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Analysis of photosynthetic picoeukaryote community structure along an extended Ellett Line transect in the northern North Atlantic reveals a dominance of novel prymnesiophyte and prasinophyte phylotypes

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ABSTRACT

Photosynthetic picoeukaryotes (PPEs) of a size $<3\,\mu m$ can contribute significantly to primary production. Here, PPE community structure was analysed along an extended Ellett Line transect, an area in the North Atlantic well studied by physical oceanographers but largely neglected in the field of microalgal ecology. Distribution patterns of specific PPE classes were determined using dot-blot hybridization analysis, while the taxonomic composition of specific PPE classes was revealed by phylogenetic analysis of plastid 16S rRNA gene sequences. In addition, we performed fluorescent *in situ* hybridization (FISH) analysis of seawater samples collected along the transect to provide a PCR-independent survey of class level PPE distribution patterns. We found the PPE community was dominated by members of the Prymnesiophyceae, Prasinophyceae and Mamiellophyceae. Interestingly, phylogenetic analysis revealed several novel Prymnesiophyceae and Prasinophyceae phylotypes (with only 85–96% identity to neighbouring sequences) within lineages for which cultured counterparts are unknown.

1. Introduction

The North Atlantic has long been a fascinating area for the study of physical oceanography due to the North Atlantic Oscillation (NAO), a feature which affects wind speeds, evaporation, rainfall and heat exchange between the ocean and the atmosphere (Hughes et al., 2008). The Ellett Line, named after the late physical oceanographer David Ellett, is part of the UK climate monitoring strategy. It originally crossed over the Rockall Trough and was repeatedly sampled from the 1970s onwards. From 1996, the area studied was extended to Iceland.

Despite the interest in this area for physicists, and the frequency of its analysis, much less is known of the phytoplankton community in this region, and particularly the picophytoplankton. This latter group, formally defined as cells $<2\,\mu m$ but more recently studied using filtration approaches that now incorporate cells $<3\,\mu m$ in diameter (see Vaulot et al., 2008), encompass both eukaryotic and prokaryotic domains (Johnson and Sieburth, 1982) and are believed to be ubiquitous, with members including cyanobacteria and PPEs (Stockner, 1988). Although eukaryotic picophytoplankton are known to contribute significantly to marine primary production (Li, 1994; Worden et al., 2004; Jardillier et al., 2010, Cuvelier et al., 2010), analysis of the distribution of specific PPE classes has mainly encompassed waters between 40°N and 40°S (e.g. see Not et al., 2008; Fuller et al., 2006a, b; Lepère et al., 2009; Shi et al., 2009; Kirkham et al., 2011).

One of the few studies in the northern North Atlantic measured pico- and nanoplankton standing stocks in areas close to part of the Ellett Line, towards the south of the Icelandic Basin (59°N, 20°W) during the PRIME cruise in 1996 (Tarran et al., 2001). In this area *Synechococcus* was the most abundant component of the picophytoplankton, reaching 2.2×10^5 cells ml^{-1} whilst PPEs did not exceed 1.2×10^4 cells ml^{-1} and *Prochlorococcus* was not detectable. Despite the much lower abundance of PPEs compared to their prokaryotic counterparts, PPEs contributed 0.9 g C m^{-2} to the biomass in this area, approximately twice that of *Synechococcus* making PPEs the most important biomass compartment of the picophytoplankton at this site.

In recent years, molecular techniques have begun to provide insights into PPE community structure in various marine environments (reviewed by Vaulot et al., 2008). In particular, cloning and sequencing of both nuclear and plastid small subunit rRNA genes has revealed a vast, previously unsuspected diversity within the

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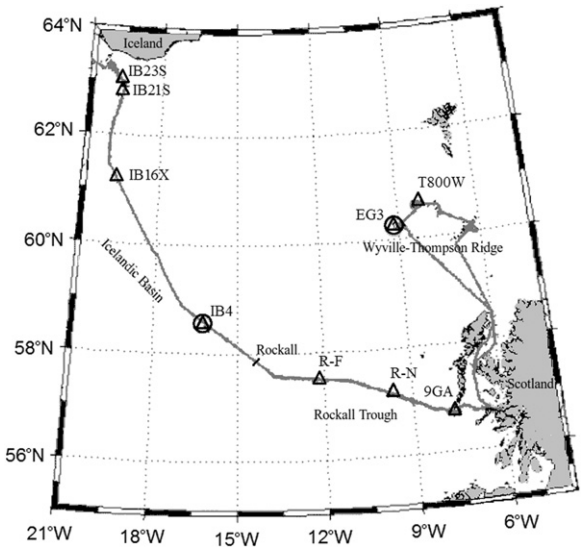

Fig. 1. Extended Ellett Line cruise track. Triangles indicate the position of stations for which samples were used for dot blot hybridization analysis at 5 or 6 depths. Clone libraries were constructed at stations IB4 and EG3, and are marked with a circle.

group (e.g. see Moon-van der Staay et al., 2001; Lepère et al., 2009; Shi et al., 2009; Liu et al., 2009). However, data illustrating the composition and structure of the PPE community is still limited, and particularly so in higher northern latitudes. Of the few studies reported thus far in this region, clone libraries constructed from partial sequences of the nuclear SSU rRNA gene in the Arctic Ocean (Lovejoy et al., 2006), and in an area of opposing water masses encompassing cold Arctic Ocean outflow water and warmer Atlantic water in Northern Baffin Bay (Hamilton et al., 2008), suggest there is a mix of uniquely Arctic species and more widely distributed photosynthetic organisms. The former includes a cold-adapted *Micromonas* ecotype (Lovejoy et al., 2007) whilst the latter includes the picoprasinophyte genera *Bathycoccus* and *Mantoniella* as well as photosynthetic stramenopile representatives. Similarly, in waters of the Norwegian and Barents Seas *Micromonas* was again an abundant component of the PPE community, particularly in coastal waters and at the polar front, whilst prymnesiophytes were more prominent in more open ocean Atlantic waters (Not et al., 2005).

Here, we performed dot-blot hybridization analysis using PPE-class-specific oligonucleotide probes to determine PPE community structure along an extended Ellett Line transect carried out in 2007 (see Fig. 1) in the northern North Atlantic. At two further sites, one in the Icelandic Basin and one at the Wyville-Thomson Ridge, the phylogenetic composition of the PPE community structure was assessed via clone libraries targeting the plastid 16S rRNA gene. In addition, we performed FISH analysis to provide a direct comparison of PPE community structure using a non-PCR-based approach. We set out to ascertain whether the PPE community along the transect was typical of more open ocean Atlantic waters seen at lower latitudes (Kirkham et al., 2011) or to what extent novel PPE lineages or phylotypes were found in this region.

2. Materials and methods

2.1. Sampling

Seven stations along the extended Ellett Line and two sites at the Wyville-Thomson Ridge (60.24°N, 9.01°W; and 60.64°N, 8.13°W) were sampled at 5 or 6 depths, ranging between 5 and 125 m, during 26th August–3rd September 2007 (Fig. 1) with a rosette of 20 L Niskin bottles. For DNA extraction 10 L of seawater was filtered first through a 3 μm pore size polycarbonate filter (Millipore MCE MF) and then onto a 0.45 μm pore size polysulfone filter (Supor450, Gelman Sciences, AnnArbor, Mich) under gentle vacuum (<10 mm Hg). The filters were transferred into 5 ml cryotubes containing 3 ml DNA lysis buffer (0.75 M sucrose, 400 mM NaCl, 20 mM EDTA, 50 mM Tris pH 9.0), flash-frozen in liquid nitrogen and stored at -80 °C until DNA extraction.

Filters for use in fluorescent *in situ* hybridization analysis were prepared according to flow cytometry data collected on board ship to calculate the volume of water expected to contain approximately 3.2×10^6 PPE cells. This volume of water was pre-filtered through a 3 μm pore-size filter and fixed with 1% (w/v) (final concentration) paraformaldehyde for 1 h at 4 °C. Cells were then collected onto 0.2 μm pore-size polycarbonate filters (Whatman) by vacuum. Samples were subsequently dehydrated using an ethanol series (3 min each of 50% (v/v), 80% (v/v) and 100% (v/v) ethanol). Filters were dried and stored at -80 °C until further analysis. Unfortunately, a number of filters prepared for FISH analysis were lost due to paraformaldehyde precipitation. Hence, a final total of 13 filters from 4 stations were analysed.

