface (Fig. 2). Chrysophyceae (mostly *Chromulina* sp.) were found from May to September, but their abundance was highest in May, in the surface layer (Fig. 2). Dinoflagellates were present from May to September at most depths (more abundant at surface in May and June), but in lower concentrations than the previous groups (Fig. 3). Dominant species included *Gyrodinium* sp., *Heterocapsa triquetra*, *Protoperidinium* sp. and *Pronoctiluca* sp. (formerly *Prorocentrum* sp.) (Table 2). These species were present during all survey months at average concentrations of 0.5 to 1.5 x 10^5 cells l^{-1} (Fig. 3); these concentrations are similar to those encountered by Sinclair (1978) and Cembella & Therriault (1989), even though *Alexandrium* sp., often the dominant dinoflagellate in this region, was virtually absent from the 1992 samples. Cryptophyceae (*Chroomonas* sp., *Rhodomonas* sp.) were also present from May to September (Fig. 3), with peaks in May and July at 1 to 5 m, but they could sometimes be found as deep as 70 m. Among 'green algae', Chlorophyceae (dominated by *Chlamydomonas* sp.) attained their highest abundance in July at 1 to 5 m, and Prasinophyceae (mostly *Pyramimonas* sp.) in June at the same depths (Fig. 4). Euglenophyceae were only occasionally present in surface waters in July. Finally, choanoflagellates and ciliates showed their maximum abundances in June and were associated with the surface layer (Sime-Ngando et al. 1995).

On a seasonal basis, Chrysophyceae and Cryptophyceae dominated in May, pennate diatoms and unidentified flagellates in June, centric diatoms in July and, at much lower abundances, Prymnesiophyceae, Chrysophyceae, Chlorophyceae and Cryptophyceae in September. Most of the cells were small (even for centric diatoms: <30 µm) and were found in the surface layer.

Taxonomically useful pigments detected include chl *b*, chl $c_{1,2}$, chl c_3, alloxanthin, diadinoxanthin, fucoxanthin, BFU and HFU, peridinin and zeaxanthin. No prasinoxanthin, siphonein, MgDVP or other pigments characteristic of Prasinophyceae were observed. Divinyl-chlorophyll derivatives could not be detected with the HPLC method used, but their presence is not suspected, as they are generally found in tropical and sub-tropical environments (Goericke & Repeta 1993). Marker pigments from cyanobacteria were also absent from the HPLC traces. Maximum concentrations of fucoxanthin (Fig. 2), diadinoxanthin and chl $c_{1,2}$ occurred

in July at 10 to 15 m. Chl c_3, BFU (Fig. 2) and peridinin (Fig. 3) showed their maxima in June at the surface. HFU was observed in only 5 samples at very low concentrations. Alloxanthin was also found at the surface, but was more evenly spread throughout the months (maximum in July; Fig. 3). Chl *b*, as well as zeaxanthin, dominated at the surface in June, although chl *b* was present in surface waters from May until September (Fig. 4).

The structure of algal communities was studied based on cell and pigment data using ordination techniques and clustering. Fig. 5 shows the dendrogram obtained from flexible-links clustering ($\beta = -0.25$) on the major algal groups encountered. Samples were clustered into 6 groups: (1) May, surface (0 to 20 m), (2) June, surface, (3) July, surface, (4) September, surface, (5) May, June and September, deep (>20 m), and (6) July, deep. These groups are clearly separated from each other when shown in the reduced space of the MDS analysis (Fig. 6a). Chrysophyceae dominated in Group 1, unidentified flagellates in Groups 2 and 4, centric diatoms in Groups 3 and 6 and dinoflagellates in Group 5 (Table 3a).

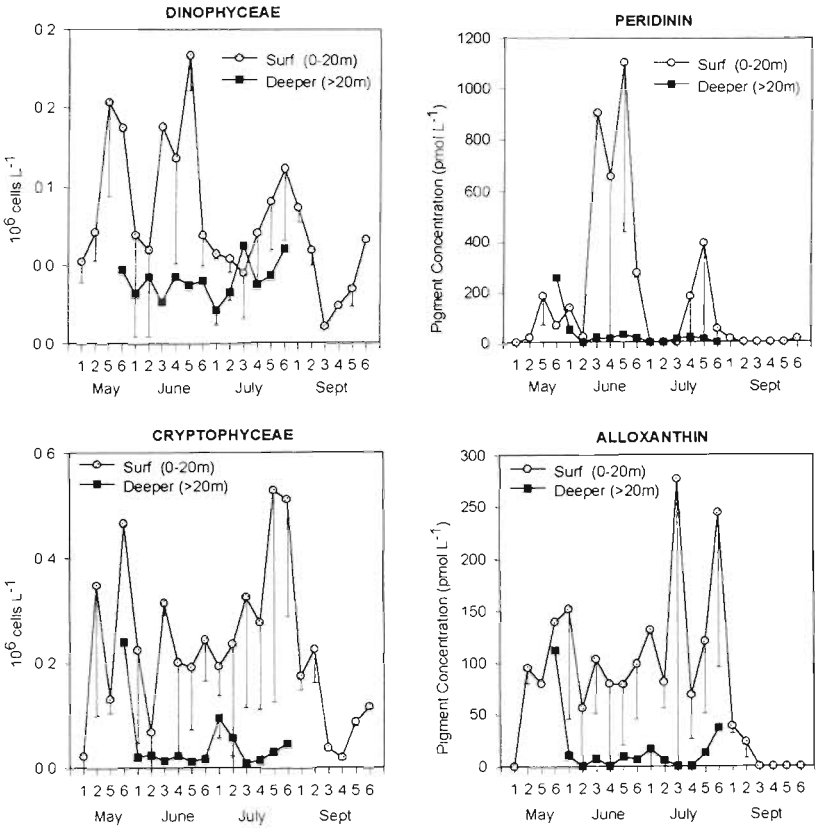

Fig. 3. Average cell counts (10^6 cells l^{-1}) for Dinophyceae and Cryptophyceae and pigment concentrations (pmol l^{-1}) for peridinin and alloxanthin in the surface (0 to 20 m) and deeper (>20 m) layers

A similar analysis run on the pigment data gave the same 6 groups (Fig. 6b). Fucoxanthin dominated in Groups 1, 3, 5 and 6, and chl *a* in Groups 2 and 4 (Table 3b). The dominance of these 2 pigments, and of chl $c_{1,2}$, which ranks third in terms of relative composition (Table 3b), is not surprising considering that most dominant algal groups in these waters possess chl *a* and $c_{1,2}$ and fucoxanthin. Although these 3 pigments dominated the pigment composition throughout the sampling period, the statistical analysis was able to identify sample groups similar to those already identified independently from the cell taxonomy data (Fig. 6b). Minor pigments (molar concentration <10% of total; Table 3b), as well as the seasonal changes in concentrations, undoubtedly played an important role in this discrimination. The 2 deeper groups differed mostly by a larger contribution of pheopigments, relative to surface groups, particularly pyropheophorbide *a* (11% of total pigments in June).

