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Distribution of eukaryotic plankton in the English Channel and the North Sea in summer

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ABSTRACT

The distribution of eukaryotic plankton was investigated in the English Channel and the North Sea during the MICROVIR cruise in summer 2007. The size distribution of autotrophic, heterotrophic eukaryotes and species composition was analyzed with a focus on two major divisions, Haptophyta and Chlorophyta, targeted by 18S rRNA probes. Picoeukaryotes (<2 μm) dominated over the larger eukaryotes at all stations. Eukaryotes larger than 5 μm were mainly composed of diatoms in the English Channel and of dinoflagellates in the North Sea. The contribution of Haptophyta was maximal in the 2 to 5 μm fraction and they appeared more abundant in the central region of the North Sea. Chlorophyta, especially *Micromonas pusilla*, generally dominated the picoplanktonic fraction in the English Channel. *Micromonas* contribution decreased between the South and the North-east of the North Sea and it was even absent at some stations. Although this species is dominant among the picoeukaryote community of the English Channel, other Chlorophyta species may also play an important ecological role in these temperate ecosystems.

1. Introduction

The North Sea is a complex marine environment characterized by different water masses. Near the coast, it consists of a mixture of North Sea water and freshwater run-off. The latter shows nutrient and biological settings completely different between areas influenced by Baltic outflow or by estuaries: along the continental coast, low salinity, nutrient rich water masses are found while the North-eastern part of the North Sea is influenced by the low salinity, nutrient poor outflow of the Baltic Sea (Ducrotoy et al., 2000). In deeper areas, relatively pure water of Atlantic origin is found. The circulation and distribution of these water masses have a major impact on their biological productivity (Otto et al., 1990; Reid et al., 1988). Previous studies carried out about various aspects of phytoplankton dynamics were limited in their spatial and temporal coverage. Nano- and micro-phytoplankton (plankton between 2 μm and 20 μm in size, and larger than 20 μm, respectively, Sieburth et al., 1978) appear to be the major contributors to algal biomass and primary production of the North Sea. More than 30 taxa have been listed as potentially forming blooms in the North Sea and adjacent waters (Reid et al., 1990). Such is particularly the case for the *Phaeocystis* and *Ceratium* genera, with blooms occurring mainly in spring and summer, respectively (Brussaard et al., 1996; Gieskes et al., 2007; Reid et al., 1990). Other studies have focused on the composition and dynamics of the nano-size fraction of the plankton but have often been restricted to specific areas of the North Sea (Druzhkov and Druzhkova, 2000; Van Duyl et al., 1990). The Continuous Plankton Recorder (CPR) survey has provided qualitative and quantitative information about the distribution of phytoplankton in different areas of the North Sea but mostly for the micro-size fraction (Beaugrand et al., 2004).

Recently, however, it has been found that picophytoplankton (<2 μm) is an ecologically important group of phytoplankton (Marañón et al., 2001; Worden et al., 2004). Little is known about picophytoplankton distribution and diversity in the North Sea, as the few studies performed so far were restricted to coastal environments. Still, it was shown that picophytoplankton in the English Channel can constitute more than 30% of the total chlorophyll biomass (Not et al., 2004). Analyses of the 18S rRNA gene on natural picoplankton communities from Helgoland and English Channel waters have revealed a high diversity among picoeukaryotes (Medlin et al., 2006; Romari and Vaulot, 2004). Picoplankton has been analyzed and quantified by epifluorescence microscopy in the Skagerrak

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(Kuylenstierna and Karlson, 1994), suggesting that the Mamelliophyceae species, *Micromonas pusilla* could be abundant in that region of the North Sea. *M. pusilla* also dominates the autotrophic picoeukaryotic population in the English Channel (Not et al., 2004). However, these studies were restricted to coastal environments and the global distribution of this important species in the North Sea remained unknown.

In the present study, epifluorescence microscopy was combined with TSA-FISH analysis of probes targeting 18S rRNA to assess the distribution of major groups of eukaryotic plankton in the English Channel and the North Sea. We grouped organisms into size classes and assessed their distribution across the different water masses that we searched. Special emphasis was put on the distribution of the picoplanktonic species *Micromonas pusilla*, expected to be well represented in this region.

2. Material and methods

2.1. Sampling and oceanographic context

The MICROVIR cruise took place on board the Dutch R/V Pelagia in the North Sea from 2nd July to 30th July 2007 from Brest (France) to Texel (The Netherlands), through the English Channel and the North Sea (Fig. 1).

Among the 23 stations occupied during the cruise, 14 of them, corresponding to long stations (24 h occupation) were selected for detailed analysis (Table 1). They were sampled at different depths (from 2 to 5 depths depending on stations) with a conductivity–temperature–depth (CTD) rosette system equipped with 12 L Niskin bottles. Generally, one sample was collected at a 10 meter-depth, one above the chlorophyll maximum, one at the chlorophyll maximum (CM), and two below it.

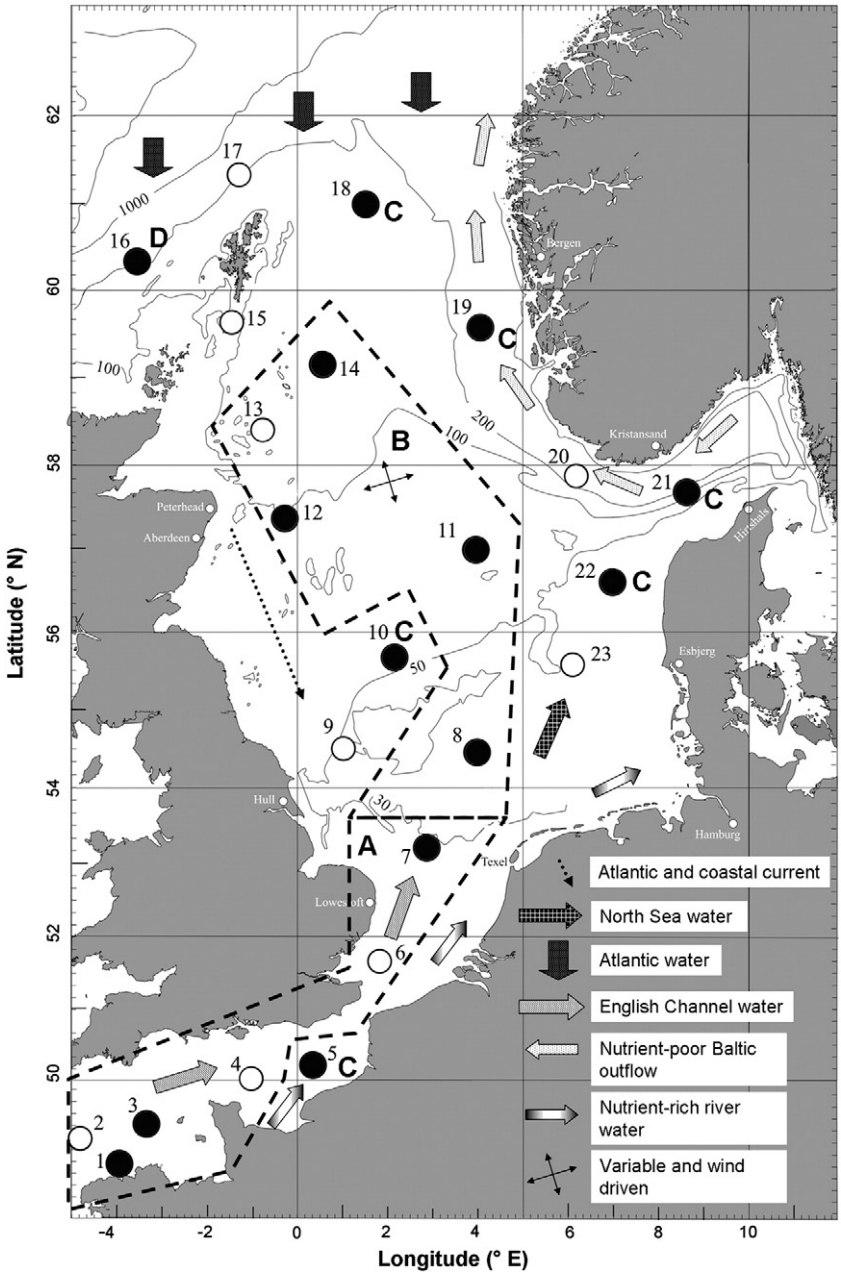

Fig. 1. Stations investigated during the MICROVIR cruise. Numbers at the bottom and on the left of the map correspond to longitudes and latitudes, respectively. Black circles correspond to the stations analyzed in this study. Isobaths are also shown on the map. The areas delimited by dotted line (A, B, C and D) correspond to the different groups of stations (Fig. 7 and Table 4). Arrows correspond to major water circulation (For more details, see Otto et al., 1990; Turrell 1992; Narayanaswamy et al., 2010).