2.2. Nutrient analysis

Micromolar nitrate, nitrite, silicate and phosphate concentrations were determined using a five-channel Bran and Luebbe segmented flow colorimetric autoanalyser as described previously (Zwirglmaier et al., 2007). Concentrations mentioned in the text are average values from seawater samples collected from surface waters down to 0.1% light levels, unless otherwise stated.

2.3. Flow cytometric analysis

Photosynthetic picoeukaryotes, *Prochlorococcus* and *Synechococcus* were enumerated using flow cytometry (FACSort, Beckton Dickinson, Oxford, UK) by their characteristic pigment autofluorescence and size (Olson et al., 1985). The flow rate was calculated by adding a known concentration of 0.5 μm multi-fluorescent latex beads (Polysciences, Eppelheim, Germany) as an internal standard (Zubkov et al., 2007). Flow cytometry data were processed using CellQuest software (Beckton Dickinson, Oxford, UK).

2.4. DNA extraction and polymerase chain reaction (PCR) amplification

DNA was extracted from filters using a previously described method (Fuller et al., 2003). PCR amplification of the 16S rRNA gene from environmental DNA and also from control strains for dot blot hybridization and/or clone library construction used the marine algal plastid biased primer PLA491F (Fuller et al., 2006a) coupled with the general oxygenic phototroph primer OXY1313R (West et al., 2001) to give an approximately 830 bp PCR product. PCR amplification was carried out using a hot-start method in a final volume of 50 μl, containing 1 mg ml^{-1} bovine serum albumin (BSA), 1 × enzyme buffer, 1.2 mM $MgCl_2$, 200 μM dNTPs, 0.8 μM primers and 2.5 U *Taq* polymerase. Amplification conditions consisted of 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 40 s, and a final extension step of 72 °C for 6 min.

2.5. Dot-blot hybridization analysis

Dot blot hybridization conditions for the various marine algal class-specific oligonucleotides were optimised previously (see Fuller et al., 2006b). Algal cultures used as controls were the same as those used by Fuller and colleagues (2006b) and were obtained from the Roscoff Culture Collection (RCC, http://www.sb-roscoff.fr/Phyto/RCC/) and the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, https://ccmp.bigelow.org/). 16S rDNA

amplicons from AMT environmental DNAs and control strains were purified, blotted onto nylon membranes and hybridized to algal class-specific oligonucleotide probes, following the method of Fuller and colleagues (2003). The oligonucleotide probes used are listed in Table 1a. Final wash (or dissociation) temperatures (Td) for each probe were determined empirically (Fuller et al., 2006b), following a previously described method (Fuller et al., 2003). Hybridization was quantified by using a Fujifilm FLA-5000 phosphorimager and Total Laboratory software (Phoretix). Relative hybridization of the PPE class-specific probes to total oxygenic phototroph 16S rDNA sequences amplified by the PLA491F OXY1313R primer pair was calculated according to the equation below

$$\text{Relative hybridization}(\%) = \left[\left(\frac{Senv}{Eenv}\right)\left(\frac{Scon}{Econ}\right)^{-1}\right] \times 100$$

where S*env* and E*env* represent hybridization to environmental DNA of the specific and eubacterial probes, respectively; S*con* and E*con* are the slopes of the specific and eubacterial probe-binding curves, respectively, calculated by hybridizing each probe to dilution series of homogenous control DNAs. The relative hybridization of a given specific probe compared with that of the eubacterial probe to the control DNAs was averaged where more than one control DNA was used. Any sample giving a signal above 2% was considered above background.

2.6. Construction of clone libraries

PCR products were cloned into the TA vector pCR2.1-TOPO (Invitrogen) and screened by restriction fragment length polymorphism (RFLP) after digestion with *Hae*III (Fermentas™). Clones with the same RFLP pattern, determined by eye observation, were considered members of the same operational taxonomic unit (OTU) and at least one member of each OTU was sequenced for phylogenetic analysis. Sequencing was carried out bidirectionally using an ABI PRISM 3130xl Genetic Analyser® (Applied Biosystems™).

2.7. Phylogenetic analysis

Sequences were assembled using Seqman software (DNASTAR™) and initially checked for the presence of chimeric artefacts using the Check Chimera programme within the Ribosomal Database Project (Cole et al., 2003). Sequences were aligned using ARB (Ludwig et al., 2004) and manually re-checked for the presence of chimeras by assessing the phylogenetic position of each half of the sequence in ARB. Sequences considered to be chimeric were excluded from further analysis. Phylogenetic relationships amongst plastid 16S rRNA gene sequences were performed in ARB using a neighbour-joining algorithm with Jukes–Cantor correction and the *Escherichia coli* sequence as a root. Short environmental sequences (<1000 bp) were added by ARB parsimony using a maximum frequency filter for plastids. Bootstrap analysis was performed with the ARB parsimony bootstrap algorithm with independent Nearest Neighbour Interchange (NNI) from 1000 replications, with 39,398 positions analysed with alignments not masked. Plastid rRNA gene sequences obtained in this work have been deposited in Genbank under accession numbers GQ863828–GQ863884.

2.8. Fluorescent in situ hybridization analyses

FISH analysis was used to determine the abundance of different PPE classes using the set of specific probes listed in Table 1b. Three probes (EUK1209R, NCHLO01 and CHLO02) were used together to target total eukaryotes, whilst for the Mamiellophyceae two probes (OSTREO01 and PRAS02) were used in combination. Filter sections were placed face-down in 10 μl hybridization buffer (HB) (40% (v/v) deionised formamide, 0.9 M NaCl, 20 mM Tris–HCl pH 7.5, 0.01% (w/v) SDS, 2 × blocking agent) for most probes except for the probe targeting the Chrysophyceae which contained 20% (v/v) deionised formamide (Jardillier et al., 2010). Filters were placed in humid hybridization chambers incubated at 35 °C for 30 min. Filters were incubated with 9 μl HB and 1 μl probe (50 ng μl^{-1} stock), prelabelled with horseradish peroxidase (see Table 1b) and hybridized at 35 °C for 3 h. Filters were washed

Table 1a
Probes used for dot blot hybridization analysis. The temperature at which stringent washes were performed (Td), probe sequence, target class and citation for each probe are given.

Probe	Td (°C)	Sequence	Target group	Reference
CHLA768	49	CCA TTC TCT CCC CTC GCT	Chlorarachniophyceae	Fuller et al. (2006a)
CHRY1037	52	GCA CCA CCT GTG TAA GAG	Chrysophyceae	Fuller et al. (2006a)
CRYP862	42	GGA TAC TTA ACG CCT TAG	Cryptophyceae	Fuller et al. (2006a)
EUB908	35	CCG TCA ATT CCT TTG AGT TT	Eubacteria	Edwards et al. (1989)
EUST985	49	CAC TTC TAG CAA ACC CTG	Eustigmatophyceae	Fuller et al. (2006a)
PAVL665	37	TAG AAA TTC CTC CTA CCC	Pavlovophyceae	Fuller et al. (2006a)
PELA1035	52	ACC ACC TGT GTG TGT CTA	Pelagophyceae	Fuller et al. (2006a)
PING1024	47	ACG TAT TCC TTA CGG CAC	Pinguiophyceae	Fuller et al. (2006a)
PRAS826	58	GAT TCG CGT ATC CCC TAG	Prasinophyceae clade VI	Fuller et al. (2006a)
PRYM666	44	CTA GAA ATT CCC TCT ACC	Prymnesiophyceae	Fuller et al. (2006a)
TREB708	44	CCT TTG GTG TTC CTC CCG	Trebouxiouphyceae	Fuller et al. (2006a)

Table 1b
Probes used for fluorescent *in situ* hybridization (FISH) analysis. The probe sequence, target organism(s) and citation are given.