Correlations between pigments and cell data within each of the 4 previously identified surface groups (Table 4) confirmed the expected marker role of alloxanthin for Cryptophyceae and of peridinin for photosynthetic Dinophyceae. However, for peridinin, significant correlations were only observed in May and June. The association between chl c_3 and Dinophyceae in September, along with the presence (but correlation not significant, probably due to the small number of data points) of fucoxanthin and diadinoxanthin, suggests that these dinoflagellates were of the fucoxanthin-containing type. Alternatively, heterotrophic (not pigmented) dinoflagellates could have been present in July and September, and the correlation with chl c_3 could be due to variations similar to chl c_3-containing cells. In the May and June surface groups, fucoxanthin was associated with Chrysophyceae, while in the July surface group it was found in diatoms. The correlation between zeaxanthin and Chrysophyceae in June (Table 4) suggests that these cells could belong to Type 1 of Bidigare (1989). Indeed, the dominant Chrysophyceae species observed here, *Chromulina* sp. (Table 2), is part of that group (Bidigare 1989). BFU was positively correlated with Prymnesiophyceae and Dinophyceae in June. Considering that this pigment has been observed in only 2 dinoflagellate species (*Gymnodinium breve*: Liaaen-Jensen 1985, Millie et al. 1995; *Gymnodinium galatheanum*: Johnsen & Sakshaug 1993), which were not seen in our samples, it

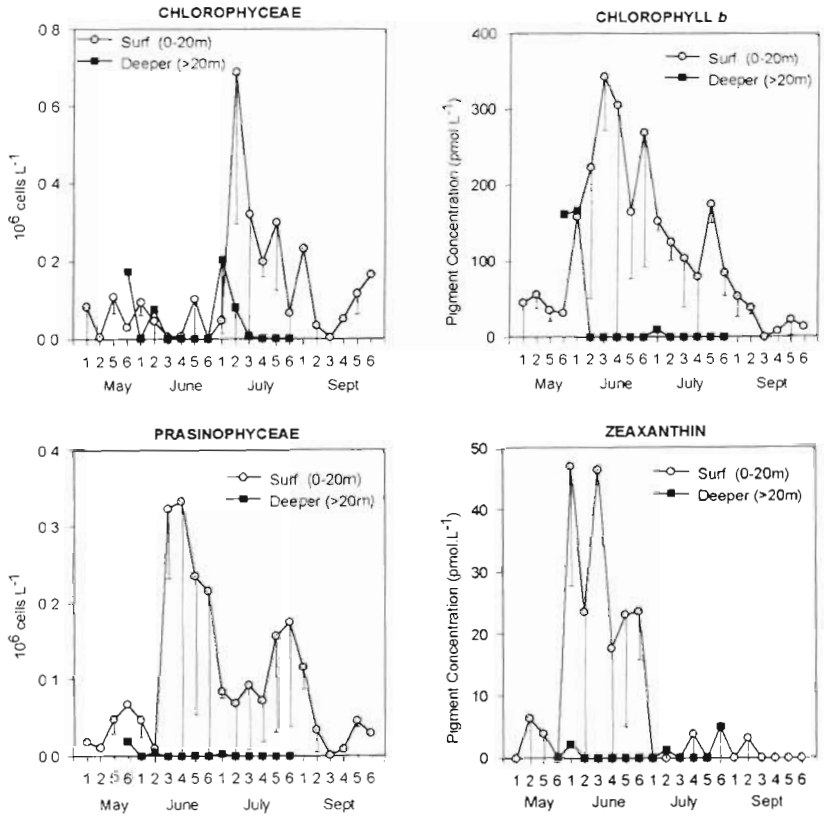

Fig. 4. Average cell counts (10^6 cells l^{-1}) for Chlorophyceae and Prasinophyceae and pigment concentrations (pmol l^{-1}) for chl *b* and zeaxanthin in the surface (0 to 20 m) and deeper (>20 m) layers

seems more likely that BFU here was a tracer for Prymnesiophyceae (Type 4 according to Jeffrey & Wright 1994). However, the dominant Prymnesiophyceae species (among those that could be identified), *Chrysochromulina* sp. (Type 3), possesses HFU rather than BFU (Jeffrey & Wright 1994), suggesting that other unidentified Prymnesiophyceae were responsible for the presence of BFU. In June chl *b* was associated with Prasinophyceae. The absence (or undetectable presence) of prasinoxanthin, MgDVP, siphonaxanthin and siphonein suggest that these cells possess a pigmentation typical of Ricketts' (1970) 'Group 1'; however, it is interesting to note the rare occurrence of lutein, even though chl *b*-containing cells were generally found near the surface (cf. Egeland et al. 1995). No significant correlations were found between chl *b* and Chlorophyceae. The pigments associated with unidentified flagellates included chl c_3, BFU, alloxanthin, diadinoxanthin, zeaxanthin and β,β-carotene in June and chl c_3 in September, suggesting possible contributions by Prymnesiophyceae, Cryptophyceae, Chrysophyceae and, perhaps, Dinophyceae and diatoms. Negative correlations, such as those between unidentified flagellates and BFU in May and fucoxanthin in July, could indicate inverse variations or prey-predator relationships (assuming these flagellates were heterotrophic).

The 4 monthly surface groups were distinct in terms of average physical characteristics of the environment (Table 5). Strong stratification and high daily irradiances in June and July favored the growth of bloom-forming diatoms in mid-summer, as observed by Levasseur et al. (1984). No nutrient limitation was observed. In September, the mixed layer became deeper than the euphotic layer. Reduced stratification was probably the major factor favoring the growth of flagellates rather than diatoms in early fall.

Algal physiology and grazing

In May, cells showed evidence of more efficient light absorption for photosynthesis, i.e. a higher average of total photosynthetic pigments (sum of chl *a*, *b*, $c_{1,2}$, c_3, HFU, BFU, fucoxanthin and peridinin) relative to chl *a* was observed for these samples (Table 6). This was principally due to an increase in the chl $c_{1,2}$:chl *a* and fucoxanthin:chl *a* ratios, indicative of more efficient light harvesting (Richardson et al. 1983, Johnsen et al. 1992, 1994). Increases in accessory pigments are often observed in cells growing under low light intensity (Richardson et al. 1983, Johnsen & Sakshaug 1993). Interestingly, photo-protective pigments (diadinoxanthin and diatoxanthin) also show higher values (relative to chl *a*) in May, decreasing thereafter throughout the summer (Table 6).

Chromatographically determined chl *a* correlated significantly with the fluorometrically determined value ($r = 0.82$, $p < 0.001$, $n = 42$). Derivatives of chl *a*, such as the allomer and the epimer of chl *a*, which generally represent around 10% of the parent compound, showed an increase with depth relative to chl *a* in June and July, as well as an increase at the surface in September (Fig 7). Some of these compounds have been related to algal senescence and zooplankton grazing (Hallegraeff & Jeffrey 1985). They can also be artefacts of storage or handling (Redden 1994), although usually adequate precautions (quick-freezing in liquid nitrogen and subsequent storage at -80°C) were taken here. The recently reported

Fig. 5. Flexible clustering of phytoplankton groups, based on a distance matrix using the Bray & Curtis (1957) coefficient. Sample codes: month-station no.-depth; M = May, J = June, K = July and S = September; A = 1 m, B = 5 m, C = 10 m, D = 25 m, E = 50 m and F = 70 m

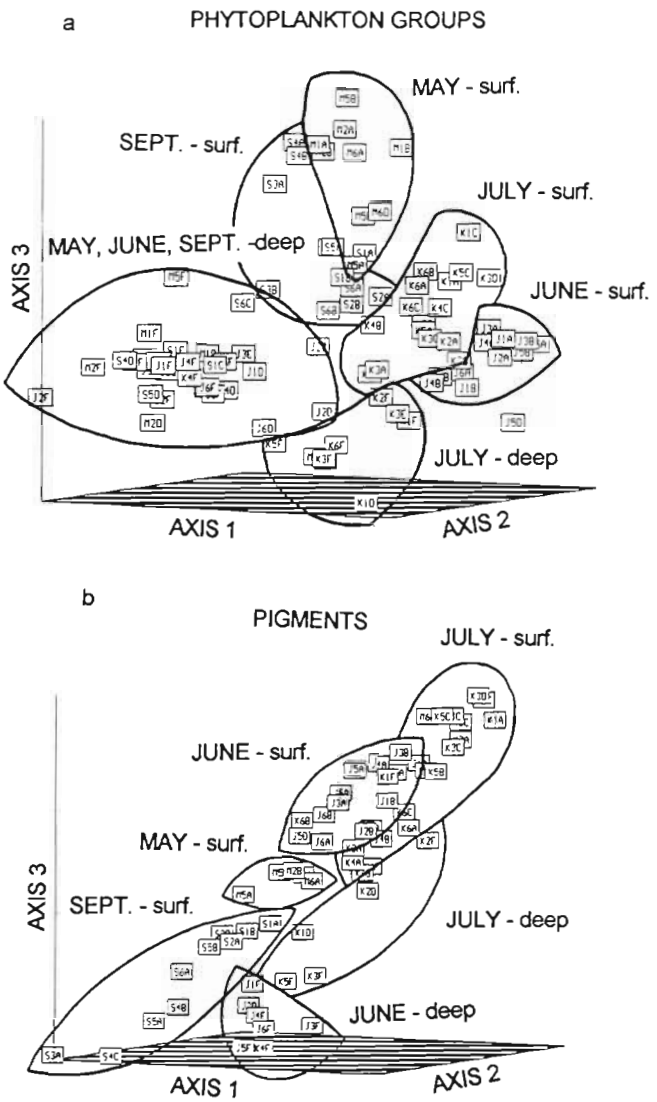

Fig. 6. Non-metric multidimensional scaling applied independently to (a) phytoplankton groups and (b) pigments. Sample codes as in Fig. 5