Table 1
Coordinates of stations analyzed in this study and sampling depths for counts with TSA-FISH probes and DAPI-staining.

Station	Latitude (°N)	Longitude (°E)	Sampling depths (m)	Maximum depth (m)
1	48.77	−3.95	10–25–50	65
3	49.33	−3.33	10–25–50	76
5	50.20	0.33	10 and 25	39
7	53.17	2.87	10 and 20	32
8	54.41	4.05	10–20–25–30	46
10	55.68	2.28	10–20–30–40–60	83
11	56.99	3.99	10–30–40	61
12	57.33	−0.33	10–20–35–50	77
14	59.17	0.67	10–20–35–50–75	124
16	60.33	−3.49	10–20–40–50	139
18	61.00	1.99	10–20–25–35–50	133
19	59.33	4.33	10–20–30–40–50	267
21	57.67	8.67	10–20–35–55–80	142
22	56.50	7.17	10–20–25	36

Water was pre-filtered through a 200 μm-mesh to remove zooplankton, large phytoplankton and particles before further filtrations in order to allow an easier visualization of smaller organisms.

2.2. Physico-chemical parameters

Samples were collected in 10 L NOEX bottles mounted on the Rosette sampler equipped with Seabird conductivity–temperature–depth (CTD). Salinity, temperature, chlorophyll *a* concentration and light intensity were obtained from the CTD mounted on the Rosette sampler equipped with a natural fluorescence detector. Nutrient samples (5 mL) were filtered through 0.2 μm pore-size polysulfone filters (Acrodisc, Gelman Sciences) and analyzed on board using a TrAAcs 800 autoanalyzer for dissolved orthophosphate, nitrate, nitrite, ammonium, and silicate as described in Baudoux et al. (2006).

2.3. DAPI-staining

Sampling, fixation and staining were performed according to Masquelier and Vaulot (2008). Counts were made using an Olympus BX51 epifluorescence microscope (Olympus Optical Co, Tokyo, Japan) equipped with a mercury light source and a ×100 UVFL objective.

We distinguished autotrophic from heterotrophic eukaryotes under blue light (490/515 nm). Autotrophic eukaryotes appeared in red due to chlorophyll autofluorescence while heterotrophic eukaryotes appeared in green. However, it was not possible to distinguish truly autotrophic organisms from organisms that had ingested chlorophyll-containing cells. Eukaryotes were classified according to three diameter ranges: (1) smaller than 2 μm, (2) between 2 μm and 5 μm, (3) larger than 5 μm (but smaller than 200 μm since samples were pre-filtered through 200 μm filters). Among eukaryotes larger than 5 μm, dinoflagellates and diatoms were counted separately. Dinoflagellates were discriminated by their shape, their size (between 5 μm and 250 μm), and the presence of a nucleus with condensed chromatin. Autotrophic and heterotrophic dinoflagellates were discriminated according to the red fluorescence of chlorophyll under blue light excitation. Some heterotrophic dinoflagellates were characterized by an intense green fluorescence under blue light, as reported previously (Shapiro et al., 1989), and counted separately. Diatoms were discriminated by their shape and size (generally >20 μm).

2.4. FISH associated with tyramide signal amplification (TSA-FISH)

Water samples (90 mL) were fixed with 1% paraformaldehyde (final concentration) at 4 °C for 1 h, and cells were poured onto 0.2 μm pore size Anodisc filters (Whatman Int. Ltd., Maidstone, Kent, UK). The filters were then dehydrated in an ethanol dilution series of 50%, 80%, and 100%, 3 min each (Amann, 1995), dried, and kept at room temperature in the dark during the cruise and at −80 °C in the lab until TSA-FISH analysis.

The oligonucleotide probes targeting 18S rRNA Euk1209, Chlo01 and NChlo01 were used as a mix in order to target all eukaryotes. Probes Chlo02 targeted Chlorophyta, Prym02, Haptophyta, Pras04, Mamiellophyceae (previously Mamiellales within class Prasinophyceae, Marin and Melkonian, 2010), Micro01, *Micromonas pusilla* (Mamiellophyceae) (Table 2).

In silico analysis of the mix probe (Euk1209 + Chlo01 + NChlo01) with the 18S rRNA gene SILVA database (133,603 eukaryote sequences; March 2010) showed that more than 99% of eukaryotes could be hybridized with at least one of the 3 probes. However, we observed that for eukaryotes between 2 and 5 μm, and for those larger than 5 μm, hybridization efficiency decreased down to 50% at some stations (from stations 7 to 16). This could be due to the presence of a larger fraction of cells refractory to permeabilization, or to a low amount of ribosomal RNA for some cells.

Whole cell *in situ* hybridization with TSA amplification was performed according to Not et al. (2002), except for additional steps of permeabilization before hybridization. To avoid cell loss during cell wall permeabilization, the filters were dipped into low melting-point Agarose (0.2% [wt/vol] in MQ water), dried face up on glass slides at 35 °C, and subsequently dehydrated in 96% (vol/vol) ethanol for 1 min. For permeabilization, 100 μL of 15 mg mL^{-1} cellulase (5.1 unit mg^{-1}, Sigma) were added to each filter and left to incubate for 10 min at 37 °C. Then, the filters were washed twice in sterile distilled water for 10 min at room temperature, dehydrated in 96% (vol/vol) ethanol for 1 min, dried, and cut into pieces with a razor blade. To avoid background fluorescence, before hybridization, 10 μL of formamide hybridization buffer (40% deionized formamide, 0.9 M NaCl, 20 mM Tris–HCl, pH 7.5, 0.01% sodium dodecyl sulfate [SDS], 10% (w:v) blocking reagent [Roche Diagnostic Boehringer]) were added to each piece of filter and incubated for 30 min at 35 °C. In order to visualize the nuclei, the cells were counterstained with DAPI (5 μg mL^{-1} final concentration) after the last wash.

Counts were performed with the same epifluorescence microscope used for DAPI-stained samples. Hybridized cells were observed under blue light (490/515 nm) to excite green fluorescein fluorescence. The parallel DAPI staining of the TSA-FISH filters allowed us to discriminate eukaryotic from prokaryotic organisms. Under UV light (360/420 nm),

Table 2
Oligonucleotide probes targeting 18S rRNA used in the present study.

Probe name	Sequence	Target group	Reference
EUK1209	5′-GGGCATCACAGACCTG-3′	Eukaryotes	Giovannoni et al. (1988); Lim et al. (1993)
CHLO01	5′-GCTCCACGCCTGGTGGTG-3′	Most Chlorophyta/some non-Chlorophyta	Simon et al. (1995)
NCHLO01	5′-GCTCCACTCCTGGTGGTG-3′	Most non-Chlorophyta/some Chlorophyta	Simon et al. (1995)
CHLO02	5′-CTTCGAGCCCCCAACTTT-3′	Chlorophyta	Simon et al. (2000)
PRYM02	5′-GGAATACGAGTGCCCCTGAC-3′	Haptophyta	Simon et al. (2000)
PRAS04	5′-CGTAAGCCCGCTTTGAAC-3′	Mamiellophyceae (except the genus *Dolichomastix*)	Not et al. (2004)
MICRO01	5′-AATGGAACACCGCCGGCG-3′	*Micromonas pusilla*	Not et al. (2004)

eukaryotic cell nuclei appeared as separate blue organelles, whereas prokaryotic cells appeared uniformly stained. For each sample, 15 to 30 fields and a minimum of 100 cells were counted.