Probe	Sequence	Target	Reference
EUK1209r	GGG CAT CAC AGA CCT G	All eukaryotes nuclear SSU rRNA	Giovannoni et al. (1988)
NCHLO01	GCT CCA CTC CTG GTG GTC	Non-chlorophyta nuclear SSU rRNA	Simon et al. (1995)
CHLO02	CTT CGA GCC CCC AAC TTT	Chlorophyta nuclear SSU rRNA	Simon et al. (2000)
PRYM02	GGA ATA CGA GTG CCC CTG AC	Prymnesiophyceae nuclear SSU rRNA	Simon et al. (2000)
CHRYSO01	GCA CCA CCT GTG TAA GAG	Chrysophyceae plastid SSU rRNA	Jardillier et al. (2010)
OSTREO01	CCT CCT CAC CAG GAA GC	Prasinophyceae clade II	Not et al. (2004)
PRAS02	CCC GTC CCG AGA CCA ACG	nuclear SSU rRNA	Biegela et al. (2003)
CRYPT13	CGA AAT ATA AAC GGC CCC AAC	Cryptophyceae nuclear SSU rRNA	Lepère et al. (2008)
PELA01	TAC CTA GGT ACG CAA ACC	Pelagophyceae nuclear SSU rRNA	Simon et al. (2000)

twice with 3–4 ml of washing buffer (WB) (56 mM NaCl, 5 mM EDTA, 0.01% (w/v) SDS, 20 mM Tris–HCl pH 7.5) and incubated at 37 °C for 20 min. Filters were then equilibrated in 3–4 ml TNT buffer (100 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% (v/v) Tween 20®) for 20 min at room temperature in the dark. The tyramide signal amplification reaction, horseradish peroxidase activation of FITC fluorochromes, was carried out by placing 20 µl TSA solution (1:1 amplification diluent and dextran sulphate with 1:50 FITC tyramide with this mixture) on filters and incubated in the dark at room temperature for 30 min. Filters were transferred to 2 successive TNT buffers at 55 °C in the dark for 20 min each. Filters were dried in the dark and then counterstained with propidium iodide (PI, 10 µg ml^{-1}) in antifading mounting solution (Citifluor). Slides were stored at 4 °C until analysed by epifluorescent microscopy. The hybridized and propidium iodide-stained filters were observed with a Zeiss Axioskop 40 (Germany) epifluorescence microscope equipped with a mercury light source and a Plan-Apochromat × 100 (Zeiss, Germany) objective. The green (FITC) and red emission (PI) fluorescence produced by the different fluorochromes was collected between 510 and 550 nm and above 585 nm. Twenty fields were chosen at random and hybridized cells were counted. When positive cell densities were lower than 10 cells per 20 fields (0.8 mm^2) two transects across the piece of filter were analysed. In cases where less than 10 cells were observed by this latter approach, the whole filter was scrutinised. This allowed a detection limit of 1 cell ml^{-1} sample filtered.

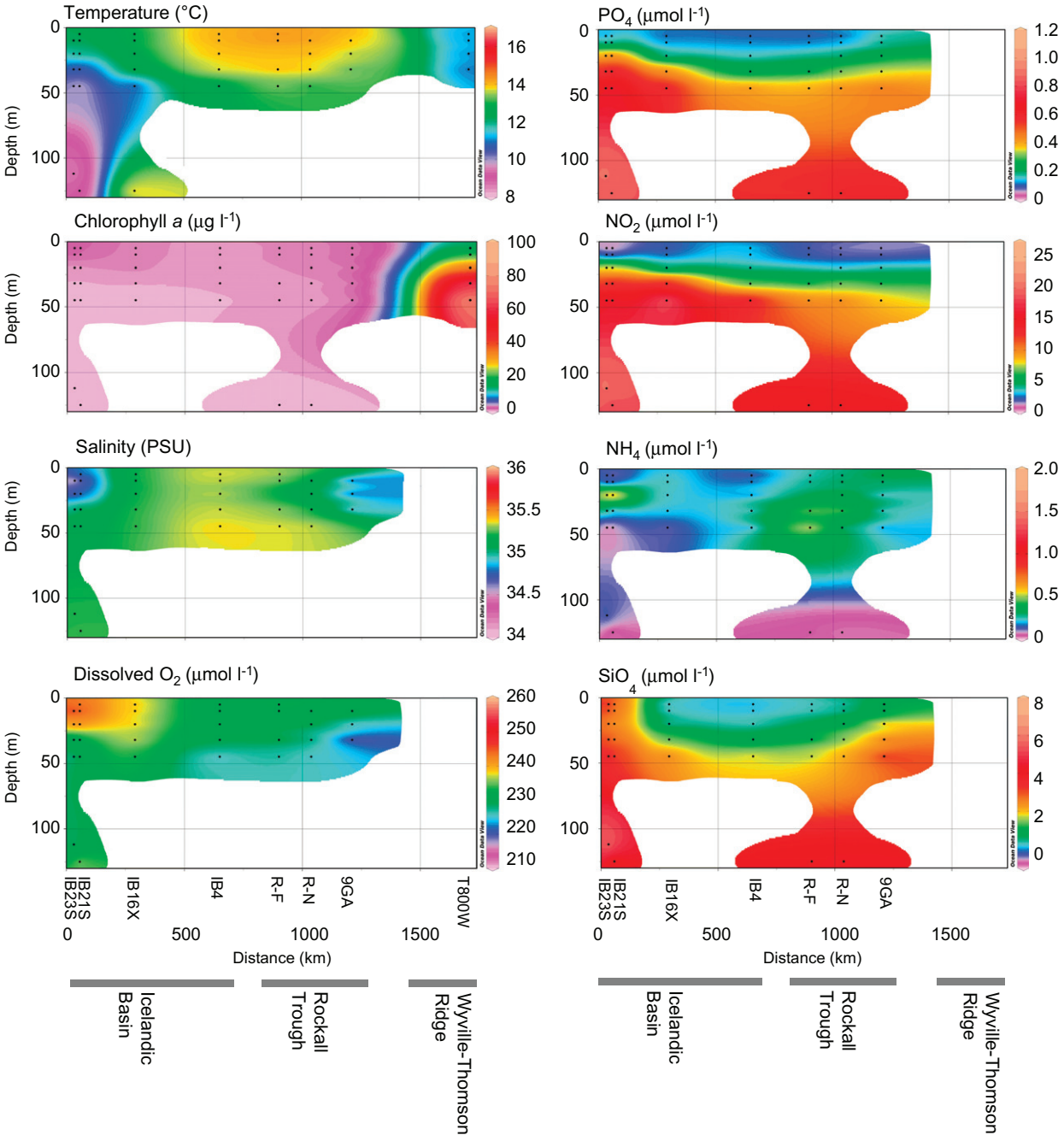

Fig. 2. Chemical and physical parameters along the extended Ellett Line cruise track. Contour plots indicate nutrient or pigment concentrations (nitrite, nitrate, phosphate, silicate, chlorophyll or dissolved oxygen) or physical measurements (temperature, salinity) plotted as a function of depth in metres (*y*-axis) along the cruise track (distance along the cruise from Iceland to the Wyville-Thompson Ridge). Black dots represent sampling points.

2.9. Statistical analyses

Multivariate statistical analyses were employed to explore relationships between the explanatory variables (environmental parameters) and the response variables (i.e. the PLA491F–OXY1313R dot blot relative hybridization values [%]). Canonical correspondence analysis (CCA) of diversity data performed using the vegan package (Legendre and Legendre, 1998) within the *R* software (http://cran.r-project.org). CCA plots were drawn using *R* software with biplot values of the environmental variables and eigenvectors of dot blot hybridization data group scores.