'dying-alga pigment', a pheophorbide *a* derivative (Head et al. 1994) with a retention time close to that of BFU, was not observed here. The presence, especially in June and July, of 4 derivatives of pheophorbide *a* (all less polar than the pigment standard produced by acidification of chlorophyllide *a*) suggests that grazing was taking place. One of these was pyropheophorbide *a*, typically dominant in copepod grazing products (Head & Harris 1992, Redden 1994). It was found mostly in June and July below the surface layer and was the only pigment increasing in concentration with depth (Fig. 7). A significant negative correlation with diatoms in June below the surface layer ($r_S = -0.88$, $n = 6$, $p < 0.05$) suggests that grazing on diatoms was the source of this pigment. All other pheophorbides had maximum concentrations in the surface layer in July, along with the diatom bloom, although their concentration relative to chl *a* increased with depth. Pheophorbide *a*3-like, eluting just before pyropheophorbide *a*, correlated negatively with Prasinophyceae ($r_S = -0.63$, $n = 17$, $p < 0.01$) and Cryptophyceae ($r_S = -0.64$, $n = 17$, $p < 0.01$) in the surface layer in July, either because of inverse variations of these algal groups with pheophorbide *a*3-like or because grazing on these cells produced this particular pheophorbide *a* derivative. Pheophytin *a* and pyropheophytin *a* were also present, with highest concentrations at the surface in July and highest ratios to chl *a* below the surface in June and July, as for most pheophorbides.

Chlorophyllide *a* and a methylated derivative presented maximum concentrations in the surface layer in July (Fig. 7). These pigments, as well as pheophorbides, have been associated with cells with an active chlorophyllase enzyme (Jeffrey & Hallegraeff 1987b), and certain diatoms, especially *Skeletonema costatum*, often cause problems upon extraction because of a very active chlorophyllase enzyme and acidic cell sap (Suzuki & Fujita 1986). Indeed, *S. costatum* was present in our samples, especially in June (30% of total centric diatoms), July (30 to 50%) and September (20 to 38%), and the presence of this alga was significantly correlated with chlorophyllide *a* and pheophorbide *a* in June ($r_S = 0.59$ and 0.80 respectively, $n = 13$, $p < 0.05$) and July ($r_S = 0.88$ and 0.70 respectively, $n = 17$, $p < 0.01$). In September, however, the correlation with chlorophyllide *a* was negative ($r_S = -0.70$, $n = 9$, $p < 0.05$), suggesting that the presence of this pigment was not always an extraction artefact. Moreover, significant correlations with Dinophyceae, Prasinophyceae and Cryptophyceae (groups known to have a low chlorophyllase activity; Jeffrey & Hallegraeff 1987b) in September further support that, for that month, chlorophyllide *a* was not related to extraction problems. Strong correlations of chlorophyllide *a* and of the allomer of chl *a* with most pheopigments (except pyropheophorbide *a*) in June and July, and the detectable presence of diadinochrome in July (an indication of acidic cell sap; Johnsen & Sakshaug 1993), suggest that most chlorophyll degradation pigments were more likely related to sloppy feeding (cell rupture without complete ingestion) on *S. costatum* and other diatoms during these months (Gieskes 1991). Conversely, increases in relative amounts of both chlorophyllide *a* and the allomer of chl *a* (Fig. 7), not accompanied by increases in pheopigments in September, suggest that these pigments were then probably related to algal senescence and not grazing.

Table 3. Cluster analysis. (A) Median values, in 10^4 cells l^{-1} (percent composition in parentheses), for each major algal group that contributes significantly ($p < 0.05$) to group discrimination. (B) Median values, in pmol pigment l^{-1}, for each pigment with a significant ($p < 0.05$, except for Pyropheophorbide *a*: $p < 0.10$) contribution

	May, surface (1)	June, surface (2)	July, surface (3)	Sept, surface (4)	May, June, Sept, deep[a] (5)	July, deep (6)
(A) Algal group						
Centric diatoms	7.3 (6)	142.2 (11)	515.8 (82)	3.0 (3)	1.2 (14)	93.4 (90)
Pennate diatoms	0.9 (1)	340.9 (25)	10.4 (2)	0.9 (1)	0.0 (0)	1.3 (1)
Dinoflagellates	7.2 (5)	12.6 (1)	6.3 (1)	4.8 (5)	3.3 (37)	3.8 (4)
Chrysophyceae	76.2 (57)	50.5 (4)	27.1 (4)	19.6 (20)	0.0 (0)	0.1 (0)
Prymnesiophyceae	6.8 (5)	67.7 (5)	5.5 (1)	9.7 (10)	0.0 (0)	0.0 (0)
Cryptophyceae	15.9 (12)	24.5 (2)	32.5 (5)	8.6 (9)	1.4 (16)	2.9 (3)
Chlorophyceae	5.4 (4)	1.0 (0)	16.6 (3)	7.0 (7)	0.1 (1)	0.4 (0)
Prasinophyceae	2.0 (2)	16.1 (1)	8.6 (1)	2.9 (3)	0.0 (0)	0.0 (0)
Choanoflagellates	1.8 (1)	6.1 (0)	2.0 (0)	2.4 (2)	0.1 (1)	0.2 (0)
Unidentified flagellates	8.6 (6)	675.8 (51)	4.5 (1)	38.8 (40)	2.5 (28)	2.3 (2)
Ciliates	0.5 (0)	0.4 (0)	0.4 (0)	0.4 (0)	0.2 (2)	0.0 (0)
(B) Pigment						
Chl c_3	74 (4)	212 (3)	0 (0)	22 (5)	9 (4)	0 (0)
Chl $c_{1,2}$	236 (13)	895 (12)	1662 (8)	52 (12)	44 (19)	209 (19)
Chl *b*	43 (2)	178 (2)	143 (1)	23 (5)	0 (0)	0 (0)
Chl *a*	492 (28)	2782 (37)	7033 (36)	155 (36)	24 (11)	257 (23)
Chl *a* derivative	23 (1)	68 (1)	174 (1)	2 (0)	0 (0)	5 (0)
Chl *a* allomer	18 (1)	72 (1)	321 (2)	15 (3)	1 (0)	12 (1)
Chl *a'* (epimer)	4 (0)	11 (0)	69 (0)	3 (1)	1 (0)	1 (0)
Chlorophyllide *a*	23 (1)	338 (5)	670 (3)	16 (4)	0 (0)	25 (2)
Pheophorbide *a*	5 (0)	69 (1)	202 (1)	6 (1)	3 (1)	38 (3)
Pheophorbide a_1	19 (1)	48 (1)	101 (1)	8 (2)	5 (2)	26 (2)
Pheophorbide a_2	2 (0)	47 (1)	115 (1)	0 (0)	1 (0)	8 (1)
Pyropheophorbide *a*	0 (0)	9 (0)	23 (0)	0 (0)	25 (11)	52 (5)
Pheophytin *a*	4 (0)	35 (0)	81 (0)	2 (0)	1 (0)	9 (1)
Pyropheophytin *a*	0 (0)	8 (0)	26 (0)	0 (0)	1 (0)	13 (1)
Peridinin	37 (2)	286 (4)	45 (0)	0 (0)	18 (8)	0 (0)
BFU	13 (1)	43 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Fucoxanthin	591 (33)	1836 (25)	8449 (43)	121 (28)	74 (33)	437 (39)
Violaxanthin	0 (0)	0 (0)	0 (0)	0 (0)	8 (4)	0 (0)
Zeaxanthin	0 (0)	33 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Diadinoxanthin	91 (5)	388 (5)	484 (2)	7 (2)	4 (2)	25 (2)
Diadinochrome	0 (0)	0 (0)	22 (0)	0 (0)	0 (0)	5 (0)
Diatoxanthin	20 (1)	15 (0)	53 (0)	0 (0)	0 (0)	0 (0)
Alloxanthin	80 (5)	82 (1)	112 (1)	0 (0)	7 (3)	6 (1)