2.5. Taxonomic identification of microphytoplankton

At each station, plankton was collected with a 10 μm-mesh size net drawn at the surface for 10 min. Samples (50 mL) were fixed with acetic formol (1% final concentration) and kept at room temperature in the dark. Acetic formol was chosen despite its impact on fragile phytoplankton species like ciliates and coccolithophores (dissolution of coccoliths) because it is the best overall choice and it is used in many surveys. The samples were gently stirred for homogenization before analysis. In the case of low cell density, the samples were centrifuged at 4000 rpm for 15 min. Qualitative observations and identifications were performed with epifluorescence microscopes (Olympus BH-2 and Olympus BX51, Olympus Optical Co, Tokyo, Japan) on several slides at 100×, 400× and 1000× magnifications. These observations provided a floristic list of the major species of nano- and microplankton present in each sample. Representative images are available from the Plankton*Net web site (http://planktonnet.awi.de//index.php?thematicid=1999).

2.6. Statistical analyses

The stations were clustered based on Bray-Curtis dissimilarities of their respective eukaryote composition. The number of groups was graphically determined based on the relative lengths of the tree branches from a hierarchical cluster analysis built using Ward's criterion as agglomerative rule (Ward, 1963). The length between two clusters is set as the one between their respective squared barycentre weighted by their size. The distance matrix was transformed thanks to the Cailliez method in order to make it Euclidean (Cailliez, 1983).

The relationships between the distribution of eukaryotes and physico-chemical and biological parameters were assessed by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). The variables taken into account to explain the distribution of small eukaryotes included temperature, salinity, silicates, ammonium, nitrates, phosphates and chlorophyll *a*. CCA was performed with the R software (R Development Core Team, 2010) using the ADE-4 package for CCA and related methods (http://www.R-project.org/). In order to avoid redundancy by expressing the same taxonomic entity several times, we subtracted recursively the abundance of lower taxonomic level units from their respective upper levels counts. For example, the abundance of Mamiellophyceae was subtracted from the abundance of total Chlorophyta, resulting in unidentified Chlorophyta (unid. Chlorophyta in Fig. 7B).

3. Results

3.1. Hydrographic data

The English Channel is one of the two major routes for Atlantic water input into the North Sea (Otto et al., 1990). Channel water was well-mixed with vertical profiles of uniform salinity, temperature and nutrient concentrations (Fig. 2 and Table 4). Chlorophyll *a* also showed constant values through the water column. At the central North Sea stations where current velocities are slower (Ducrotoy et al., 2000), salinity profiles were constant but temperature and nutrient profiles indicate stratified conditions. A CM (chlorophyll maximum) appeared progressively from the surface-level at station 8 to a 35 m-depth at station 14. At the north western station 16, temperature stratification was weaker than elsewhere and nutrient concentrations were not depleted at the surface as elsewhere in the North Sea. This is clearly the result of Atlantic water influence (Otto et al., 1990). In contrast to stations in the vicinity (stations 14 and 18), the chlorophyll maximum was observed at the surface with a value up to 9-fold higher than at the other stations. For Norwegian coastal stations, stratification was observed for both physical and chemical parameters. Furthermore, at these stations salinity was lower at the surface revealing the influence of nutrient-poor freshwater from the Baltic Sea (Ducrotoy et al., 2000). For these deep coastal stations, the CM was observed between 20 m and 35 m depending on stations, near the thermocline. In contrast, at stations 5 and 22, which are shallow stations located near the continental coast, we observed well-mixed waters and nutrient concentrations ranging from two to 35-times higher than at the surface of the Norwegian stations due to a nutrient-rich freshwater input from large rivers (Rhine, Meuse, Elbe) (Narayanaswamy et al., 2010).

3.2. Abundance of eukaryotes

Total eukaryote concentrations, determined by DAPI counts, ranged from 345 cell mL^{-1} at station 19 (50 m deep) to $9.2 \cdot 10^3$ cell mL^{-1} at station 7 (10 m deep) (Fig. S1). Eukaryotes smaller than 2 μm dominated at each station, except at station 16 (10 m deep) where eukaryotes between 2 and 5 μm dominated (Fig. S2). Concentrations of picoeukaryotes ranged from 126 cells mL^{-1} at station 12 (35 m deep) to $8 \cdot 10^3$ cells mL^{-1} at station 7 (10 m deep) while concentrations of eukaryotes between 2 and 5 μm, and larger than 5 μm ranged from 64 cells mL^{-1} at station 19 (50 m deep) to $5.7 \cdot 10^3$ cells mL^{-1} at station 16 (10 m deep), and 64 cells mL^{-1} at station 14 (75 m deep) to $0.7 \cdot 10^3$ cells mL^{-1} at station 5 (25 m deep), respectively (Fig. S2). Because of differences in maximum depth between the stations, whole water column integration provided minima and maxima at stations (Fig. 3A) different from those of vertical profiles. Total eukaryote abundance ranged from $0.5 \cdot 10^7$ cells cm^{-2} at station 22 to $3.7 \cdot 10^7$ cells cm^{-2} at station 3. Picoeukaryote abundances ranged from $2.5 \cdot 10^6$ cells cm^{-2} at station 12 to $30.6 \cdot 10^6$ cells cm^{-2} at station 3 while those of eukaryotes between 2 and 5 μm and larger than 5 μm ranged from $1.2 \cdot 10^6$ cells cm^{-2} at station 5 to $14.5 \cdot 10^6$ cells cm^{-2} at station 16, and $8.5 \cdot 10^5$ cells cm^{-2} at station 7 to $2.4 \cdot 10^6$ cells cm^{-2} at station 21, respectively. Altogether, the dominance of picoeukaryotes was observed for both vertical profiles and integrated data at all stations, except at station 16 where eukaryotes between 2 μm and 5 μm dominated.

Generally, autotrophic eukaryotes accounted for half or more of total eukaryote abundance. This also held true for the different size classes. However, at stations 5, 10, 16, 19, 21 and 22 autotrophic picoeukaryotes accounted for less than 50% (from 10% to 48%) of total picoeukaryotes (Fig. 3B). At station 5, autotrophs accounted for 44% of total eukaryotes larger than 5 μm while at the other stations they accounted for 60% to more than 90% of total eukaryotes larger than 5 μm.

3.3. Distribution and abundance of the major eukaryote lineages

Diatom (<200 μm) abundances ranged from $0.1 \cdot 10^5$ cells cm^{-2} at station 22 (5 cells mL^{-1} at a 25 meter-depth) to $5.5 \cdot 10^5$ cells cm^{-2} at station 18 (325 cells mL^{-1} at a 25 meter-depth) as maximal value (Fig. 4).

Dinoflagellate (<200 μm) abundances were generally higher than those of diatoms and increased progressively from the English Channel ($2 \cdot 10^5$ cells cm^{-2}; 29 cells mL^{-1}) to the Skagerrak ($9 \cdot 10^5$ cells cm^{-2}; 238 cells mL^{-1}). On average, 58% of the dinoflagellates were autotrophic, with a minimum value of 32% at station 7, and a maximum value of 90% at station 1 (Fig. 4). Among heterotrophic dinoflagellates, we observed a maximum of 22% of green-fluorescing dinoflagellates at station 10. Elsewhere, they were either absent (stations 1 and 3) or below 20%.