Rarefaction analyses were carried out for the two clone libraries constructed using Analytic Rarefaction 1.3 (Holland, 2003). Coverage values were calculated for each clone library as an estimate of the proportion of RFLP types from a sample that are represented in a clone library. This was calculated using $C=1-(n_1/N)$ where n_1 is the number of RFLP types appearing in a library and N is the total number of clones analysed in the library. The non-parametric richness estimate, S_{Chao1} (Hughes et al., 2001), was calculated for suspected PPE clones from libraries using the formula

$$S_{Chao1} = S_{obs} + F_1^2/[2\times(F_2+1)] - (F_1\times F_2)/[2\times(F_2+1)]$$

where S_{obs} is the number of different RFLP types observed; F_1 the number of RFLP types to which only one clone was assigned and F_2 the number of RFLP types to which only two clones were assigned.

3. Results

3.1. Physico-chemical characteristics of the extended Ellet Line

The cruise transect comprised seven stations along the extended Ellett Line and two sites at the Wyville-Thomson Ridge (Fig. 1). Physical and chemical variables measured along the extended Ellett Line are shown in Fig. 2. The Icelandic Basin, between 63.32°N 20.21°W and 58.5°N 16°W, was characterised by nitrate concentrations ranging between 0.6 and 20.5 μM, ammonium levels ranging from undetectable to 0.8 μM, phosphate concentrations between 0.08 and 0.9 μM, silicate ranging from undetectable levels to 5.9 μM and chlorophyll *a* concentrations between 0.02 and 2.3 mg m^{-3}. For the Rockall Trough, between 57.51°N 12.25°W and 56.81°N 7.35°W, nutrient concentrations ranged between 0.1 and 14.6 μM nitrate, 0.01–0.98 μM ammonium, 0.06–0.6 μM phosphate, 0.005–4.2 μM silicate and chlorophyll *a* concentrations between 0.02 and 1.2 mg m^{-3}. Nutrient concentrations were not measured at the Wyville-Thomson Ridge, between 60.25°N 9.01°W and 60.64°N 8.13°W, but chlorophyll *a* concentrations were the highest recorded along the transect ranging between 12.1 and 78.3 mg m^{-3}. Temperature ranged between 8.5 and 14.4 °C across the transect with the highest values from the southern end of the Icelandic basin across most of the Rockall Trough.

3.2. Picophytoplankton community structure

PPEs and *Synechococcus* showed similar distribution patterns along the transect (Fig. 3) with PPE cell numbers highest in surface waters of the coastal Icelandic Basin site and at the easterly end of the Rockall Trough peaking at 3.5×10^4 and 3.9×10^4 cells ml^{-1}, respectively. *Synechococcus* reached 3.6×10^5 cells ml^{-1} in surface waters of the coastal Icelandic Basin site, but peaked at over 5×10^5 cells ml^{-1} towards the southern end of the Icelandic Basin. At the two Wyville-Thomson Ridge sites, EG3 and T800W, PPE and

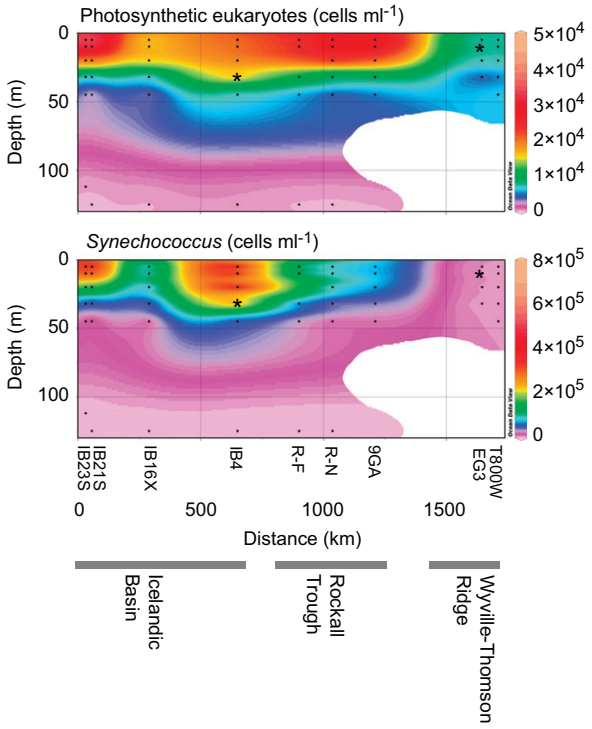

Fig. 3. Photosynthetic picoplankton distribution patterns along the extended Ellett Line. Assessment of the abundance (cells ml^{-1}) of PPEs (top), *Synechococcus* (bottom) as determined by flow cytometry. For each panel the *y*-axis is the depth (m) and the *x*-axis the distance (km) along the cruise transect (left to right from Iceland to the Wyville-Thompson Ridge). Black dots represent sampling points. The location of samples used for construction of clone libraries is indicated with an asterisk.

Synechococcus cell numbers were much lower reaching only 8×10^3 and 5×10^3 cells ml^{-1}, respectively.

3.3. PPE community structure

Of the ten class-specific probes used in dot blot hybridization analysis, five reached values above background. These were probes targeting Prymnesiophyceae, Chrysophyceae, Cryptophyceae, Pelagophyceae and Pinguiophyceae (Fig. 4). Amongst these five classes Prymnesiophyceae dominated, with an average relative hybridization value of 25% over the whole transect, reaching a maximum value of 65% at 32 m depth at station F towards the western end of the Rockall Trough. Cryptophyceae, on average, represented 8% of the hybridization signal, reaching a maximum relative hybridization value of 65% at 32 m, station IB21S in the Icelandic Basin, but was below background at the Wyville-Thomson Ridge. Chrysophyceae reached a maximum value of 46% relative hybridization at 32 m at station IB4 towards the southern end of the Icelandic Basin but values were much lower over the rest of the transect (average 9.65%). Pelagophyceae reached a maximum relative hybridization value of 24% towards the southern end of the Icelandic Basin, but with an average relative hybridization value of only 4.5% over the whole transect. Pinguiophyceae reached 3% relative hybridization in the Rockall Trough but was below the 2% background level throughout most of the transect.

Thirty-two samples, for which dot blot hybridization, chemical, physical and flow cytometry data were available, were analysed by canonical correspondence analysis (CCA) to identify environmental parameters that might explain the distribution of specific PPE classes (Fig. 5). This analysis demonstrated that 66% of the variation in dot blot hybridization data could be associated with the measured environmental variables. Up to 65% of this