[a]No pigment data for May and September deep samples (concentrations too low)

DISCUSSION

Phytoplankton communities

Many of the chromophyte groups reported in this study have seldom been mentioned in earlier published studies of the region, probably because of their relatively low abundances, small sizes and the fact that all flagellated cells were often reported as 'flagellates'. Chrysophyceae have been reported in early fall (Sinclair 1978) and unidentified flagellates mostly in May and June (Levasseur et al. 1984) at concentrations (Therriault & Levasseur 1985) similar to those reported in this study for Chrysophyceae, Prymnesiophyceae and Cryptophyceae. Chlorophyceae, often of freshwater origin, have been observed from May to October (Cardinal & Lafleur 1977, Sinclair 1978). Abundances of Chlorophyceae and Prasinophyceae reported here are clearly higher (by a factor of 10^3) than those of previous reports (Sinclair 1978). Small sizes probably affected counting efficiency for these groups. Dinoflagellates in the LSLE have historically attracted interest because of the presence of toxic species (*Alexandrium* sp.; Therriault et al. 1985, Cembella & Therriault 1989). Blooms generally occur in June and/or August (Cembella & Therriault 1989, Desbiens et al. 1990), but high abundances can also be found in July (Sinclair 1978). In 1992, low abundances of *Alexandrium* sp. were found, both in this study and in toxic algal surveys done by government agencies (DFO-Canada: M. Lev-

Table 4. Spearman rank order correlations between potential marker pigments and cell abundances in various algal groups from surface waters. Only significant ($p < 0.05$) coefficients are listed

Pigment	Algal group	May	June	July	Sept
Chl *b*	Prasinophyceae		0.69		
Chl c_3	Dinophyceae				0.85
	Unidentified flagellates		0.57		0.75
Alloxanthin	Cryptophyceae	0.95	0.81	0.56	0.85
	Unidentified flagellates		0.64		
BFU	Prymnesiophyceae		0.57		
	Diatoms (pennate)		0.56		
	Dinophyceae		0.67		
	Unidentified flagellates	−0.89	0.68		
Fucoxanthin	Diatoms (centric)			0.70	
	Chrysophyceae	0.75	0.65		
	Unidentified flagellates			−0.52	
Peridinin	Dinophyceae	0.85	0.77		
Zeaxanthin	Chrysophyceae		0.69		
	Unidentified flagellates		0.64		

asseur pers. comm.). Instead, dinoflagellates were dominated by *Gyrodinium* sp., *Heterocaspa triquetra*, *Pronoctiluca* sp. (formerly *Prorocentrum* sp.) and *Protoperidinium* sp., most of these potentially bloom-forming and toxic (Johnsen & Sakshaug 1993). The predominance of pennate diatoms in June surface waters is probably related to increased turbulence during spring runoff (Table 5). Other local studies reported the presence of these algae either in mid-summer (Sinclair 1978) or early fall (Levasseur et al. 1984). Mixing events probably explain these different patterns. The dominant centric diatoms found here have been frequently reported in summer in the LSLE (Cardinal & Lafleur 1977, Sinclair 1978, Levasseur et al. 1984) with similar abundances.

Phytoplankton succession follows the general pattern reported for this area by Sinclair (1978) and Levasseur et al. (1984) and for other northern temperate coastal waters (Smayda 1980). The combination of cell and pigment taxonomy used here, however, provided greater details. The presence of various groups of nanoflagellates in May and June in the LSLE is characteristic of springtime mixed surface waters in cold environments (cf. Nõmmann & Kaasik 1992 for the Baltic Sea).

Both daily primary production and chlorophyll values from this study fall within the range of values published for the LSLE (Sinclair 1978, Therriault & Levasseur 1985, Vézina 1994). Although small cells often constituted a significant fraction of chlorophyll biomass (Table 1), they seemed to play a more important role with respect to primary production. The contribution of small algal cells (<5 µm) to the total chl *a* biomass in the euphotic layer was always less than 50%, while the contribution of small cells to the total primary production varied between 10 and 90% (Fig. 8). Plotting these size fractions one against the other (Fig. 8), as in Tremblay & Legendre (1994), shows that large cell biomass accumulated in the euphotic layer in May and September while their production was low, suggesting intensive grazing on small cells during these months. The share of biomass and production for each size fraction was more equilibrated in summer. Overall it appears that primary production supported a microbial food web in spring and fall and probably a more classical food web in summer in this environment. This is consistent with the pheopigment profiles (Fig. 7), which were highest in the 2 summer months, and with the maximum abundances of the major copepod *Calanus finmarchicus* (S. Roy unpubl. data) and of herbivorous ciliates (Sime-Ngando et al. 1995) in June.

Comparisons between pigment and cell taxonomy

Three elements of comparisons were examined in this study: (1) similarity of groups based on clustering with both cells and pigments, (2) correlations between

Table 5. Average principal physical and chemical characteristics (SD in parentheses) for the surface groups identified by the statistical analysis done on cell counts. Depth of the euphotic layer defined as the depth receiving 1% of surface irradiance; depth of the mixed layer defined as the depth where the gradient in σ_t $m^{-1} \geq 0.2$ (Therriault & Levasseur 1985); salinity stratification index defined as (salinity in the deep layer – salinity at the surface)/average salinity (Dyer 1973); na: not available

Group	Temperature (°C)	Salinity	Daily incident ($E\ m^{-2}\ d^{-1}$)	Euphotic layer depth (m)	Mixed layer depth (m)	Salinity strat. index	Amonium conc. (µM)
May, surface	6.0 (2.1)	na	51.3 (4.1)	12.5 (2.7)	na	na	0.70 (0.43)
June, surface	9.2 (2.9)	25.7 (1.6)	40.6 (2.3)	13.5 (2.5)	12.5 (2.3)	0.28 (0.03)	0.26 (0.22)
July, surface	7.9 (1.4)	26.9 (1.1)	48.4 (2.2)	15.1 (2.8)	14.5 (6.1)	0.31 (0.13)	0.12 (0.08)
Sept, surface	5.2 (0.9)	27.4 (2.0)	15.0 (2.2)	17.2 (2.9)	25.7 (13.6)	0.21 (0.07)	0.34 (0.28)

marker pigments and cell data from appropriate algal groups, and (3) conversion of pigments into relative algal abundances that can then be compared with microscopic values. The first 2 elements, presented above, show generally good agreement between pigments and cells, although correlations point out that some associations vary seasonally, e.g. fucoxanthin mostly with Chrysophyceae in May and with diatoms in July.

Converting pigment data into relative abundances of various algal groups was done by estimating the contribution to total chl *a* by various pigment markers characteristic of different algal groups. Thus, if dinoflagellates have a certain chl *a*:peridinin ratio, the peridinin concentration found is multiplied by this ratio and expressed relative to total chl *a* to give the percent composition of dinoflagellates in the samples. For multiple pigment markers, multiple regression analysis is applied: (Total chl *a*) = constant + *a*(marker 1) + *b*(marker 2) + ... where *a*,*b*,... represent various chl *a*:marker *x* ratios.