Although plankton net samples demonstrated the presence of diatom species at almost all stations (Table 3), epifluorescence microscopy revealed that their abundance was low at stations 3, 12 and 14. This low abundance could be partly due to the pre-filtration step of the epifluorescence samples through 200 μm filters which

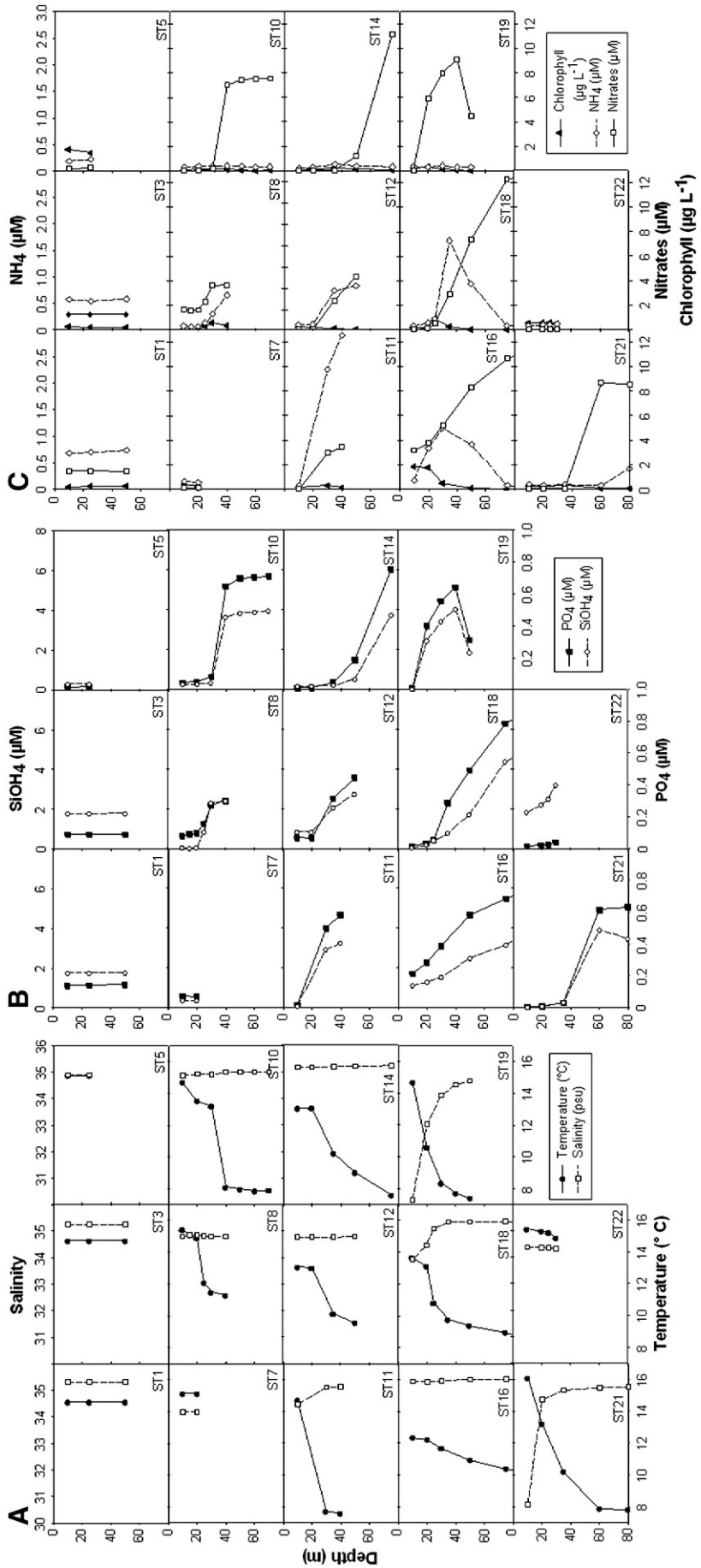

Fig. 2. Physico-chemical and biological parameters at the stations analyzed in the present study. Vertical profiles of temperature (°C) (●) and salinity (psu) (□) (A), concentrations of PO_4 (µM) (■) and $SiOH_4$ (µM) (○) (B), chlorophyll *a* (µg L^{-1}) (▲), concentrations of NH_4 (µM) (◊) and concentrations of nitrates plus nitrites (NO_3+NO_2) (µM) (□).

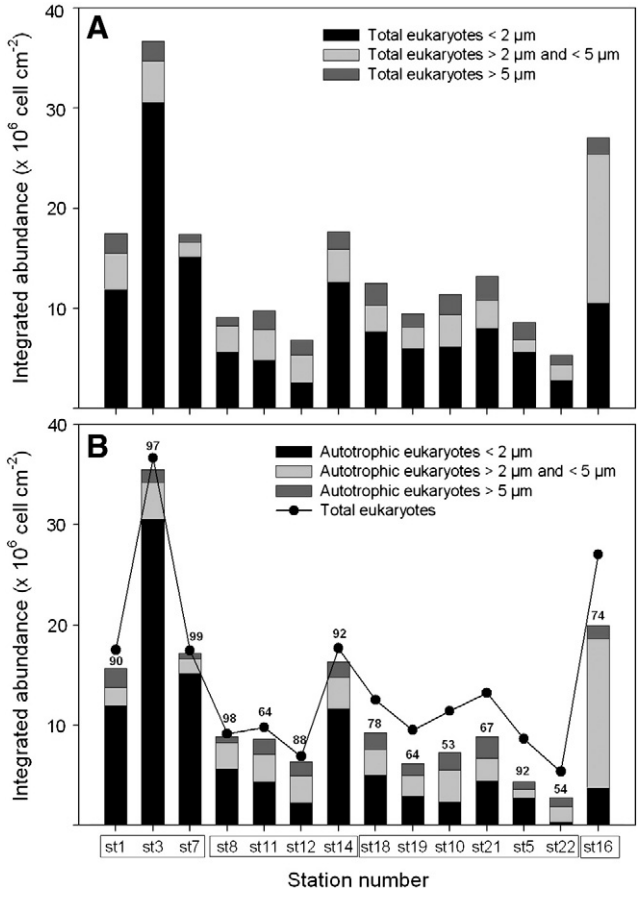

Fig. 3. Whole water column-integrated abundance ($\times 10^6$ cells cm^{-2}) of total (A) and autotrophic (B) eukaryotes measured by DAPI-staining with the contribution of the different size ranges for the stations that were analyzed. Numbers at the top of the vertical bars correspond to the contribution (in percentage) of autotrophic eukaryotes to the total eukaryotes. On the abscissa, stations are clustered into the groups defined in the present study (see Fig. 1).

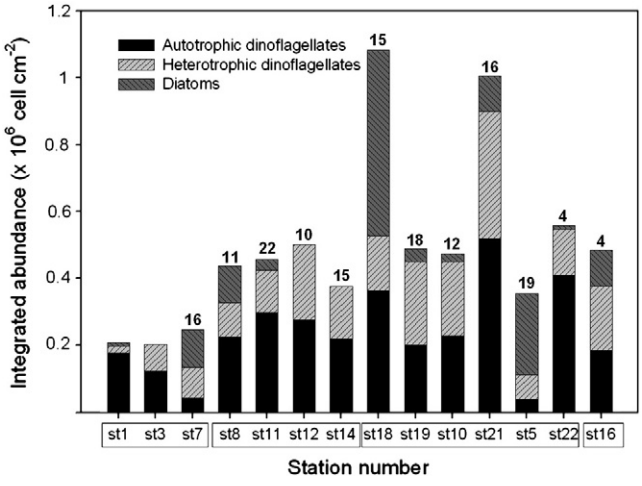

Fig. 4. Whole water column-integrated abundance ($\times 10^6$ cells cm^{-2}) of diatoms (hatched gray), and dinoflagellates measured by DAPI-staining with contributions of autotrophic (black) and heterotrophic (hatched light gray) dinoflagellates. Numbers above the vertical bars correspond to the percentages of green dinoflagellates among heterotrophic dinoflagellates. No number means an absence of green dinoflagellates. On the abscissa, stations are clustered into the groups defined in the present study (see Fig. 1).

removed large or chain-forming diatoms like *Proboscia alata* or *Chaetoceros* sp.

