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SHORT NOTE

Succession of picophytoplankton during the spring bloom 2012 in Disko Bay (West Greenland)—an unexpectedly low abundance of green algae

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Abstract Picoplankton are an ecologically important component of pelagic Arctic marine ecosystems that may be heavily impacted by climate change. In order to assess potential impacts of a changing environment on this group, it is necessary to develop a better understanding of their population dynamics and seasonal distribution. This study, carried out in Disko Bay, West Greenland, during spring 2012, demonstrates that fuco-algae (e.g. chrysophytes, cryptophytes, diatoms and pelagophytes) dominated the picophytoplankton during the spring bloom with minor contributions from haptophytes. In the post-bloom phase, fuco-algae were replaced by haptophytes. In contrast to total chlorophyll *a*, which varied dramatically over the study period, the picoplanktonic chlorophyll *a* remained relatively stable despite the variability in picophytoplankton community composition. Based on mostly molecular studies, a general picture has emerged from the literature that mamiellophytes (a group within the green algae) dominate Arctic picophytoplankton. Here, however, green algae were found to contribute with only about 10 % of the picoplanktonic chlorophyll *a*. We suggest here that differences in cell size may offer a plausible explanation for the contrast between results obtained from molecular studies and those obtained from pigment- and microscopy-based studies.

Keywords CHEMTAX · GC content · HPLC · Picoeukaryotes · 19′-hex-fucoxanthin · Real-time qPCR

Introduction

Phytoplankton research in the Arctic has typically focused on the larger microalgae, especially diatoms, that dominate during the spring bloom (Wassmann et al. 1999; von Quillfeldt 2001; Hodal et al. 2011). Nevertheless, it is well known that small phytoplankton play an important role in the Arctic marine carbon cycle: cells <10 µm contributed 46 % of primary production in the northern Barents Sea in July and August (Hodal and Kristiansen 2008), picoplankton (<2 µm) contributed 36 % of autotrophic biomass at selected stations in the Arctic Ocean from June to September (Booth and Horner 1997) and 11–72 % of total chlorophyll *a* was attributable to the <1 µm size-fraction in a shallow basin in the Canadian Arctic from August to September (Smith et al. 1985). Furthermore, in the Canadian Arctic, the chlorophyll *a* contribution of picoplankton has been observed to increase, and nanophytoplankton to decrease, as increasing temperatures, ice melt and river run-off strengthen stratification and decrease the supply of nutrients to the upper layer of the water column (Li et al. 2009).

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Despite the importance of these organisms elsewhere in the Arctic, picophytoplankton have received only limited attention in the coastal waters of Greenland (Booth and Smith 1997; Lett et al. 2011), although total phytoplankton, protozoans and copepods have all been examined extensively in association with the spring bloom there (Nielsen and Hansen 1995; Levinsen et al. 2000; Madsen et al. 2008). Succession of Arctic picoeukaryotes during the spring bloom has also only received limited attention to date (Sørensen et al. 2011). In addition, current knowledge of picophytoplankton population dynamics is mostly based on bulk measurements. Bulk measurements of picophytoplankton abundance or chlorophyll *a* have shown that picophytoplankton vary less than larger microalgae, both spatially and temporally, across many marine regions (Larsson and Hagström 1982; Magazzù et al. 1996; Wright et al. 2009). However, the population dynamics within the picophytoplankton community are not well understood.

The most common approach for studying the diversity of Arctic picophytoplankton until now has been based on molecular data. These studies generally identify picoplanktonic mamiellophytes (previously prasinophytes, Marin and Melkonian 2010), especially *Micromonas pusilla*, to be widespread and abundant in the Arctic. Indeed, it has often been suggested that *M. pusilla* is the most important picophytoplankter in Arctic systems (Lovejoy et al. 2007; Sørensen et al. 2011; Terrado et al. 2011). It has been shown, however, that molecular methods can overestimate mamiellophyte and underestimate haptophyte abundances when compared to non-molecular approaches such as HPLC (van der Staay et al. 2000). This observation has led to the so-called 19′-hex-fucoxanthin paradox where 19′-hex-fucoxanthin, a pigment found in haptophytes, is abundant despite the fact that haptophytes appear to have low abundances in molecular surveys. A proposed mechanism, which could explain this paradox, is a high GC-ratio of haptophyte 18S rDNA. This might lead to poor amplification during PCR (Liu et al. 2009; Marie et al. 2010) as a high GC content may prevent disassociation during melting or cause formation of secondary structures serving as termination sites during elongation (McDowell et al. 1998; Aird et al. 2011).