This approach was used successfully in earlier studies (Gieskes & Kraay 1983, Gieskes et al. 1988, Everitt et al. 1990, Barlow et al. 1993, Letelier et al. 1993) and was applied here to the various cluster groups identified previously. Multiple regression analysis provided a number of statistically significant coefficients (chl *a*:marker *x*) which were used as initial values in a curve fitting procedure (SigmaPlot computer program, using the Marquardt-Levenberg algorithm) with the same equation as above. Curve fitting forced the overall contributions to add up to 100% and avoided solutions containing negative contributions. This approach is especially useful when multicolinearity is present (correlations between marker pigments) and permitted the inclusion of less abundant groups which did not necessarily show up as significant variables in the multiple regression. The final marker pigment coefficients (Table 7) were used to calculate percent contribu-

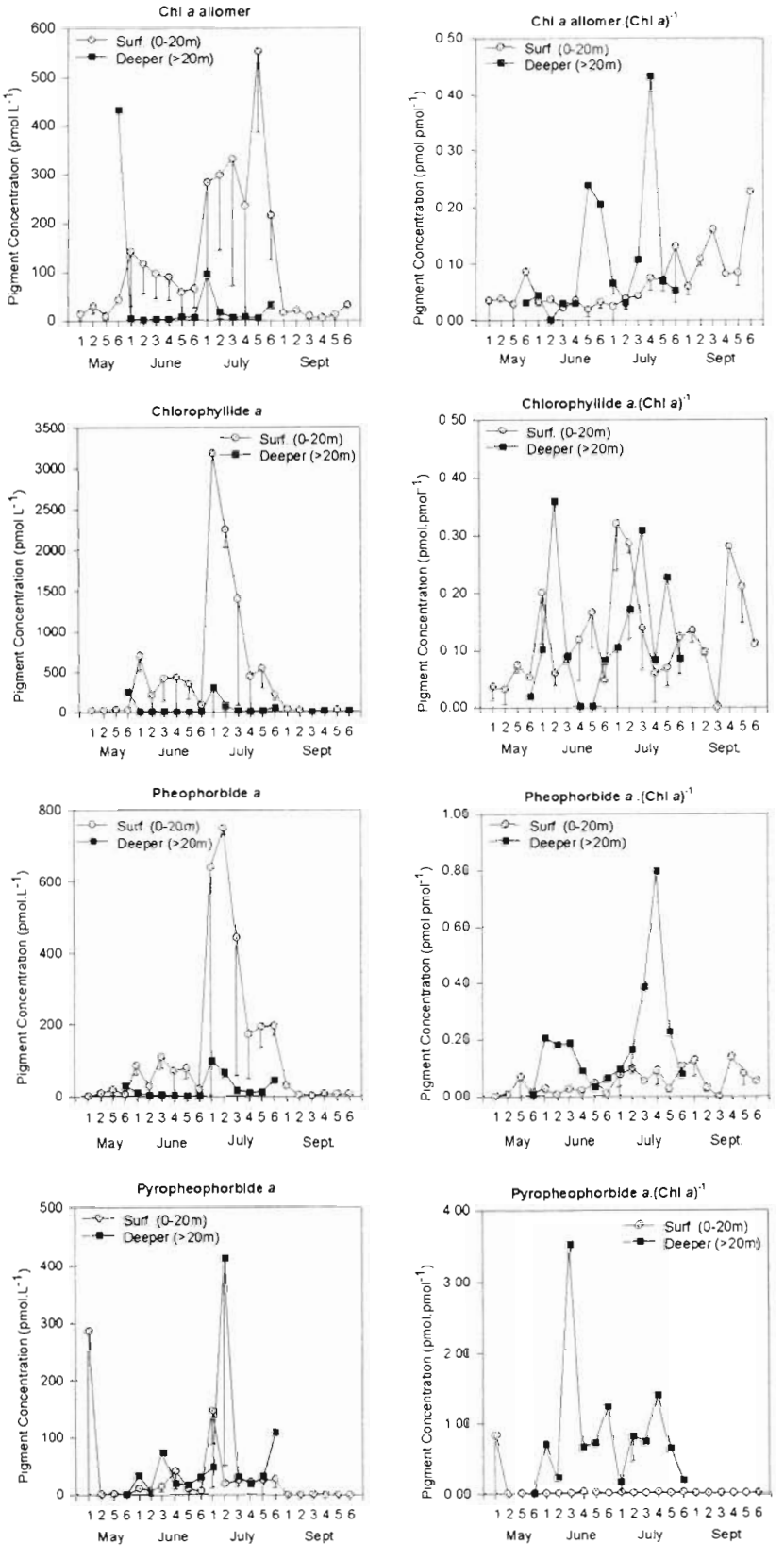

Fig. 7 Average pigment concentrations (pmol l^{-1}) (left column) and ratio to chl *a* (right column) for various chl *a* degradation pigments

Table 6. Average surface values (SD in parentheses) of HPLC-determined chl *a*, of the sum of photosynthetic pigments (SPP = chl *a*, chl *b*, chl c_{1+2}, chl c_3, BFU, HFU, fucoxanthin and peridinin) and of the ratios of SPP and photo-protective pigments (PRP = diadinoxanthin + diatoxanthin) to chl *a*

Month	Chl *a* (nmol l^{-1})	SPP (nmol l^{-1})	SPP:chl *a*	PRP:chl *a*
May	0.63 (0.39)	1.88 (0.86)	3.37 (0.95)	0.25 (0.14)
June	3.15 (1.94)	7.72 (4.27)	2.60 (0.59)	0.14 (0.06)
July	6.97 (4.91)	18.29 (13.97)	2.53 (0.58)	0.10 (0.06)
Sept	0.14 (0.10)	0.37 (0.26)	2.73 (0.56)	0.04 (0.03)

tions, as described above. In cases where a pigment occurred in more than 1 group, contributions from each group were tentatively calculated on the basis of another discriminating pigment, when possible. In June (surface), for example, correlations suggested that fucoxanthin and zeaxanthin were associated with Chrysophyceae (Type 1), and chl c_3 and BFU with Prymnesiophyceae (Type 4) (Table 4). Both algal groups possess fucoxanthin, as do the abundant pennate diatoms found in that month. The fucoxanthin contribution from Prymnesiophyceae was calculated from the mean fucoxanthin:chl c_3 ratio for Type 4 Prymnesiophyceae (0.96; Jeffrey & Wright 1994) and subtracted from total fucoxanthin to give Chrysophyceae + diatom-associated fucoxanthin. If microscopic cell information and correlations between pigments and algal groups had not been available, this exercise would have been more difficult. The marker pigment coefficients fit relatively well with published values in the cases of alloxanthin, peridinin, chl c_3 and fucoxanthin, although the peridinin coefficients are somewhat lower (Table 7). For chl *b*, 2 of the 4 values were comparable with previously published values while the 2 others gave excessively large values that were constrained in the curve-fitting process. The small number of observations within each group probably led to this situation. The fucoxanthin coefficient shows a seasonal increase from May to July, with a decrease in September. These changes could be taxonomically-related, the highest value (1.23) in July reflecting the chl *a*:fucoxanthin ratio in summer bloom diatoms while lower values in May and June could be related to the presence of other algal groups containing fucoxanthin. However, ratios of chl *a*:fucoxanthin in Chrysophyceae and Prymnesiophyceae are generally as high or higher as those of diatoms (Vesk & Jeffrey 1987, Bidigare 1989, Jeffrey & Wright 1994). The lower ratios observed here in May and September are thus more likely due to low-light acclimated cells. Indeed for September, decreased stratification and daily irradiance could account for this, and nanoflagellates from early spring are often characterized by lower compensation light intensity (Takahashi et al. 1978). Decreases in many ratios of chl *a*:photosynthetically competent pigments have been associated with decreases in irradiance (Richardson et al. 1983, Sukenik et al. 1987, Johnsen et al. 1992, 1994, Johnsen & Sakshaug 1993). Low ratios are also found in the deeper layers in June and July; again, this can be interpreted as an indication of photo-acclimation. Low values of most chl-*a*-per-marker-pigment coefficients in May are also reflected in the higher values of total photosynthetically competent pigments per chl *a* (Table 6). Higher relative amounts of photo-protective pigments in May (Table 6) are difficult to explain in this context; maybe they are indicative of frequent vertical movements, bringing cells close to surface light. Cells exposed to fluctuating light intensities sometimes resemble high-light photo-acclimated cells (accumulating photo-protective pigments) and sometimes low-light cells (low relative amounts of photo-protective pigments; Vignault 1996).