Plankton net samples highlighted a transition pattern from diatoms to dinoflagellates. The three stations sampled in the English Channel were dominated by large chain-forming diatoms (*Guinardia flaccida, G. delicatula,* and *G. striata*) with only a few dinoflagellates (*e.g. Prorocentrum* spp.). At station 8, large diatoms were still observed but dinoflagellates started to appear, especially some species of *Ceratium*. The next four stations (stations 10, 11, 12 and 14) were dominated mostly by dinoflagellates, especially *Ceratium* spp. and *Scrippsiella trochoidea*, whereas just a few diatoms were observed sporadically. Diatoms were more abundant at station 16 along with large *Ceratium* species (*e.g. C. fusus* and *C. trichoceros*). Station 18, situated at the extreme North of the transect, was dominated by small dinoflagellates and small chain-forming diatoms. Larger dinoflagellates reappeared in the next three stations: *Ceratium* spp. and heterotrophic species (*e.g. Protoperidinium* spp at station 21). At station 22, a bloom of *Ceratium* spp. was observed with an abundance reaching 90 cells mL^{-1} throughout the water column. Although some ciliates were observed by microscopy, we did not count them because fixation and storage methods were not appropriate for their quantification (Leakey et al., 1994).

The distribution of Chlorophyta and Haptophyta was obtained using group-specific probes targeting 18S rRNA revealed by TSA-FISH. The ratio between the cells hybridized with a mix of 18S rRNA probes (Euk1209 + Chlo01 + NChlo01) targeting all eukaryotes and the DAPI-stained eukaryotes counted under UV light (called hybridization ratio below) ranged from 70% (for station 19) to 97% (for station 1) with an average value of 86%, suggesting that most eukaryotes could be labeled with the 18S rRNA probes. On average, 47% and 19% of the total number of eukaryotes were hybridized with Chlorophyta and Haptophyta probes, respectively. Maximum values (Fig. 5) were observed in the English Channel for the Chlorophyta (92%), and at station 16 for the Haptophyta (62%).

For eukaryotes smaller than 2 µm, the hybridization ratio varied from 71% (for station 19) to 100% (for stations 1, 7 and 8) with an average value of 93% (Fig. 6A). 19% (station 22) to 100% (stations 1, 7, and 12) of these cells were chlorophytes, while no haptophytes were detected in this fraction. Among chlorophytes, we observed an average of 55% of Mamiellophyceae with minima and maxima observed at station 19 (less than 20%), and at station 5 (more than 90%), respectively. Counts with the probe Micro01, targeting *M. pusilla*, labeled an average of 39% of the Mamiellophyceae smaller than 2 µm. Maximum values (100%) were observed at stations 1, 7 and 12. *M. pusilla* was not detected at stations 11, 18, 19 and 21.

For eukaryotes between 2 and 5 µm in size, lower hybridization ratios were observed: from 51% (for stations 14 and 16) to 93% (for station 21) with an average value of 68% (Fig. 6B). Outside the English Channel, a majority of Haptophyta were observed in this size range (from 60% to 96%), except at station 8 where 71% of eukaryotes were chlorophytes. Among eukaryotes between 2 and 5 µm belonging to the Chlorophyta, we observed less than 30% of Mamiellophyceae at each station, except at station 3 where all Chlorophyta belonged to the Mamiellophyceae.

Eukaryotes larger than 5 µm showed hybridization ratios from 59% (for station 7) to 97% (for station 1) with an average value of 82% (Fig. 6C). Chlorophytes and haptophytes contributed from less than 5% up to 37% of eukaryotes. Generally, haptophyte contribution dominated over chlorophyte, except at stations 1, 5 and 8. Mamiellophyceae larger than 5 µm were observed only at stations 3, 8, 14, 16 and 21 where they counted for less than 15% of chlorophytes.

3.4. Relation between physico-chemical parameters and eukaryote plankton composition

Three broad groups and one isolated site can be distinguished from Ward's clustering based on Bray-Curtis distances computed from

Table 3
Floristic list of the microplankton observed on plankton net samples for each station. Points mean that the species was present at the station. Nutrition mode: A for autotrophy, M for mixotrophy, H for heterotrophy. Estimated dimensions are mostly from Tomas (1997) and iconographic data (http://planktonnet.awi.de/).

Station Species				3	5	7	8	10	11	12	14	16	18	19	21	22
	Nutrition mode	Shape	Estimated dimensions (μm)	English Channel			North Sea									
Bacillariophyceae																
Chaetoceros decipiens	A	Chains	Apical axis 9–84				•									
Chaetoceros densus	A	Chains	Apical axis 10–55	•												
Chaetoceros didymus	A	Chains	Apical axis 10–40												•	
Chaetoceros spp.	A	Chains	–		•	•							•	•	•	
Corethron hystrix	A	Single	Pervalvar axis 20–200						•			•				
Coscinodiscus radiatus	A	Single	Diameter 30–180	•												
Dactyliosolen fragilissimus	A	Chains	Pervalvar axis 42–300		•											
Diploneis bombus	A	Single	Apical axis 70–80	•												
Diploneis littoralis	A	Single	nd	•												
Eucampia zodiacus	A	Chains	Apical axis 8–80			•										
Guinardia delicatula	A	Chains	Apical axis 30–60		•	•										
Guinardia flaccida	A	Chains	Apical axis 50–200	•	•	•						•			•	
Guinardia striata	A	Chains	Apical axis 100–250		•	•										
Leptocylindrus danicus	A	Chains	Diameter 5–16				•									
Meuniera membranacea	A	Chains	Apical axis 50–90	•												
Paralia sulcata	A	Chains	Diameter 8–130	•	•	•								•		•
Pleurosigma normanii	A	Single	Apical axis 90–220	•												
Podosira stelliger	A	Single	Apical axis 30–50		•											
Proboscia alata	A	Chains	Diameter 2.5–13				•		•		•	•	•	•	•	
Pseudo-nitzschia sp.	A	Chains	–		•							•				
Rhizosolenia imbricata	A	Chains	Apical axis 2.5–57		•	•	•							•		•
Rhizosolenia setigera	A	Single	Apical axis 150–200	•		•										
Rhizosolenia styliformis	A	Single	Diameter 23–90				•					•				
Thalassiosira spp.	A	Chains	–			•										
Dinophyceae																
Ceratium furca	M	Single	Length 200–300				•		•			•		•	•	•
Ceratium fusus	M	Single	Length 200–600						•	•	•	•		•	•	•
Ceratium horridum	M	Single	Length 200–300				•		•		•			•	•	
Ceratium lineatum	M	Single	Length 80–120								•					•
Ceratium longipes	M	Single	Length 250–350						•		•			•	•	
Ceratium macroceros	M	Single	Length 300–400				•							•	•	
Ceratium massiliense	M	Single	Length 500–600				•		•							
Ceratium spp.	M	Single	–							•						
Ceratium trichoceros	M	Single	Length 500–700								•	•				
Ceratium tripos	M	Single	Length 200–300									•		•		
Dinophysis acuminata	H	Single	Length 30–50			•			•		•	•			•	•
Pyrocystis lunula	M	Single	Length 150–200			•										
Gonyaulax digitale	M	Single	Length 30–50			•										
Gonyaulax spinifera	M	Single	Length 30–50				•									
Gonyaulax spp..	M	Single	–							•						
Phalacroma rotundata	H	Single	Length 30–50												•	•
Prorocentrum gracile	M	Single	Length 20–30	•											•	
Prorocentrum micans	M	Single	Length 30–50	•			•			•				•	•	•
Protoperidinium bipes	H	Single	Length 20–30				•		•			•				
Protoperidinium claudicans	H	Single	Length 40–70												•	
Protoperidinium depressum	H	Single	Length 150–200						•		•				•	
Protoperidinium steinii	H	Single	Length 30–50												•	•
Protoperidinium pellucidum	H	Single	Length 30–50		•											
Protoperidnium spp.	H	Single	–			•				•	•					
Scrippsiella trochoidea	M	Single	Length 20–30				•	•	•	•	•	•			•	
Dictyochophyceae																
Dictyocha sp.	A	Single	–				•									
Dictyocha speculum	A	Single	Length 19–34	•	•									•		

eukaryote abundances (Fig. 7A). Group C reveals sub-groupings which correspond to the shallow coastal stations (stations 5 and 22) on the one hand, and to the deeper coastal stations (stations 10, 18, 19 and 21) on the other hand. Group D is composed of only one station (station 16) which seems to be very different from the other stations regarding its eukaryote composition. Groups A and B correspond to English Channel stations (stations 1, 3 and 7) and central North Sea stations (stations 8, 11, 12 and 14), respectively.