In this study, the composition and succession of the picophytoplankton community before, during and after the 2012 spring bloom in Disko Bay, West Greenland, were investigated using a pigment-based method (HPLC-CHEMTAX). In addition, the hypothesis that haptophytes are underrepresented in molecular studies (compared to pigment-based studies) due to reduced amplification caused by their high GC content was tested in a real-time qPCR experiment.

Methods

Sample collection

A total of 14 water samples were collected at 20 m in Disko Bay (69°N, 53°W), Greenland. For HPLC analysis, 500–1000 mL were pre-filtered on a 3-µm Nucleopore filter (Whatman) and the microbial biomass was collected on a GF/F filter (Whatman) and stored at −20 °C during the field period and afterwards at −80 °C until extraction. For total chlorophyll *a*, 250 mL was filtered directly on to a GF/F filter (Whatman) and extracted in 96 % ethanol for 12–24 h (Jespersen and Christoffersen 1987), and chlorophyll *a* was measured on a TD-700 Turner fluorometer calibrated against a chlorophyll *a* standard before and after acidification (Yentsch and Menzel 1963). For ice cover and nutrient measurements, see the supplementary material.

HPLC

Picoplankton pigments were extracted from GF/F filters in 2.5 ml 100 % methanol, sonicated on ice for 15 min and left to extract for 24 h at 4 °C prior to filtering (0.2 µm) 1 ml extract into HPLC vials and mixing with 300–400 µl water. HPLC analyses were performed on a Shimazu LC 10A system with a Supercosil C18 column (250 × 4.6 mm, 5 µm). Pigments were indentified by retention times and absorption spectra identical to those of authentic standards and quantified against standards purchased from DHI (Hørsholm, Denmark). The concentrations of chlorophyll c_3, 19′-but-fucoxanthin, fucoxanthin, 19′-hex-fucoxanthin, alloxanthin, chlorophyll *b* and chlorophyll *a* were determined.

CHEMTAX

The contributions of different phytoplankton classes to total chlorophyll *a* were calculated using CHEMTAX 1.95 (Mackey et al. 1996). For measurements below the detection level of the instrument (Table S1, Online Resource 1), the detection limit was used as the measured value to ensure that green algae were not underestimated as chlorophyll *b* was often below the detection limit. Initial pigment ratios used in CHEMTAX are listed in Table S2. Inspired by Not et al. (2005), three chemotaxic groups were created: (1) green algae, (2) fuco-algae (fucoxanthin-containing algae such as chrysophytes, cryptophytes, diatoms, pelagophytes and haptophytes without 19′-hex-fucoxanthin) and (3) haptophytes with 19′-hex-fucoxanthin. Input ratios for the CHEMTAX matrix were taken from Higgins et al. (2011) preferentially using pigment ratios from field studies when available: the green algae chemotaxonomic

group was based on prasino-3 (which has *M. pusilla* as example species), fuco-algae on the average pigment ratios of crypto-1, diatom-1, synuro-1 and pelago-1, and for the haptophytes, hapto-7 and hapto-8 were used. Dinoflagellates were omitted as no picoplanktonic dinoflagellate species are known and their presence in molecular studies of picoplankton has been suggested to stem from a filtration artefact (Sørensen et al. 2013). Cyanobacteria were not included as they are virtually absent from Arctic marine waters (Gradinger and Lenz 1995; Li 1998; Sherr et al. 2003).