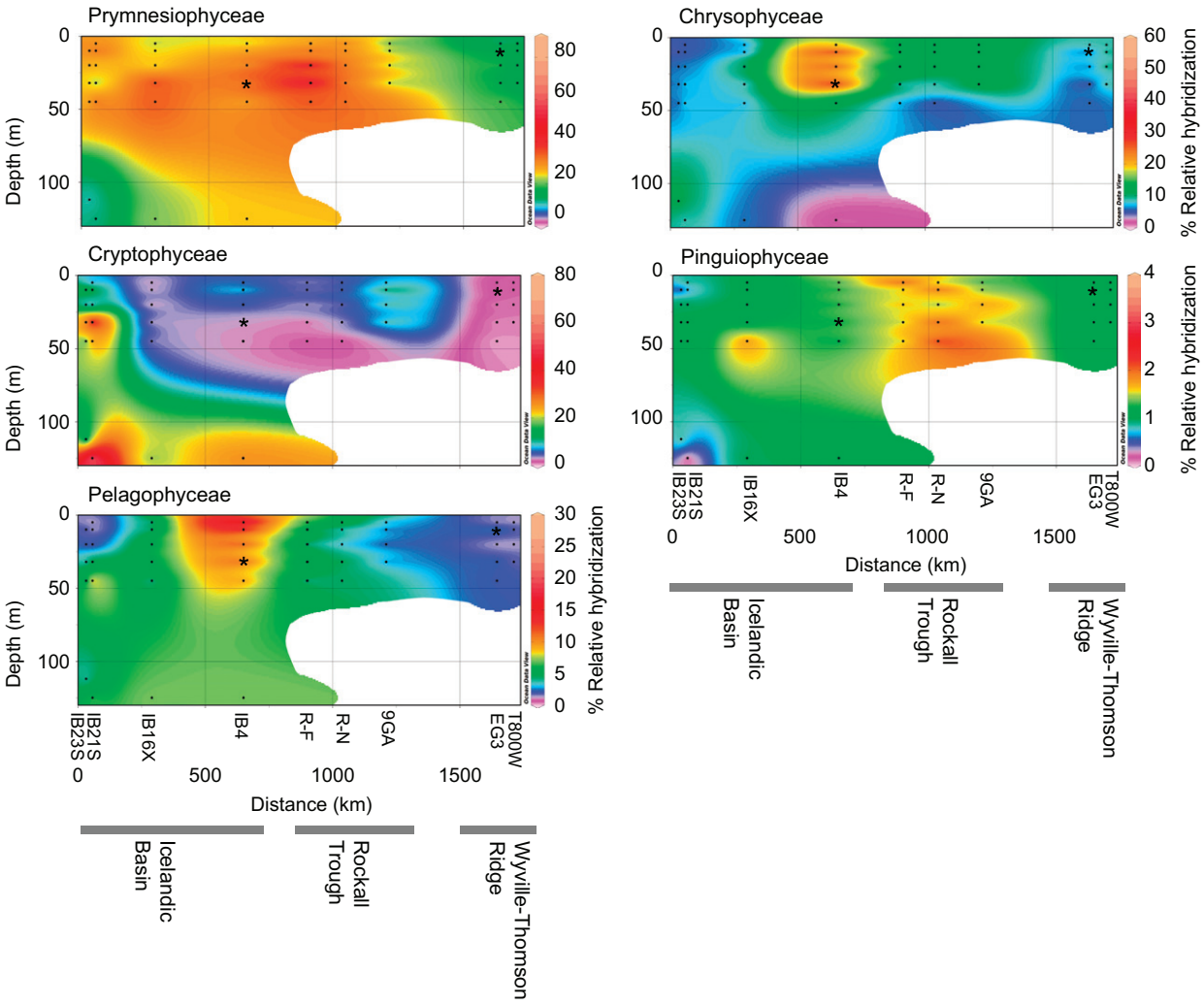

Fig. 4. Dot blot hybridization data showing the distribution of specific PPE classes along the extended Ellett Line transect. Contour plots indicate the % relative hybridization (as a proportion of all amplified by primers PLA491F and OXY1313R). *x*- and *y*-axes are as described in Fig. 4. Black dots represent sampling points. The location of samples used for clone library construction is indicated with an asterisk.

variation was explained by a 9-variable model including the parameters temperature, ammonium concentration, chlorophyll *a* (Chl *a*), the mixed layer depth (MLD), phosphate concentration, nitrate/nitrite concentration (measured together), depth, silicate concentration and salinity. Alone, the most important variable was temperature, which could explain up to 24% of the variation. Addition of ammonium, Chl *a*, and MLD explained a further 12%, 6% and 6%, respectively. Additional variables only provided $\leq 5\%$ increments in the amount of variance in dot blot hybridization data that was explained.

3.4. Taxonomic composition of the PPE community

Two clone libraries were constructed targeting the plastid 16S rRNA gene using samples from this transect. One sample, from the Icelandic Basin (station IB4) at 32 m depth, was selected due to the noticeably high relative hybridization signals for Chrysophyceae (46%) and Pelagophyceae (16%) at this site (see Fig. 4). The other clone library was constructed using 16S rRNA gene amplicons from station EG3 at the Wyville-Thomson Ridge at 10 m depth, for which only two classes reached above background hybridization levels, Prymnesiophyceae and Pelagophyceae, and the total relative hybridization signal for this sample was only 27%. PPE classes represented in these two clone libraries are shown in Table 2.

Rarefaction analysis of these clone libraries (Suppl. Fig. 1) is suggestive of both libraries reaching a plateau indicating that analysis of further clones would yield only a few novel RFLP types. This is confirmed by the high coverage values calculated for both clone libraries, 90% for the IB4 library and 89% for the EG3 library. Both libraries were comparatively species rich but with EG3 notably richer than IB4 as shown by S_{chao1} values, of 57.1 and 26.3, respectively.

Both libraries were dominated by RFLP types whose corresponding sequences could be assigned phylogenetically to Prymnesiophyceae (Fig. 6) (representing 62% and 68% of the clones for the IB4 and EG3 libraries, respectively). This contrasts with the dot blot hybridization analysis for station IB4 which indicated that chrysophytes were more abundant than the prymnesiophytes. Thus, the clone libraries suggest this class is more abundant than predicted by dot blot hybridization analysis (Table 2). Indeed, four of the prymnesiophyte-affiliated sequences obtained from these libraries had a one base pair deletion within the target sequence for the Pymnesiophyceae dot blot hybridization probe, which might explain this underestimation of the Prymnesiophyceae community by dot blot hybridization analysis. However, it is difficult to predict the proportion of prymnesiophytes that were not effectively targeted by the Prymnesiophyceae-specific dot blot hybridization probe since RFLP screening was used. For example, for one particular RFLP type, of two clones sequenced,

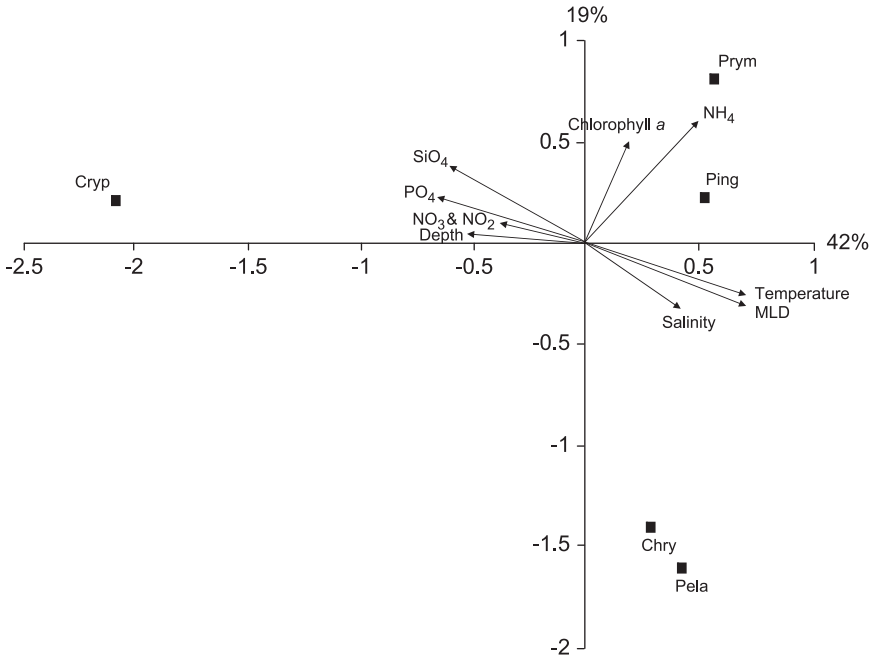

Fig. 5. CCA plot for PPE class distributions along the extended Ellett line in relation to environmental variables, from dot blot hybridization results for classes: Prymnesiophyceae (Prym), Chrysophyceae (Chry), Cryptophyceae (Cryp), Pinguiophyceae (Ping), Pelagophyceae (Pela). Arrows pointing in roughly the same direction indicate a high positive correlation, arrows crossing at right angles indicate a near-zero correlation, and arrows pointing in the opposite direction have a high negative correlation.

Table 2
Number of RFLP types and clones (the latter in brackets) within each algal class observed in the two plastid 16S rRNA gene clone libraries constructed from the extended Ellett Line transect at the Iceland Basin station IB4, 32 m depth and the Wyville-Thomson Ridge station EG3, 10 m depth.

Class/Group	Number of RFLP types (clones) in libraries	
	IB4, 32 m	EG3, 10 m
Prasinophyceae	3 (34)	6 (42)
Prymnesiophyceae	6 (123)	9 (153)
Chrysophyceae	1 (5)	–
Cryptophyceae	2 (2)	2 (5)
Dictyochophyceae	3 (9)	3 (7)
Pelagophyceae	3 (26)	–
Bacillariophyceae	–	1 (8)
Cyanobacteria	2 (5)	3 (7)
Heterotrophic bacteria	–	1 (3)

IB4_32m_109 and IB4_32m_206, the former clone contained this deletion but the latter did not.