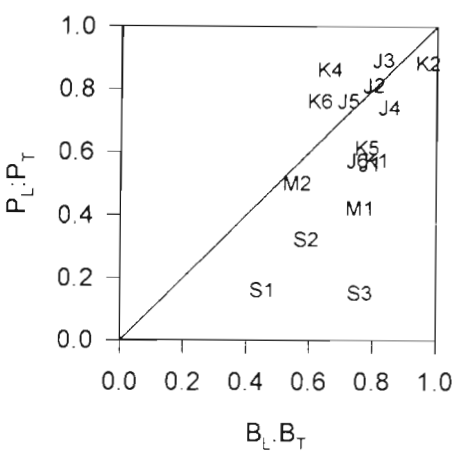

Fig. 8. P-B diagram. Abscissa: ratio of integrated (over the euphotic layer) chlorophyll biomass of large (>5 µm) cells (B_L) over total biomass (B_T); ordinate: ratio of integrated (over the euphotic layer) primary production by large cells (P_L) over production by all cells present (P_T). Sample codes as in Fig. 5

Relative contributions of algal groups determined from pigment taxonomy were finally compared with those obtained from microscopy (Table 8). Results generally show a good fit between them. However, algae containing chl *b* are underrated in microscopic counts, particularly in the May, June and September surface groups. A number of Prasinophyceae and/or Chlorophyceae were probably missed in the counts. High numbers of unidentified flagellates in microscopic counts of June and September contribute to a lack of match between pigments and cells for these samples. It is generally difficult to distinguish between diatoms,

Chrysophyceae, Prymnesiophyceae and fucoxanthin-containing Dinophyceae using pigments, although attempts made here gave reasonable results, except for September. Contributions of various algal groups to

Table 7 (A) Marker pigment coefficients (final values after curve fitting; mol chl *a* per mol marker pigment) used to transform concentrations of marker pigments into contributions of various algal groups to total chl *a*. (B) Comparison with a selection of literature values of the same coefficients (transformed to molar values)

	Chl *b*	Chl c_3	Alloxanthin	Fucoxanthin	Peridinin
(A) This study					
May, surface	3.00[a]	0.74	0.40	0.65	–
June, surface	2.87	1.16	5.37	0.96	0.38
July, surface	3.00[a]	–	1.35	1.23	–
Sept, surface	2.17	3.66	–	0.65	–
June, deep	–	–	1.20[b]	0.23	0.42
July, deep	–	–	1.20[b]	0.53	–
(B) Other sources					
Wood (1979)	3.05				
Gieskes et al. (1988)	3.00			1.27	
Hooks et al. (1988)	1.13				
Everitt et al. (1990)	0.76		1.27		1.82
Johnsen et al. (1992)		3.33			
Jeffrey & Wright (1994)		4.0–12.6			
Hager & Stransky (1970)			1.17	1.03	
Gieskes & Kraay (1983)			3.86–4.19	1.32–1.89	
Wilhelm et al. (1991)			2.54	1.18	1.05
Bidigare (1989)				1.23	
Jeffrey et al. (1975)					1.66

[a]Ratio constrained to 3.00 to prevent values >10
[b]Ratio constrained to 1.20 to prevent values <0.10

Table 8. Relative contributions of major algal groups (in percent) determined from pigments (P) compared with those obtained from microscopy (M). Values shown are averages for each surface and deeper group determined by the cluster analysis. Numbers in bold: significant pigment variable in multiple regression equation

Algal group (marker pigment)	May		June		July		September	
	P	M	P	M	P	M	P	M
(A) Surface								
Chlorophyceae, Prasinophyceae (chl *b*)	**19**	6	19	1	5	4	**27**	10
Chrysophyceae	59[a] (fucox.)	57		4		4	27[b] (fucox.)	20
Prymnesiophyceae	11 (chl c_3)	5	9 (chl c_3)	5		1		10
Diatoms		7	**49**[a] (fucox.)	36	**82** (fucox.)	84		4
Cryptophyceae (alloxanthin)	5	12	**13**	2	5	5		9
Dinophyceae (peridinin or indicated)		5	6	1		1	37 (chl c_3)	5
Unidentified flagellates		6		51		1		40
(B) Deeper								
Diatoms, Chrysophyceae, Prymnesiophyceae (fucoxanthin)			**55**	14	**83**	90		
Dinophyceae (peridinin)			**25**	37		4		
Cryptophyceae (alloxanthin)			19	16	4	3		
Unidentified flagellates				28		2		

[a]Based on fucoxanthin and could include Chrysophyceae + diatoms
[b]Based on fucoxanthin and could include Chrysophyceae + Prymnesiophyceae + diatoms
[c]No chl c_3 in deep groups (Table 3b), thus no dinoflagellates containing fucoxanthin

Table 9. Contribution of various algal groups to primary production (mg C m^{-3} d^{-1}) based on the pigment approach of Claustre et al. (1994): relative contributions of major algal groups based on pigments (Table 8) were transformed into chl *a* values for each group, then multiplied by appropriate C:chl *a* ratios and by typical growth rates for these algal groups (from Claustre et al. 1994). For fucoxanthin, when the algal origin of this pigment was not clear, calculations were made assuming the presence of nanoflagellates (n), and assuming the presence of diatoms (d). Only surface groups are presented. Total estimated primary production is the sum of the contributions by the various algal groups. Total measured primary production is taken from Table 1

	May	June	July	September
Chl *b*	4.7	24.5	12.9	2.0
Chl c_3	2.1	11.7		3.1
Alloxanthin	1.0	17.9	7.6	
Fucoxanthin	13.9	60.6 (n)	173.7	2.9 (n)
		31.8 (d)		1.5 (d)
Peridinin		7.6		
Total estimated primary production	21.7	122.3 (n)	194.2	8.0 (n)
		93.5 (d)		6.6 (d)
Total measured production (Table 1)	19.9	115.9	261.9	3.7

primary production, calculated as in Claustre et al. (1994), were used to examine how our pigment-based interpretation of algal groups corresponded with total primary production values. Data from Table 9 suggest that fucoxanthin belonged mostly to nanoflagellates in June and to diatoms in September. Unidentified flagellates from September seemed to be mostly made up of Chlorophyceae and Dinophyceae, while in June (surface) Chlorophyceae and Cryptophyceae contribute to these cells.

In conclusion, the seasonal phytoplankton pattern observed in this study involved a major centric diatom bloom event in July, preceded by an increase in pennate diatoms in June, likely caused by bottom resuspension due to spring runoff. Grazing in June and July was indicated by the presence of pyropheophorbide *a*, a copepod grazing product tracer, and chlorophyll degradation pigments, likely associated with sloppy feeding and with the presence of cells (diatoms) with high chlorophyllase activity and acidic cell sap. Pheopigments and degradation products of chl *a* were also mostly found in these 2 months. This information concurs with maximum abundances of major copepod species and of herbivorous ciliates in June preceding the summer diatom bloom. May was characterized by small flagellates from Chrysophyceae, Cryptophyceae and Chlorophyceae and lower values of algal biomass and production but higher light harvesting efficiency. Mixing prevented the establishment of vertical fluorescence patterns in May and September and probably lowered the effective daily light exposure of algae, which translated into lower light acclimation than in summer, with higher ratios of photosynthetic pigments to chl *a*. Low-light acclimation was also observed in the deep (>20 m) June and July populations, affecting marker pigment coefficients used to calculate relative algal contributions. Increases in relative amounts of chlorophyllide *a* and the allomer of chl *a* in September were interpreted as signs of algal senescence. The fall populations were composed of a number of chlorophyte and chromophyte (fucoxanthin-containing) algae. Low pigment concentrations and low numbers of observations complicated the identification task for September.