In total, 100 different input matrices were used, where one employed the ratios listed in Table S2 and the other 99 varied by a random amount of up to 10 %. Ratio limits for accessory pigments were set at 200 %, and the mean values from the 10 runs with the lowest root-mean-square error (RMSE) were used. CHEMTAX analysis was not performed on samples from March due to very low pigment concentrations. The Excel file used for the CHEMTAX analysis can be found as Online Resource 2.

Cluster analysis

A cluster analysis was performed on pigment ratios to divide sampling sites into clusters having similar pigment composition and, thereby, similar picophytoplankton communities, using the same method as Fujiwara et al. (2014). Data were scaled and clustering performed with Ward's hierarchical clustering using Euclidean distance, and approximately unbiased *p* values and bootstrap probability values were calculated using the R package, pvclust (Suzuki and Shimodaira 2006). This was done to get another assessment of picophytoplankton community composition independent of the CHEMTAX analysis. As with CHEMTAX, this analysis was only done on samples from April and May. The pigment ratios 19′-but-fucoxanthin/chlorophyll *a*, fucoxanthin/chlorophyll *a*, 19′-hex-fucoxanthin/chlorophyll *a*, fucoxanthin/19′-hex-fucoxanthin and fucoxanthin/19′-but-fucoxanthin were used. Chlorophyll *b* and alloxanthin were not used for this analysis as they were often below the detection limit.

GC content

DNA sequences of Arctic picoplankton from published studies have previously been collated in a meta-study (Terrado et al. 2012). Haptophyte and mamiellophyte sequences were extracted from that meta-study and GC contents calculated.

Real-time qPCR

The amplification efficiencies of haptophyte and mamiellophyte 18S rDNA were compared in a real-time qPCR experiment. Plasmids containing inserts of haptophyte (HQ156821) or mamiellophyte (HQ156812) 18S rDNA obtained in a previous study of Arctic picoeukaryotes were used as templates (Sørensen et al. 2011). The primers 457f (5′-AAACSATGCCGACTAGGG-3′) and 529R (5′-TTTC AGCCTTGCGACCAT-3′), designed for this study, were used. They produce a 109-bp amplicon with a GC content of 49 and 47 % for the haptophyte and mamiellophyte plasmids, respectively (similar to the average GC content of Arctic picoplanktonic haptophytes and mamiellophytes, see results). As the primers are only intended for amplification of the same 18S region with different GC content for the two plasmids, their specificity was not tested. Plasmids were linearised (Hou et al. 2010) using the restriction enzyme FastDigest NotI (Thermo Scientific) following the manufacturer's protocol (using FastDigest buffer), and standard curves for each plasmid were created using two-fold dilution series, only using replicates with a Cq range $\leq$0.5.

The following PCR protocol was employed: 95 °C for 15 min, 40 cycles of 95 °C for 15 s, 52 C for 20 s and 72 °C for 20 s followed by a melt curve analysis from 72 to 95 C at 0.2 °C increments of 10 s. Experiments were run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

Statistical analyses and data collection

Statistical analyses were carried out in R (R Core Team 2013), and Datathief III version 1.6 (Tummers 2006) was used to extract data from the literature.

Results

Pigments

Fuco-algae were the primary contributors to picophytoplanktonic chlorophyll *a* (Fig. 1). However, haptophytes were also present. This community profile for the picophytoplankton extended until mid-May, at which time haptophytes replaced the fuco-algae. A similar community shift is indicated by the cluster analysis of pigment ratios where the samples from mid- and late May cluster separately from earlier dates (Fig. 2). Over the investigated period, fuco-algae contributed with 50 % of picoplankton chlorophyll *a*, haptophytes 40 % and green algae only 10 %.