The next most important class after the Prymnesiophyceae was the Prasinophyceae, representing 16% and 19% of the sequences in the IB4 and EG3 libraries, respectively. This class comprises several clades but only Prasinococcales (clade VI) is currently targeted by the suite of dot blot hybridization probes so far developed (Table 1a). These two classes were more diverse in the EG3 library than the IB4 library, as evidenced by the higher number of RFLP types assigned to these classes in the former (Table 2).

Aside from the two major classes, community structure at the two sites varied considerably, with sequences affiliated to Chrysophyceae and Pelagophyceae only detected in the IB4 library (see Table 2). This is despite Pelagophyceae being shown by dot blot hybridization analysis to be present in both samples, representing up to 16% and 4% relative hybridization in the Icelandic Basin and Wyville-Thomson Ridge samples, respectively. The absence of Chrysophyceae at the Wyville-Thomson Ridge site is thus evidenced by both clone library and dot blot hybridization approaches. A small number of clones related to Dictyochophyceae and Cryptophyceae were also present in both libraries (Table 2).

Phylogenetic analysis of the two clone libraries revealed several novel phylotypes within the Prasinophyceae and Prymnesiophyceae, the two most abundant classes detected along the transect based on both FISH data and representation of RFLP types in clone libraries (Figs. 6 and 7). For example, an extensive and long-branching cluster derived from five Ellett Line sequences within Prasinophyceae clade 16S-VIII possessed only 85–90% identity to the nearest published sequence (Fig. 6). Similarly, a novel lineage within the Prymnesiophyceae, also comprising five Ellett Line sequences, showed only 89–93% identity to the nearest published sequences (Fig. 7). Two more sequences, EG3_10m_101 and IB4_32M_213, cluster alone and have only 96% identity to their nearest cultured neighbours. Furthermore, within Prymnesiophyceae clades 16S-I and 16S-II and Prymnesiales *Chrysochromulina* clade B2, sequences were found with as little as 95%, 94% and 96% identity, respectively, to their nearest cultured neighbours.

FISH analysis detected Prymnesiophyceae, Mamiellophyceae (formerly Prasinophyceae clade II, see Marin and Melkonian, 2010), Chrysophyceae, Cryptophyceae and Pelagophyceae along the transect (Table 3). Prymnesiophyceae cells were again well represented using this PCR-independent approach, though up to one-third of labelled cells appeared larger than 3 μm in diameter, despite samples being pre-filtered through a 3 μm pore-size mesh during sample preparation. However, cells which labelled with other class-specific probe combinations were all <3 μm in size. Interestingly, in six of 13 samples members of the Mamiellophyceae were the most abundant PPE group, highlighting the lack of a dot blot hybridization probe targeting this group. In contrast, cells attributable to Chrysophyceae, Cryptophyceae and Pelagophyceae appeared to be only minor components of the PPE community based on FISH analysis.

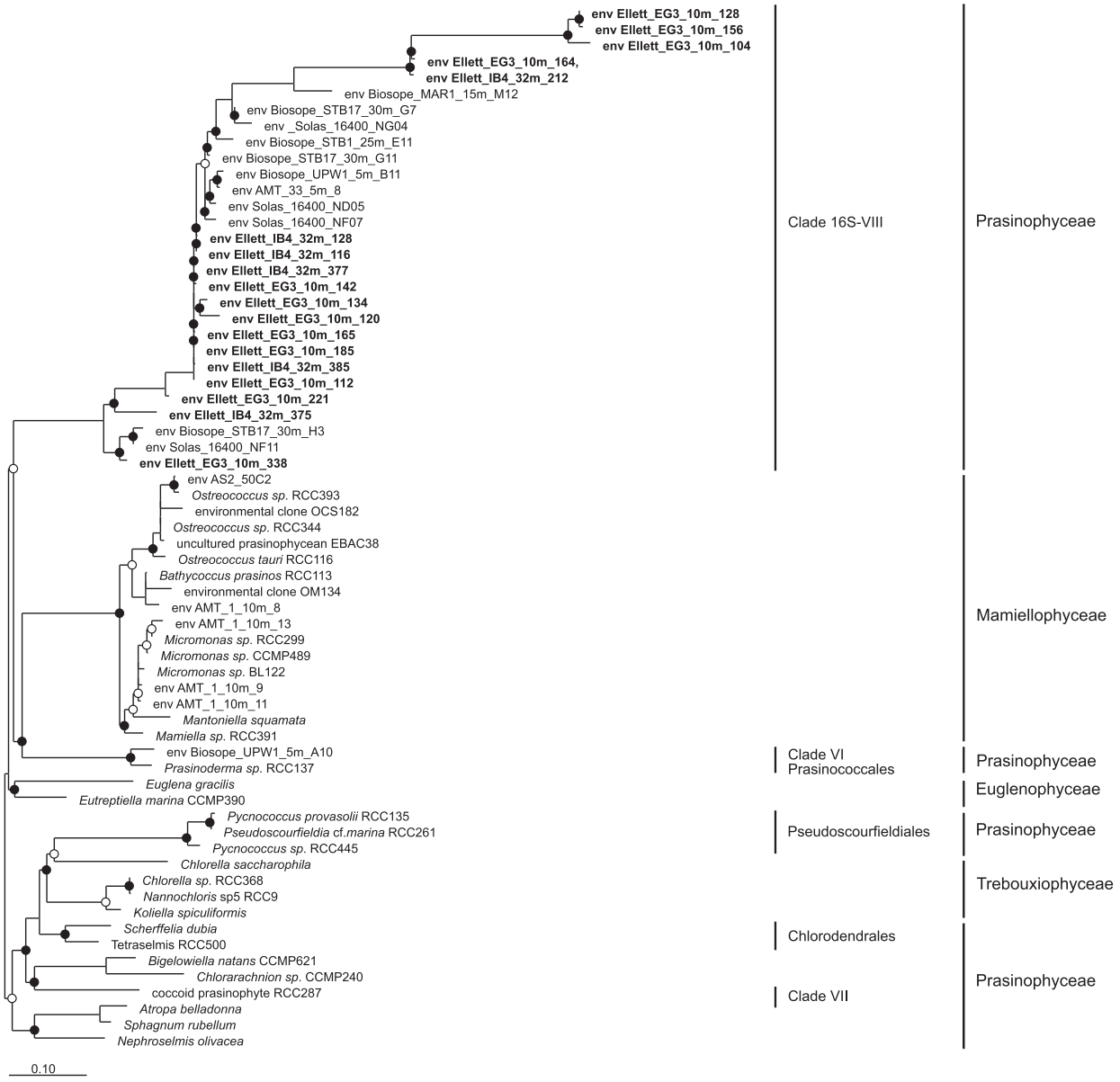

Fig. 6. Phylogenetic relationships amongst Prasinophyceae plastid 16S rRNA gene sequences constructed using a neighbour-joining algorithm. This tree is extracted from one comprising all marine algal plastid sequences and rooted to *Escherichia coli*. Environmental sequences (600–800 nucleotides in length) were added by parsimony using a maximum frequency filter for plastids. Bootstrap values $>70\%$ and $<90\%$ are marked with an open circle, values $>90\%$ are marked with a filled circle. Environmental sequences from the extended Ellett line cruise are indicated in bold.

4. Discussion

4.1. Diversity and distribution of major PPE classes

The photic zone of the extended Ellett Line was characterised by high nutrient concentrations and high cell numbers for both *Synechococcus* and PPEs compared to other published data from cruises in the Atlantic Ocean, Pacific Ocean and Arabian Sea (Fuller et al., 2006a, b; Lepère et al., 2009; Kirkham et al., 2011) as well as the globally widespread samples analysed by Cuvelier et al. (2010). However, these densities are similar to those observed in coastal waters (e.g. see Worden et al., 2004; Not et al., 2004; McDonald et al., 2007). The three methodologies used in this study, namely dot blot hybridization and FISH analyses, as well as phylogenetic analysis of sequences retrieved here, showed that members of the Prymnesiophyceae, Prasinophyceae and Mamiellophyceae were the major components of the PPE community in these high latitude Atlantic waters.