Comparisons of the pigment and microscopic approaches showed different characteristics between the two: the presence of small cells was often a problem for microscopic identifications, while the lack of specificity of some markers (e.g. fucoxanthin) reduced taxonomic precision from the pigment approach. Combining both was certainly advantageous, in that cell-pigment correlations helped in the assignment of a number of pigment markers. Pigments also helped in ascribing taxonomic identities for unidentified flagellates which were numerous in June and September. If only pigments had been available, and if only significant coefficients had been retained from the multiple regression analysis used to calculate algal group contributions, results would have been much less detailed (see Table 8, bold numbers). On the other hand, major trends would still have been detected. Thus, the choice between using the methods singly or together will depend partly on the degree of taxonomic detail needed.

Acknowledgements. This research was supported by grants from FODAR (Fonds pour le Développement et l'Avancement de la Recherche, Université du Québec) to M.G., S.R. and J.P.C., by NSERC individual grants to S.R. and M.G., by FCAR individual grant to M.G., by FIR-UQAR (Fonds Institutionnel de Recherche, Université du Québec à Rimouski) to J.P.C. and M.G. and by the program 'Actions structurantes' of the Québec Ministry of higher education and science to M.G. and T.S.N. We thank B. Ramirez for algal counts and identification, L. Lorrain for pigment analysis, L. Bérard-Therriault for confirmations of cell identifications, technical assistants C. Belzile and N. Blouin and summer students A. Bélanger, L. Cormier and H. Lambert for help with ship work. Special thanks to the captains (S. St-Louis and A. Richard) and crew of the RV 'A.C. Horth' for ship work. We also thank 4 referees for their helpful comments. This study is a contribution to the research program of the GREC (Groupe de Recherche en Environnement Côtier).

LITERATURE CITED

Barlow RG, Mantoura RFC, Gough MA, Fileman TW (1993) Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep Sea Res 40:459–477

Barlow RG, Mantoura RFC, Peinert R, Miller AEJ, Fileman TW (1995) Distribution, sedimentation and fate of pigment biomarkers following thermal stratification in the western Alboran Sea. Mar Ecol Prog Ser 125:279–291

Bathman U, Liebezeit G (1986) Chlorophyll in copepod fecal pellets: changes in pellet numbers and pigment content during a declining Baltic spring bloom. PSZN I: Mar Ecol 7:59–73

Bidigare RR (1989) Photosynthetic pigment composition of the brown tide alga: unique chlorophyll and carotenoid derivatives. Coastal Estuarine Stud 35:57–75

Bidigare RR, Kennicutt MC II, Ondrusek ME, Keller MD, Guillard RRL (1990) Novel chlorophyll-related compounds in marine phytoplankton: distributions and geochemical implications. Energy & Fuels 4:653–657

Bjørnland T, Pennington FC, Haxo FT, Liaaen-Jensen S (1984) Carotenoids of Chrysophyceae and Dinophyceae 'Coc. Min Haltenbanken' and *Gymnodinium breve* (Florida red tide). In: Abstract 7th Int IUPAC Symposium on Carotenoids, Munich, p 21

Bjørnland T, Tangen K (1979) Pigmentation and morphology of a marine *Gyrodinium* (Dinophyceae) with a major carotenoid different from peridinin and fucoxanthin. J Phycol 15:457–463

Bray RJ, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27: 325–349

Cardinal A, Lafleur PE (1977) Le phytoplancton estival de l'estuaire maritime du Saint-Laurent. Soc Phycol de France Bull 22:150–160

Cembella AD, Therriault JC (1989) Population dynamics and toxin composition of *Protogonyaulax tamarensis* from the St. Lawrence estuary. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides: biology, environmental science and toxicology. Elsevier Science Publishing Co Inc, New York, p 81–84

Chisholm SW, Olson RO, Zettler ER, Goericke R, Waterbury JB, Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334: 340–343

Claustre H, Kerhervé P, Marty JC, Prieur L, Videau C, Hecq JH (1994) Phytoplankton dynamics associated with a geostrophic front: ecological and biogeochemical implications. J Mar Res 52:711–742

Davies BH (1976) Carotenoids. In: Goodwin TW (ed) Chemistry and biochemistry of plant pigments, 2nd edn, Vol 2. Academic Press, London, p 38–165

Demers S, Roy S, Gagnon R, Vignault C (1991) Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photo-protection mechanism. Mar Ecol Prog Ser 76: 185–193

Desbiens M, Coulombe F, Gaudreault J, Cembella AD, Larocque R (1990) PSP toxicity of wild and cultured blue mussels induced by *Alexandrium excavatum* in Gaspé bay (Canada): implications for aquaculture. In: Graneli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier Science Publishing Co Inc, New York, p 459–462

Dyer KR (1973) Estuaries: a physical introduction. John Wiley & Sons, London

Egeland ES, Eikrem W, Throndsen J, Wilhelm C, Zapata M, Liaaen-Jensen S (1995) Carotenoids from further Prasinophytes. Biochem Syst Ecol 23:747–755

Everitt DA, Wright SW, Volkman JK, Thomas DP, Lindstrom EJ (1990) Phytoplankton community compositions in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distributions. Deep Sea Res 37: 975–997

Gieskes WWC (1991) Algal pigment fingerprints: clue to taxon-specific abundance, productivity and degradation of phytoplankton in seas and oceans. In: Demers S (ed) Particle analysis in oceanography. NATO ASI G27, Springer-Verlag, Berlin, p 61–99

Gieskes WWC, Kraay GW (1983) Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. Limnol Oceanogr 28: 757–766

Gieskes WWC, Kraay GW, Nontji A, Setiapermana D, Sutomo (1988) Monsoonal alternation of a mixed and layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprints. Neth J Sea Res 22:123–137

Goericke R, Repeta DJ (1992) The pigments of *Prochlorococcus marinus*: the presence of divinyl chlorophyll *a* and *b* in a marine procaryote. Limnol Oceanogr 37:425–433

Goericke R, Repeta DJ (1993) Chlorophylls *a* and *b* and divinyl chlorophylls *a* and *b* in the open subtropical North Atlantic Ocean. Mar Ecol Prog Ser 101:307–313

Goodwin TW (1980) The biochemistry of the carotenoids. Chapman and Hall, London

Gratton Y, Mertz G, Gagné JA (1988) Satellite observations of tidal upwelling and mixing in the St. Lawrence estuary. J Geophys Res 93(C6):6947–6954

Hager A, Stransky H (1970) Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. V Einzelne Vertreter der Cryptophyceae, Euglenophyceae, Bacillariophyceae, Chrysophyceae und Phaeophyceae. Arch Mikrobiol 73: 77–89

Hallegraeff GM (1981) Seasonal study of phytoplankton pigments and species at a coastal station off Sidney: importance of diatoms and the nanoplankton. Mar Biol 61: 107–118

Hallegraeff GM, Jeffrey SW (1985) Description of new chlorophyll *a* alteration products in marine phytoplankton. Deep Sea Res 32:697–705

Head EJH, Hargrave BT, Subba Rao DV (1994) The accumulation of a phaeophorbide *a*-like pigment in sediment traps during the late stages of a spring bloom: a product of dying algae? Limnol Oceanogr 39: 176–181

Head EJH, Harris LR (1992) Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar Ecol Prog Ser 86:229–238

Hertzberg S, Liaaen-Jensen S (1969) The structure of myxoxanthophyll. Phytochemistry 8:1259–1280

Holm-Hansen O, Lorenzen CJ, Holmes RN, Strickland JDH (1965) Fluorometric determination of chlorophyll. J Cons Int Explor Mer 30:3–15

Hooks CE, Bidigare RR, Keller MD, Guillard RRL (1988) Coccoid eukaryotic marine ultraplankters with four different HPLC pigment signatures. J Phycol 24:571–580

Jeffrey SW (1981) Algal pigment systems. In: Falkowski PG (ed) Primary productivity in the sea. Plenum Press, New York, p 33–58

Jeffrey SW, Hallegraeff GM (1987a) Phytoplankton pigments, species and light climate in a complex warm-core eddy of the East Australian Current. Deep Sea Res 34:649–673

Jeffrey SW, Hallegraeff GM (1987b) Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar Ecol Prog Ser 35:293–304