Canonical Correspondence Analysis (CCA, Fig. 7B) suggests that environmental data explain 63% of the total variability of the biological data. The first and second eigenvalues represent respectively 43% and 16% of the total inertia. The first plane of the analysis displays a good separation of the four groups established by clustering, although some groups such as group A or C display further sub-groups (7 *vs.* 1 and 3 for A; 5 *vs.* the other stations for group C). The first CCA axis is negatively linked to an ammonium gradient while the second axis is negatively linked to a temperature gradient. Both axes suggest a common gradient for nitrates, phosphates, silicates and salinity which is orthogonal to the chlorophyll *a* gradient. Some of the station groups can be linked to some of the eukaryotic population gradients. For example stations 1 and 3 (but not 7 from the same group A) are clearly linked to high values of *Micromonas* while station

16 is linked to Mamelliophyceae >5 μm and nano (2–5 μm) Haptophyta; coastal stations (group C except station 5) are linked to Dinoflagellates and Diatoms.

4. Discussion

Our study showed that the distribution of the different eukaryote groups within the plankton was clearly influenced by the different water masses (Table 4 and Fig. 7). As confirmed by the CCA, there is a well-defined separation between English Channel, North Sea and Atlantic waters.

In the English Channel (area A), *M. pusilla* dominated, in particular at station 3 as previously shown (Foulon et al., 2008; Not et al., 2004). We can hypothesize that this dominance is linked to the absence of stratification since these stations are the only ones which are fully mixed vertically (see Fig. 2A). Such vertical mixing imposes constant changes in irradiance. *Micromonas* may be better adapted to these variable conditions. However, in coastal North Sea stations (stations 5 and 22) which were also well mixed, *M. pusilla* contribution to the pico-eukaryotic community was low, suggesting that other pico-eukaryotes could dominate. Knefelkamp et al. (submitted for publication) indeed observed that in summer, more than 40% of the pico-eukaryotes monitored in Helgoland waters did not belong to Chlorophyta. The difference observed between stations 1 and 3 *vs.* 7 is clearly linked to the location of station 7 at the transition between the English Channel and the North Sea, characterized by higher temperature and lower salinity, probably due to the freshwater input from the Scheldt (Ducrotoy et al., 2000), as well as the nutrient depletion at the surface. In the rich and well-mixed waters of the English Channel, microphytoplankton was dominated by the *Chaetoceros* and *Guinardia* diatom genera which are usually observed in summer (Jouenne et al., 2007).

Based on CCA, area B is the least discriminated area. This could be due to its central position and to the complexity of currents occurring in this area (Fig. 1). The eukaryote composition in area B is poorly explained by the chemical and physical parameters used in the CCA. However, we observed that Haptophytes >2 μm dominated at these open sea stations. This gradient in haptophyte abundance from coastal to pelagic waters had already been noticed by Thomsen et al. (1994) and Not et al. (2008). Furthermore, the CCA highlights a higher abundance of Mamiellophyceae between 2 and 5 μm in this area. It suggests that while pico-Mamiellophyceae are preferably found in coastal waters, larger ones are found in open sea water. In contrast

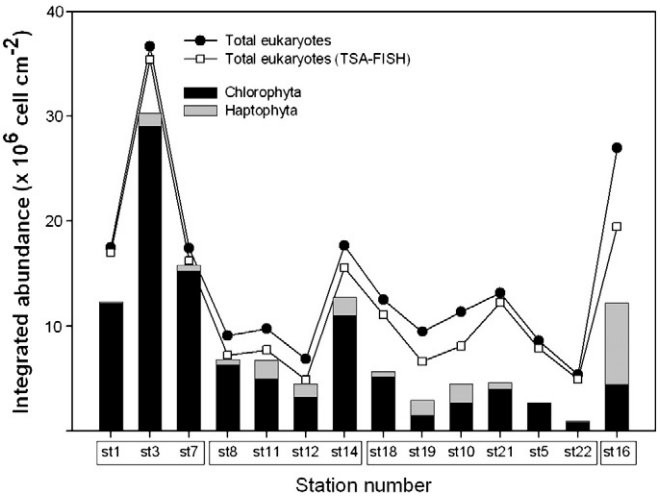

Fig. 5. Whole water column-integrated abundance ($\times 10^6$ cells cm^{-2}) of total eukaryotes counted by DAPI-staining (●) and total eukaryotes obtained by TSA-FISH (□) with the contributions of Chlorophyta (in black) and Haptophyta (in gray). On the abscissa, stations are clustered into the groups defined in the present study (see Fig. 1).

with the English Channel, the larger eukaryote community of the stratified North Sea waters was dominated by the dinoflagellates (Table 3). Such a dominance switch between diatoms and dinoflagellates has been clearly established in the past (Cloern and Dufford, 2005; McQuatters-Gollop et al., 2007).

Station 16 has chemical characteristics close to stations 1 and 3, except for ammonium concentrations which are higher in the English Channel (Fig. 7B). This similarity is probably linked to the common origin of these Atlantic water masses occurring in the English Channel and near the Shetland Islands (Ducrotoy et al., 2000; Narayanaswamy et al., 2010; Otto et al., 1990). However the eukaryote community is different at Station 16: it is mainly dominated by nano haptophytes between 2 and 5 μm, inducing a relative decrease of the picophytoplankton as hypothesized previously (Iriarte and Purdie, 1994). A similar trend was reported by Riegman and Kraay (2001) in the Faroe–Shetland Channel during the summer of 1999. Haptophyte dominance at station 16 is probably linked to stratification since haptophyte pigment signature is found to dominate in most stratified oceanic waters (Liu et al., 2009). The use of acetic formalin for preservation of the phytoplankton samples that were observed by microscopy induced the dissolution of coccoliths and therefore prevented from verifying whether these haptophytes were coccolithophorids or rather *Chrysochromulina* like.

In the present study, no haptophytes smaller than 2 μm were observed. This fits with the fact that the smallest Haptophyta species (*Chrysochromulina minor*) described so far has a minimum size of 2.5 μm (Vaulot et al., 2008). Flow cytometer sorted populations of picoplankton from the English Channel do not contain haptophyte 18S rRNA gene sequences in contrast to nanoplankton where haptophytes dominate (Marie et al., 2010). However, in other oceanic regions, in particular oligotrophic ones, several evidences point to the existence of pico-sized haptophytes (Liu et al., 2009). The dominant pigment in the size fraction below 3 μm in the Equatorial Pacific is 19′-hexanoyloxyfucoxanthin, characteristic of haptophytes (Moon-van der Staay et al., 2000). Genetic analysis of the 18S rRNA gene on flow cytometry sorted picoplankton cells from the South-East Pacific revealed novel clades of haptophytes (Shi et al., 2009). This suggests that pico-haptophytes, although not yet isolated in culture, exist but are probably restricted to more oligotrophic waters. This could be explained by their mixotrophic feeding mode which could confer them a competitive advantage in low nutrient waters (Worden and Not, 2008).