A central role for prymnesiophytes is consistent with pigment and inverted microscope data of the >2 μm fraction from the same area in early summer 2001 (Moore et al., 2005). The distribution of this group was linked to the concentration of ammonium and Chl *a* (Fig. 5), contrasting with other studies (e.g. Lepère et al., 2009; Kirkham et al., 2011) that have shown no correlation with measured environmental variables. We cannot rule out though that Prymnesiophyceae community structure is also influenced by factors that have not been measured here, including predators, viruses or trace metal availability, whilst stochastic processes (e.g. chance and random immigration) may also play a part (Ofitero et al., 2010). Moreover, since a very broad phylogenetic group is encompassed within the Prymnesiophyceae, potentially equating to taxa with dissimilar physiologies that respond differently to environmental variables, this could also explain why correlations are not obvious and hence why the relationships observed here differ from previous investigations. The dominance of Prymnesiophyceae in the PPE community along the extended Ellet Line transect mirrors that

Fig. 7. Phylogenetic relationships amongst Prymnesiophyceae plastid 16S rRNA gene sequences. Constructed using a neighbour-joining algorithm. This tree is extracted from one comprising all marine algal plastid sequences and rooted to *Escherichia coli*. Environmental sequences (600–800 nucleotides in length) were added by parsimony using a maximum frequency filter for plastids. Bootstrap values $>70\%$ and $<90\%$ are marked with an open circle, values $>90\%$ are marked with a filled circle. Environmental sequences from the extended Ellett line cruise are indicated in bold. Sequences highlighted with an asterisk belong to a novel cluster containing only environmental sequences.

found in other parts of the Atlantic Ocean (Kirkham et al., 2011) as well as more generally in other oceanic regions e.g. the pelagic Arabian Sea (Fuller et al., 2006b), Indian Ocean (Not et al., 2008), the coastal and eastern Mediterranean Sea (McDonald et al., 2007; Man-Aharonovich et al., 2010) and the ultra-oligotrophic South-East Pacific Ocean (Lepère et al., 2009) using plastid gene targeted molecular approaches. This contrasts with the situation using nuclear SSU rRNA approaches where, despite obtaining sequences from a range of environments e.g. Pacific Ocean (Moon-van der Staay et al., 2001; Worden, 2006), Weddell Sea, Scotia Sea, Arctic Ocean and Arctic-Atlantic convergence area (Díez et al., 2001; Lovejoy et al., 2006; Hamilton et al., 2008), English Channel (Romari and Vaulot 2004) or Mediterranean Sea (Díez et al., 2001; Massana et al., 2004) such sequences never dominate clone libraries unless such libraries are constructed from DNA derived from flow sorted PPE cells (Shi et al., 2009; Marie et al., 2010). The dominance of Prymnesiophyceae using plastid gene approaches is matched by pigment studies using the diagnostic pigment 19′-hexanoyloxyfucoxanthin (Moon-van der Staay et al., 2000; Not et al., 2008; Ras et al., 2008; Liu et al., 2009) as well as recent FISH and CO_2 fixation data that reveals this class not only to be cosmopolitan in oceanic waters as a picoplanktonic component but also to be a major player in marine CO_2 fixation, accounting for significantly higher rates of CO_2 fixation than other picoplankton groups (Jardillier et al., 2010; Cuvelier et al., 2010). Our observation of a high relative abundance of Prymnesiophyceae in high latitude Atlantic waters thus further highlights the global importance of this class in marine systems across waters encompassing a range of trophic status.

Phylogenetic analysis (Fig. 7) showed that sequences related to the Prymnesiophyceae were phylogenetically diverse in the extended Ellett Line clone libraries, with as little as 89% similarity to previously published sequences. Other sequences were closely related to cultured species, particularly those related to *Phaeocystis* spp., a genus widely distributed in oceanic waters (e.g. see Schoemann et al., 2005) but with sequences here seemingly more closely related to known temperate species i.e. *Phaeocystis globosa*. A number of sequences from the clone libraries (each highlighted with an asterisk in Fig. 7) fell within a cluster containing only environmental sequences from the Pacific, Mediterranean Sea and equatorial Atlantic Ocean (McDonald et al., 2007; Lepère et al., 2009; Jardillier et al., 2010). This cluster is, however, relatively closely related to various *Chrysochromulina* spp. a diverse group but with only a few described picoplanktonic species (Vaulot et al., 2008). In contrast, several sequences from the IB3 library fell within a previously described lineage Prym16S-II (Lepère et al., 2009), again containing no cultured representatives (Fig. 6) but similar to environmental sequences from the Pacific, Arabian Sea and Atlantic Ocean (Fuller et al., 2006a; Lepère et al., 2009; Kirkham et al., 2011). Whilst these lineages encompassing solely environmental sequences may partly be due to an incomplete inventory of plastid rRNA genes from cultured representatives of this class it more likely highlights the extensive level of diversity contained within this group, as was recently seen using the LSU rRNA gene (Liu et al., 2009). Thus, a continued effort to culture pico-sized prymnesiophytes, and to sequence both their 18S and 16S rRNA genes, is required if we want to fully identify, and subsequently characterize, these major components of the PPE community.

FISH analysis revealed that members of the Mamelliophyceae were also abundant along the Ellet Line transect. Even so, the abundance of this class may still be underestimated since it has been reported elsewhere (Demir-Hilton et al., in press) that the OSTREO01 probe, used in this study in combination with the PRAS02 probe, does not hybridize well on all field samples. This group is well known for its dominance in coastal and

Table 3
Results of fluorescent *in situ* hybridization (FISH) along the extended Ellett Line transect. For each sample total eukaryotes (EUKS) cells ml^{-1} are counted using a combination of FISH probes: EUK1209r, NCHLO01 and CHLO02. Class-specific FISH data is expressed in cells ml^{-1} (bold, top line) and as a percentage of total eukaryotes by FISH (middle line). The bottom line for each sample shows the corresponding % relative hybridization value obtained for each sample.

Station	Depth (m)	Total	Results = cells ml^{-1} and % of total eukaryotes				
		EUKS	Pras II	Prym	Chry	Cryp	Pela
IB21S	**5**	**2.01×10^4**	**1.02×10^4**	**6.66×10^3**	**< 1**	**10**	**10**
			50.6%	33.1%	< 0.1%	< 0.1%	< 0.1%
				28%	5%	4%	1%
IB21S	**10**	**2.85×10^4**	**2.07×10^3**	**2.32×10^3**	**28**	**9**	**17**
			7.3%	8.1%	0.1%	< 0.1%	0.1%
				39%	6%	10%	0%
IB21S	**20**	**2.18×10^4**	**2.74×10^3**	**1.00×10^3**	**25**	**18**	**14**
			12.6%	4.6%	0.1%	0.1%	0.1%
				24%	7%	4%	1%
IB21S	**32**	**7.19×10^3**	**9.51×10^2**	**6.31×10^2**	**< 1**	**5**	**16**
			13.2%	8.8%	< 0.1%	0.1%	0.2%
				16%	9%	65%	7%
Rockall F	**5**	**1.13×10^4**	**3.11×10^2**	**1.96×10^3**	**4**	**1**	**70**
			2.8%	17.4%	< 0.1%	< 0.1%	0.6%
				23%	9%	1%	3%
Rockall F	**10**	**9.53×10^3**	**4.14×10^2**	**3.52×10^3**	**< 1**	**4**	**95**
			4.3%	37%	< 0.1%	< 0.1%	1%
				36%	7%	1%	1%
Rockall F	**20**	**1.67×10^4**	**2.13×10^2**	**4.80×10^3**	**12**	**2**	**39**
			1.3%	28.7%	0.1%	< < 0.1%	0.2%
				61%	0%	1%	1%
Rockall N	**5**	**2.47×10^4**	**1.11×10^4**	**1.19×10^4**	**96**	**7**	**63**
			44.8%	48%	0.4%	< 0.1%	0.3%
				39%	14%	3%	8%
Rockall N	**10**	**1.80×10^4**	**5.55×10^3**	**3.68×10^2**	**7**	**2**	**24**
			30.9%	2%	< 0.1%	< 0.1%	0.1%
				33%	10%	1%	2%
Rockall N	**20**	**1.99×10^3**	**1.75×10^2**	**32**	**5**	**< 1**	**3**
			8.8%	1.6%	0.3%	< 0.1%	0.2%
				25%	13%	1%	2%
9GA	**5**	**1.30×10^3**	**0**	**44**	**< 1**	**< 1**	**1**
			0	3.4%	< 0.1%	< 0.1%	0.1%
				11%	12%	10%	1%
9GA	**10**	**3.25×10^3**	**62**	**41**	**7**	**1**	**< 1**
			1.9%	1.3%	0.2%	< 0.1%	< 0.1%
				14%	12%	20%	1%
9GA	**20**	**3.84×10^2**	**16**	**16**	**< 1**	**< 1**	**2**
			4.2%	4.2%	< 0.1%	< 0.1%	0.5%
				14%	12%	15%	1%