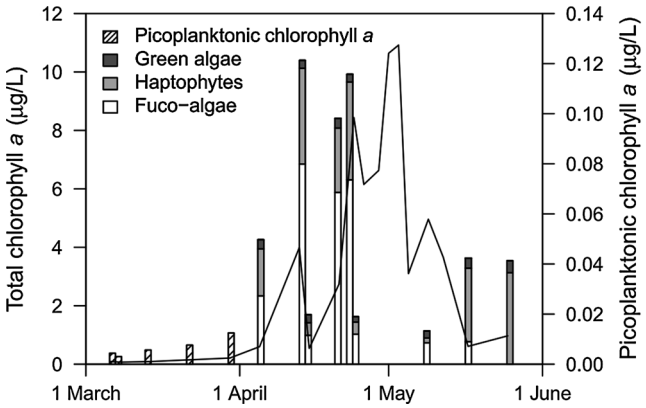

Fig. 1 Total chlorophyll *a* (*line*) and picoplanktonic chlorophyll *a* from different algal groups (*bars*) at 20 m depth. CHEMTAX analysis was not performed on samples from March due to very low pigment concentrations

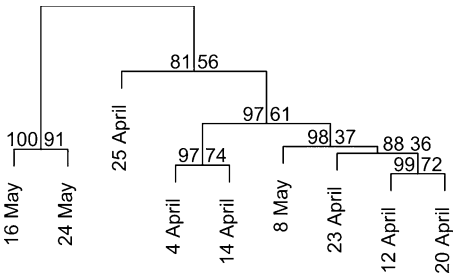

Fig. 2 Cluster analysis based on pigment ratios of samples from April to May. At each branch, approximately unbiased *p* values (*left*) and bootstrap probability values (*right*) are given

Picoplanktonic chlorophyll *a* showed a temporal pattern similar to that of total chlorophyll *a*, although with a smaller relative amplitude (Fig. 1): while total chlorophyll *a* varied by a factor of 20 during April and May, picoplanktonic chlorophyll *a* varied only ninefold. The variability of specific picophytoplankton groups was higher than that of bulk-picophytoplankton chlorophyll *a*: fuco-algae contributed from 0 to 70 % of picoplanktonic chlorophyll *a*, while haptophytes varied 21-fold during the same period.

GC content and real-time qPCR

The 49 % GC content of Arctic picoplanktonic haptophyte DNA sequences was significantly higher than that of Arctic picoplanktonic mamiellophyte DNA sequences (47 %, *t* test, $t = 9.65$, $df = 45$, $p < 0.0001$). The amplification efficiency of haptophytes (107 %) was not significantly lower than that of mamiellophytes (102 %). These amplification efficiencies were within the recommended limit of 90–110 % for the Touch Real-Time PCR Detection System (Taylor et al. 2009). For both mamiellophytes and haptophytes, the explained variation of the standard curve was $R^2 = 0.99$ and the melting points were practically identical between all runs (83.4–83.6 °C).

Discussion

Previous investigations of the spring bloom in the Disko Bay have mainly focused on the overall bloom dynamics of phytoplankton, i.e. total chlorophyll *a*, without quantitatively resolving the underlying diversity (Nielsen and Hansen 1995; Levinsen et al. 2000; Madsen et al. 2008). This study is unique as it focuses explicitly on picophytoplankton during the spring bloom in the coastal waters of Greenland. In addition, the use of HPLC-CHEMTAX allows determination of the taxonomic origin of picoplankton chlorophyll *a*. To date, only a few studies have investigated Arctic picoplankton using HPLC-CHEMTAX (Not et al. 2005; Lovejoy et al. 2007). In the current study, fuco-algae and haptophytes were found to dominate the picophytoplankton community before and during the spring bloom, while haptophytes dominated in the post-bloom phase.

Picophytoplankton succession in Disko Bay

Compared to total chlorophyll *a*, picoplankton-derived chlorophyll *a* varied less during the study period. Variability in total chlorophyll *a* is normally attributable to larger phytoplankton, while picophytoplankton contribute with a more or less stable background concentration; this pattern is seen both temporally and spatially in a range of marine habitats (e.g. Larsson and Hagström 1982; Magazzù et al. 1996; Wright et al. 2009).