In the stratified area, especially at station 18, small chain-forming diatoms belonging to the *Chaetoceros* genus were observed. Much of the water in the North Sea enters the Southern part of the Skagerrak and leaves it again through the Northern part along the Norwegian South coast (Lange et al., 1992). This South–North current could explain the abundance of diatoms like *Chaetoceros spp.*, in the stratified waters of station 18 where it could be transported from Southern well-mixed waters where it proliferates. Concerning dinoflagellates, a higher occurrence of mixotrophic dinoflagellates, especially belonging to the *Ceratium* genus, was observed in the Southern part of the study area (well-mixed and shallow ecosystems). Heterotrophic dinoflagellates, like *Protoperidinium* and *Dinophysis*, were abundant at stations 19 and 21, in the Skagerrak where freshwater impact was observed at the surface (Beaugrand et al., 2004; Reid et al., 1988; Reid et al., 1990). This suggests a link between salinity and *Protoperidinium* distribution, as observed for other dinoflagellates by France and Mozetic (2006).

The presence of green-fluorescing dinoflagellates, initially observed by Shapiro et al. (1989) in the North-West Atlantic and reported by Masquelier and Vaulot (2008) to account for 5 to 50% of heterotrophic dinoflagellates in the South-Eastern Pacific was striking. These organisms have rarely been reported so far and mostly in the open ocean. Therefore it is quite surprising to observe them in the central North Sea. We hypothesize that this lack of previous reporting is due to the fact that studies made on larger cell-size

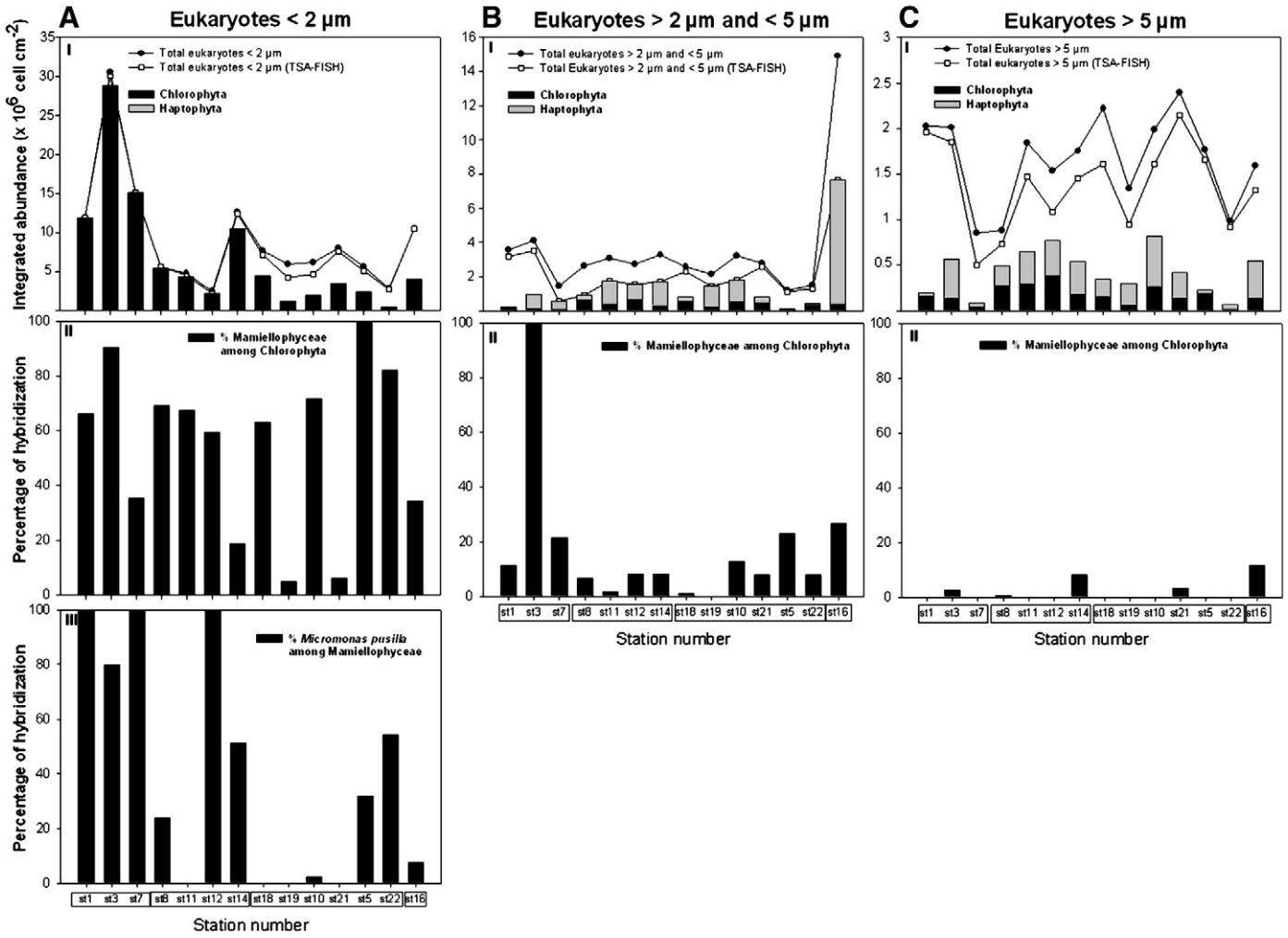

Fig. 6. Whole water column-integrated abundance ($\times 10^6$ cells cm^{-2}) of eukaryotes smaller than 2 µm (A), between 2 and 5 µm (B), and larger than 5 µm (C). Top panels (I): eukaryotes counted by DAPI-staining (●) and eukaryotes obtained by TSA-FISH (□) with the contributions of Chlorophyta (in black) and Haptophyta (in gray). Middle panels (II), percentages of Mamiellophyceae among Chlorophyta obtained by comparing the eukaryotes hybridized with the Pras04 probe with the eukaryotes hybridized with the Chlo02 probe. Bottom panel (III), for eukaryotes smaller than 2 µm (A), percentage of *Micromonas pusilla* among Mamiellophyceae obtained by comparison of eukaryotes smaller than 2 µm hybridized with the Micro01 probe and eukaryotes smaller than 2 µm hybridized with the Pras04 probe . Note the different y scales for top panels (I) of the figure. On the abscissa, stations are clustered into the groups defined in the present study (see Fig. 1).

fractions do not usually use epifluorescence. In the North Sea, green-fluorescing dinoflagellates accounted for as much as 22% of the heterotrophic dinoflagellates (station 11) and were not observed at stations 1 and 3 of the English Channel. Although Shapiro et al. (1989) suggested that their presence was generally positively correlated with chlorophyll *a*, in the present study, green-fluorescing dinoflagellates and chlorophyll *a* had opposite patterns (data not shown). The same trend was observed by Masquelier and Vaulot (2008) in the South-eastern Pacific where green-fluorescing dinoflagellates accounted for up to 50% and between 5% and 25% of heterotrophic dinoflagellates in the oligotrophic and meso-eutrophic zones, respectively. Despite a probable higher abundance of green-fluorescing dinoflagellates in oligotrophic waters, we think that this heterotrophic group could be widespread in most areas and may therefore play an important role that was neglected in previous work. This difference in fluorescence among dinoflagellates could be linked to differences in physiological and ecological characteristics, but unfortunately no cultures of these organisms seem to exist at the present time and detailed investigations are lacking.