nutrient-rich areas (Not et al., 2004, 2005, 2008), a feature contrasting with the more open ocean environments occupied by members of the Prymnesiophyceae (see references above). The fact that these two classes co-occur at similar levels along the transect suggests physical and chemical parameters demonstrate features of both coastal and open ocean waters. Previous work has shown the plastid primers used here are biased against members of the Mamelliophyceae (McDonald et al., 2007), whilst a dot blot hybridization probe for the group is currently not developed. However, the clone libraries constructed in this study did identify several phylotypes within Prasinophyceae clade 16S-VIII, a lineage containing no known cultured representatives but comprising other environmental sequences from the Pacific (Lepère et al., 2009) and equatorial Atlantic (Kirkham et al., 2011). This is despite the class accounting for the largest proportion of picoeukaryote cultures currently maintained (Vaulot et al., 2008). It is possible that the clade 16S-VIII sequences in the libraries represent prasinophytes that could be detected by our Mamiellophyceae FISH probes. Culturing and sequencing effort is required to find the 18S rRNA source of the plastid 16S rRNA sequences. There are lineages defined by sequencing of the nuclear SSU gene e.g. VIII and IX that also contain no known cultured counterparts (see Viprey et al., 2008). Hence, one or more of these may correspond to the lineages detected here (Fig. 6).

Members of the Cryptophyceae also appeared abundant along the transect, as evidenced by dot blot hybridization data, with highest relative hybridization values occurring towards the coast of Iceland. Along an extended Atlantic Ocean transect (AMT15), this class was also found to be restricted to upwelling waters and coastal areas (Kirkham et al., 2011) based on a similar molecular approach. Various other methods have also shown cryptophytes to be abundant at a number of coastal sites (Romari and Vaulot, 2004; McDonald et al., 2007; Not et al., 2007). Their increased abundance in upwelling and coastal sites is likely to be related to the elevated nutrient concentrations encountered in these regions (see Fig. 5). Indeed the CCA analysis showed this class was strongly linked to the nutrient variables nitrate, silicate and phosphate, and hence also with depth (Fig. 5). However, FISH analysis detected only small numbers of cryptophytes (Table 3), values that are likely close to background. In particular, station IB21S showed a relative hybridization value of 65% at 32 m depth whereas FISH analysis revealed only 0.1% in this sample. Similarly, a study of phytoplankton diversity off the north-west Iberian coast found that using the same Cryptophyceae FISH probe as used here, only a minor fraction of cells were labelled, although pigment analysis indicated that cryptophytes were a major contributor to chlorophyll *a* concentration in the region (Not et al., 2007). Plastid sequences could derive from bursting or

lysed dinoflagellate cells, particularly those of the genus *Dinophysis*, which are known to harbour plastids of cryptophyte origin (Hackett et al., 2003).

Similarly, FISH data indicated that the Chrysophyceae were a minor class along the extended Ellett line whereas dot blot hybridization data suggests the class contributes more significantly to the PPE population. However, filters collected at station IB4, where relative hybridization values for the Chrysophyceae were highest, were not used for FISH analysis due to paraformaldehyde precipitation. Along the rest of the transect, relative hybridization values for the class were lower. The use of a plastid targeted Chrysophyceae FISH probe supported findings of Jardillier et al. (2010) of the presence of two plastids in chrysophyte cells, and suggests that plastid dot blot hybridizations overestimate Chrysophyceae abundance.

Pelagophyte abundance appears to be comparatively high in the Icelandic Basin and Rockall trough, reaching up to 24% relative hybridization. In other cruises, pelagophytes were only occasionally detected by dot blot hybridization analysis in the Pacific Ocean, Arabian Sea and Atlantic Ocean (Lepère et al., 2009; Fuller et al., 2006b; Kirkham et al., 2011). FISH analysis also indicated the presence of pelagophytes, at concentrations greater than those of cryptophytes and chrysophytes. Furthermore, the clone library constructed for station IB4 contained two pelagophyte lineages, one containing two RFLP types related to *Pelagomonas calceolata* and one containing one RFLP type related to *Ankylochrysis lutea* (data not shown). Both lineages were also detected in the Pacific Ocean (Lepère et al., 2009).

4.2. PPE community structure and measured environmental variables

Our dataset suggests relationships between certain classes and environmental variables, corresponding putatively to the different requirements of these PPEs. Indeed, CCA showed the variation in PPE community structure to be fairly strongly influenced by temperature, salinity, MLD, depth, ammonium, Chl *a*, phosphate, nitrate and silicate concentrations. More precisely, chrysophyte and pelagophyte distribution patterns revealed peaks in regions with the highest temperature and salinity values, which is supported by the link between these parameters along axis one of the CCA (Fig. 5). In contrast, cryptophyte distributions were lowest in these regions but peaked in the samples with the highest phosphate and nitrate concentrations (see Figs. 2, 4 and 5), a feature again supported by the CCA analysis. Temperature and salinity have been shown to be important in influencing other planktonic communities. For example, assemblages of the planktonic photosynthetic nanoeukaryotes coccolithophores and dinocysts, inferred from fossils contained within sediments from an area similar to that studied here, were strongly related to these factors at the sea surface (Solignac et al., 2008). Salinity and temperature are indicators of different water masses, and many water masses converge in the hydrographically complex Rockall channel. In particular, warm saline water of the Gulf Stream crosses the channel from the south in addition to North Atlantic central water, cooler, lower salinity north western oceanic polar front water and higher salinity water of Mediterranean origin (Ellett et al., 1986). Similarly, factors including salinity were important in determining the PPE community structure of a region of convergence of water masses in the Arctic (Hamilton et al., 2008). Salinity may have an increased effect on the ecology of the northern north Atlantic as climate change in the Arctic causes reduced ice cover and discharge of low salinity, colder water into the Atlantic. This has been shown to expand the biogeographic ranges of phytoplankton as well as fish species northwards from the subtropics in the east and southwards for boreal species in the western north Atlantic (Greene et al., 2008). The mixed layer depth also significantly explained the distribution of PPEs along the Ellett Line transect (Fig. 5). Water column mixing/stratification status thus also seems to be important in explaining the occurrence of specific PPE classes, as was recently suggested for subtropical marine picophytoplankton communities (Bouman et al., in press)

In summary, this work has revealed the PPE community structure in previously understudied high latitude north Atlantic waters. The community was characterised by putatively autochthonous, potentially cold-adapted but high nutrient requiring groups, as well as groups found more generally in marine systems. PPEs were dominated by members of the Prymnesiophyceae, Prasinophyceae and Mamiellophyceae, with sequences with low identity (as little as 85% identity) to published sequences identified in two of these classes, Prymnesiophyceae and Prasinophyceae. More minor classes contributing to the PPE community included the Cryptophyceae, Chrysophyceae, Pelagophyceae and Pinguiophyceae.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2011.05.004.

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