Jeffrey SW, Haxo FT (1968) Photosynthetic pigments of dinoflagellates (Zooxanthellae) from corals and clams. Biol Bull 135:149–165

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining Chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194

Jeffrey SW, Sielicki M, Haxo FT (1975) Chloroplast pigment patterns in dinoflagellates. J Phycol 11:374–384

Jeffrey SW, Wright SW (1994) Photosynthetic pigments in the Haptophyta. In: Green JC, Leadbeater BSC (eds) The Haptophyte algae. Systematics Association Special Vol no. 51. Clarendon Press, Oxford, p 111–132

Jensen A (1966) Algal carotenoids. V. Iso-fucoxanthin-rearrangement product of fucoxanthin. Acta Chem Scand 29:1728–1730

Johnsen G, Nelson NB, Jovine RVM, Prézelin BB (1994) Chromoprotein- and pigment-dependent modeling of spectral light absorption in two dinoflagellates, *Prorocentrum minimum* and *Heterocapsa pygmaea*. Mar Ecol Prog Ser 114:245–258

Johnsen G, Sakshaug E (1993) Bio-optical characteristics and photoadaptive responses in the toxic and bloom-forming dinoflagellates *Gyrodinium aureolum*, *Gymnodinium galatheanum*, and two strains of *Prorocentrum minimum*. J Phycol 29:627–642

Johnsen G, Sakshaug E, Vernet M (1992) Pigment composition, spectral characterization and photosynthetic parameters in *Chrysochromulina polylepis*. Mar Ecol Prog Ser 83: 241–249

Klein B, Sournia A (1987) A daily study of the diatom spring bloom at Roscoff (France) in 1985. II. Phytoplankton pigment composition studied by HPLC analysis. Mar Ecol Prog Ser 37:265–275

Lean DRS, Burnison BK (1979) An evaluation of errors in the ^{14}C method of primary production measurement. Limnol Oceanogr 24:917–928

Legendre L, Legendre P (1984) Écologie numérique. Tome 2. La structure des données écologiques. Masson, Paris, et Les Presses de l'Université du Québec

Letelier RM, Bidigare RR, Hebel DV, Ondrusek M, Winn CD, Karl DM (1993) Temporal variability of phytoplankton community structure based on pigment analysis. Limnol Oceanogr 38:1420–1437

Levasseur M, Therriault JC, Legendre L (1984) Hierarchical control of phytoplankton succession by physical factors. Mar Ecol Prog Ser 19:211–222

Liaaen-Jensen S (1978) Marine carotenoids. In: Scheuer PJ (ed) Marine natural products. Academic Press, New York, p 1–73

Liaaen-Jensen S (1985) Carotenoids of lower plants—recent progress. Pure Appl Chem 57:649–658

Lund JWG, Kipling C, Lecren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170

Millie DF, Kirkpatrick GJ, Vinyard BT (1995) Relating photosythetic pigments and *in vivo* optical density spectra to irradiance for the Florida red-tide dinoflagellate *Gymnodinium breve*. Mar Ecol Prog Ser 120:65–75

Millie DF, Paerl HW, Hurley JP (1993) Microalgal pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications. Can J Fish Aquat Sci 50:2513–2527

Nõmmann S, Kaasik E (1992) Hydrodynamical control of phytoplankton succession during the vernal light-limited phase in the Baltic Sea. Mar Ecol Prog Ser 84:279–292

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Pennington FC, Strain HH, Svec WA, Katz JJ (1964) Preparation and properties of pyrochlorophyll *a*. methyl pyrochlorophyllide *a*, pyropheophytin *a*, and methyl pyropheophorbide *a* derived from chlorophyll by decarbomethoxylation. J Am Chem Soc 86:1418–1426

Redden A (1994) Grazer-mediated chloropigment degradation and the vertical flux of spring bloom production in Conception Bay, Newfoundland. PhD thesis, Memorial University of Newfoundland, St. John's

Richardson K, Beardall J, Raven JA (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol 93:157–191

Ricketts TR (1970) The pigments of the Prasinophyceae and related organisms. Phytochemistry 9:1835–1842

Rowan KS (1989) Photosynthetic pigments of algae. Cambridge University Press

Sime-Ngando T, Gosselin M, Roy S, Chanut JP (1995) Significance of ciliated protozoa in the lower St. Lawrence estuary: comparison with bacterial, phytoplankton and particulate organic carbon. Aquat Microb Ecol 9:243–258

Sinclair M (1978) Summer phytoplankton variability in the lower St. Lawrence estuary. J Fish Res Bd Can 35: 1171–1185

Smayda TJ (1980) Phytoplankton species succession. In: Morris I (ed) The physiological ecology of phytoplankton. University of California Press, Berkeley, p 493–570

Stauber JL, Jeffrey SW (1988) Photosynthetic pigments in fifty-one species of marine diatoms. J Phycol 24:158–172

Sukenik A, Wyman KD, Bennet J, Falkowski PG (1987) A novel mechanism for regulating the excitation of photosystem II in a green alga. Nature 327:705–707

Suzuki R, Fujita Y (1986) Chlorophyll decomposition in *Skeletonema costatum*: a problem in chlorophyll determination of water samples. Mar Ecol Prog Ser 28:81–85

Takahashi M, Barwell-Clarke J, Whitney F, Koeller P (1978) Winter conditions of marine plankton populations in Saanich Inlet, B.C., Canada. I. Phytoplankton and its surrounding environment. J Exp Mar Biol Ecol 31:283–301

Tangen K, Bjørnland T (1981) Observations on pigments and morphology of *Gyrodinium aureolum* Hulburt, a marine dinoflagellate containing 19'-hexanoyloxyfucoxanthin as the main carotenoid. J Plankton Res 3:389–401

Therriault JC, Levasseur M (1985) Control of phytoplankton production in the lower St. Lawrence estuary: light and freshwater runoff. Nat Can (Rev Ecol Syst) 112:77–96

Therriault JC, Painchaud J, Levasseur M (1985) Factors controlling the occurrence of *Protogonyaulax tamarensis* and shellfish toxicity in the St. Lawrence estuary: freshwater runoff and the stability of the water column. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates, Proc Third Int Conf on Toxic Dinoflagellates. Elsevier Science Publishing Co Inc, New York, p 141–146

Tremblay JE, Legendre L (1994) A model for the size-fractionated biomass and production of marine phytoplankton. Limnol Oceanogr 39:2004–2014

Vesk M, Jeffrey SW (1987) Ultrastructure and pigments of two strains of the picoplanktonic alga *Pelagococcus subviridis* (Chrysophyceae). J Phycol 23:322–335

Vézina AF (1994) Mesoscale variability in nitrogen uptake rates and the f-ratio during a coastal phytoplankton bloom. Limnol Oceanogr 39:854–868

Vignault C (1996) Photo-acclimatation chez le phytoplancton marin: implication du cycle xanthophyllien et influence sur la fluorescence algale. MSc thesis, Université du Québec à Rimouski

Welschmeyer NA, Lorenzen CJ (1985) Role of herbivory in controlling phytoplankton abundance: annual pigment budget for a temperate marine fjord. Mar Biol 90:75–86

Wilhelm C, Rudolph I, Renner W (1991) A quantitative method based on HPLC-aided pigment analysis to monitor structure and dynamics of the phytoplankton assemblage—a study from Lake Meerfelder Maar (Eifel, Germany). Arch Hydrobiol 123:21–35

Withers NW, Fiksdahl A, Tuttle RC, Liaaen-Jensen S (1981) Carotenoids of the Chrysophyceae. Comp Biochem Physiol 68B:345–349

Wood AM (1979) Chlorophyll *a*:*b* ratios in marine planktonic algae. J Phycol 15:330–332

Wright SW, Jeffrey SW (1987) Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. Mar Ecol Prog Ser 38:259–266

Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjornland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar Ecol Prog Ser 77: 183–196

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Res 10:221–231

This article was submitted to the editor

Manuscript first received: December 27, 1995
Revised version accepted: July 2, 1996