This first detailed analysis of the eukaryotic plankton summertime community throughout the English Channel and the North Sea allowed us to determine general trends for its composition in the different hydrological regions. However, many questions remain open. In particular, it would be interesting to determine the distribution of key genera/species, similar to what we obtained for *Micromonas*, as this genus was found abundant only in particular environmental conditions and not throughout the whole area as could have been expected from previous work (Foulon et al., 2008; Not et al., 2004). Moreover, the present study was conducted during the summer of 2007 which was characterized by quite low surface temperatures (an average 2°°C lower than in 2006 and 2008; http://meteocentre.com/) that followed quite high spring temperatures (2 °C to 4 °C higher than in 2006 and 2008, depending on regions). Therefore, it would be necessary to assess eukaryote community structures under more standard summer conditions to determine whether the features we observed are really typical of the North Sea in summer. Knefelkamp et al. (submitted for publication) have monitored the detailed dynamics of picoplankton in the German North Sea (Helgoland) between March 2005 and March 2006. They showed a strong correlation between the change in water temperature and the abundance of picoeukaryotes in the German Bight, while their composition was quite uniform throughout the year with a dominance of Chlorophyta. Despite the occasional occurrence of a stratification in the German Bight (Huthnance, 1991), we can admit

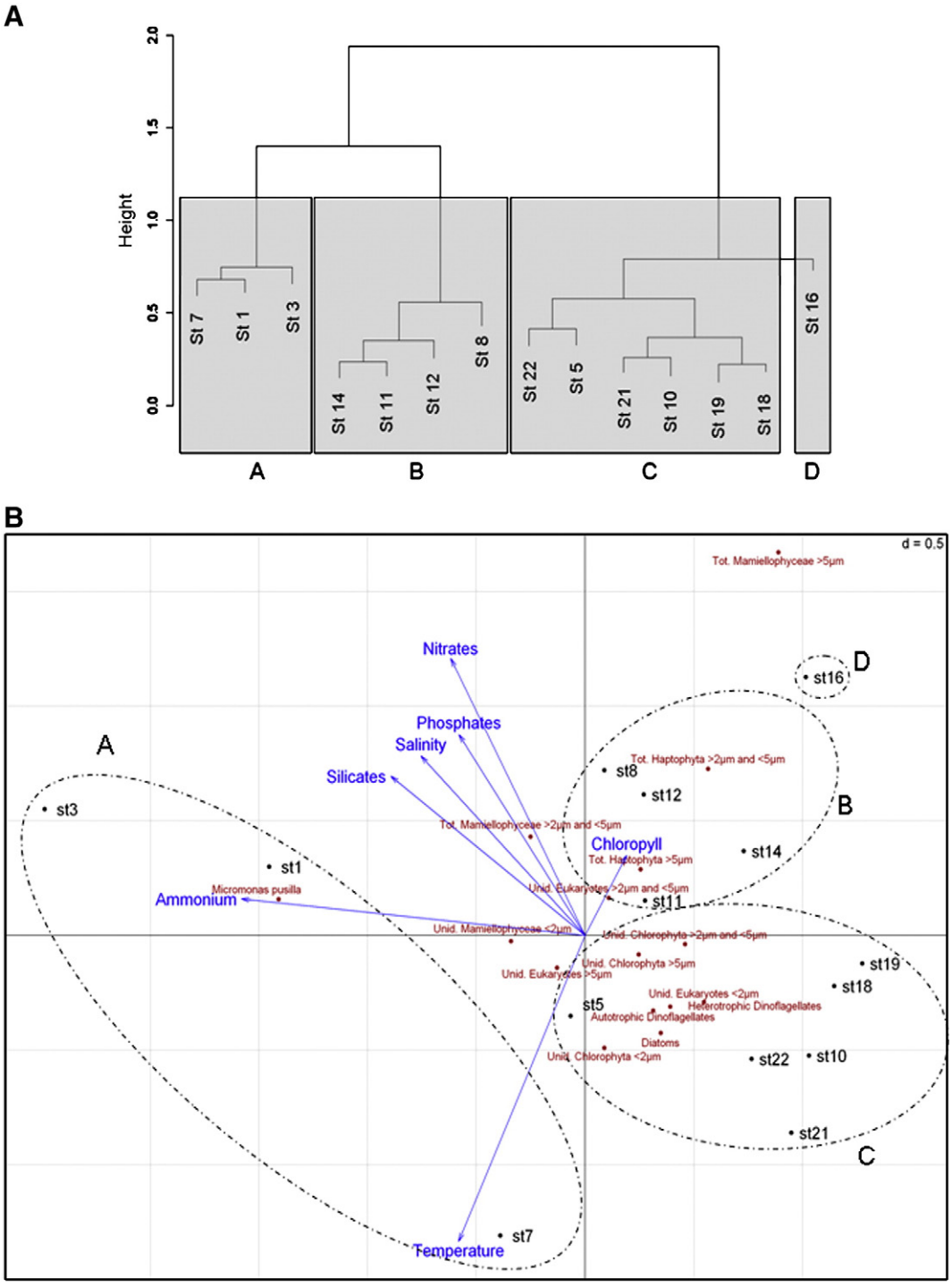

Fig. 7. Statistical analyses: (A) Ward's clustering upon sites based on Bray-Curtis distances computed from eukaryote abundances; (B) canonical correspondence analysis (CCA) plot performed with whole water column-integrated values of eukaryote abundances and physico-chemical parameters. The first (abscissa axis) and second (ordinate axis) eigenvalues represent 43% and 16% of the CCA total inertia, respectively. Chlorophyll means chlorophyll *a*.

that the shallower Helgoland waters are well-mixed all year round (Reid et al., 1988; Tian et al., 2009). Therefore, we can hypothesize that the interannual dynamics of picoeukaryotes in well-mixed areas around the English Channel and the North Sea are influenced by the temperature while areas showing stratification during summer could be more influenced by the different water masses as suggested by Kuylenstierna and Karlson (1994) in the Skagerrak.

Changes in phytoplankton composition have probable ecological consequences with respect to foodwebs. Although it is generally accepted that heterotrophic nanoflagellates are the major grazers of picoplankton (Mackey et al., 2002; Sato et al., 2007), predation by heterotrophic dinoflagellates could also be important (Sanders et al., 2000; Sherr et al., 1991). Furthermore, other studies have shown that grazers could select their prey (Hansen et al., 1996; Reckermann and Veldhuis, 1997), suggesting that the composition of the pico-and nano planktonic population which is influenced by physico-chemical conditions could have an impact on the grazer population like heterotrophic dinoflagellates, and therefore explain in part, the dominance of some genera over others between the different areas investigated here.

Table 4
Characteristics of the sampled areas. For stratification, the − symbol means that the stations of the area were composed of well-mixed waters; the + symbol means that the stations of the area were stratified; the ± symbol means that the area was composed of both stratified and well-mixed stations. For nutrients and salinity, ranges (min–max) are given; the percentage of *Micromonas pusilla* and Haptophytes were obtained for whole water column-integrated values relative to total eukaryote abundances.

Area	Stations	Stratification	Temperature (°C)	Salinity (p.s.u.)	Phosphates (μM)	Nitrates (μM)	Ammonium (μM)	Silicates (μM)	Water masses	% *Micromonas pusilla*	% Haptophytes	Microplankton dominant group
A	1–3–7	−	14.6–15.1	34.2–35.3	0.07–0.15	0.1–1.5	0.1–0.7	0.4–1.8	English Channel	43–78	0.6–3.4	Diatoms *Chaetoceros* *Guinardia*
B	8–11–12–14	+	7.6–15.4	34.5–35.2	0.01–0.8	0.04–11.1	0.05–2.9	0.03–3.74	North Sea	0–23	7–27	Dinoflagellates *Ceratium*
C	5–10–18–19–21–22	±	7.4–16.1	30.2–35.4	0.01–0.8	0.03–12.3	0.05–1.68	0.06–5.74	Freshwater	0–9	1–22	Diatoms *Chaetoceros Guinardia* Dinoflagellates *Ceratium Protoperidinium* *Dinophysis*
D	16	−	10–12.3	35.3–35.4	0.2–0.8	3.2–11.8	0.05–1.2	1.14–4.31	Atlantic	0.3	38	Dinoflagellates *Ceratium*

Supplementary materials related to this article can be found online at doi:10.1016/j.seares.2011.05.004.

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