Although chlorophyll *a* from picoplankton was relatively stable compared to that from larger algae, the contribution of specific picophytoplankton groups changed dramatically during the study (Fig. 1). The reduction of

picoplanktonic fuco-algae chlorophyll *a* in mid-May was partly offset by a relative increase in haptophytes; thus, succession kept picoplanktonic chlorophyll *a* at a relatively stable level. So although picoplanktonic chlorophyll *a* concentrations may appear to be relatively stable when compared to total chlorophyll *a*, the components of this community may exhibit temporal variation comparable to that of larger phytoplankton.

The results of a CHEMTAX analysis can be highly dependent on the input matrices used. Therefore, such results may not be reliable when regional data on the pigment ratios of phytoplankton groups are not available as is the case here. However, the observed change in community composition observed in late May by the CHEMTAX analysis (Fig. 1) was supported by the cluster analysis of pigment ratios as these two dates clustered separately (Fig. 2), indicating that their community composition was distinct.

Unexpectedly low abundance of picoplanktonic green algae

The finding of a low contribution of green algae to picoplankton chlorophyll *a* (10 %, Fig. 1) is in marked contrast to molecular studies of Arctic picoplankton in Kongsfjorden (Luo et al. 2009), Adventfjorden and Billefjorden (Sørensen et al. 2011), Franklin Bay (Terrado et al. 2011), the Beaufort and Bering Seas (Potvin and Lovejoy 2009; Balzano et al. 2012) and from the Canadian to the European Arctic (Lovejoy et al. 2006; 2007). In all these studies, mamiellophytes have been found to be dominant and widespread picophytoplankton.

In contrast, a study from the Barents, Greenland and Norwegian Seas using TSA-FISH epifluorescence microscopy indicated that *M. pusilla* only contributed 7 % of picoplankton chlorophyll *a*, despite its numerical dominance, due to its small size (Not et al. 2005). In addition, HPLC-CHEMTAX in the same study found mamiellophytes to contribute less to picoplankton chlorophyll *a* than both fucoxanthin-containing algae and haptophytes at all stations and depths. In another study, however, HPLC-CHEMTAX indicated that mamiellophytes contributed 55 % of picoplankton chlorophyll *a* in the Canadian Arctic (Lovejoy et al. 2007). It is currently difficult to conclude whether the abundance of mamiellophytes elsewhere in the Arctic is a reflection of a methodological bias associated with molecular methods or whether the composition of the picophytoplanktonic community of West Greenland is fundamentally different compared to other Arctic regions. In either case, mamiellophytes are unlikely to be as dominant throughout the entire Arctic as previously thought. This should be examined further by including molecular methods when studying samples from West Greenland.

The 19′-hex-fucoxanthin paradox

For clone libraries of Arctic picoplankton, mamiellophyte sequences are, on average, 23-fold (range 2–41) more abundant than haptophyte sequences (Potvin and Lovejoy 2009; Sørensen et al. 2011; Terrado et al. 2011). In comparison, the CHEMTAX analysis presented here indicates haptophytes to have an average contribution to picoplanktonic chlorophyll *a* 6 times greater than that of green algae. This paradox has previously been reported, as 19′-hex-fucoxanthin (a marker pigment for haptophytes) is ubiquitous in the world oceans, while haptophyte 18S rDNA is relatively rare (Liu et al. 2009). One suggestion that has been put forward to explain this discrepancy is that a high GC content of haptophyte 18S rDNA may cause amplification bias and underrepresentation of haptophyte DNA sequences in molecular studies (Liu et al. 2009; Marie et al. 2010).

In the current study, real-time qPCR did not indicate a decreased amplification efficiency of GC-rich (49 %) haptophyte rDNA compared to GC-poor (47 %) mamiellophyte rDNA. This is in agreement with other studies which did not find differences in amplification efficiencies for sequences with a GC content of 40–55 % (Benjamini and Speed 2012) and 11–56 % (Aird et al. 2011). However, a high GC content has been demonstrated to decrease amplification efficiency under certain circumstances: a study using real-time qPCR found amplification efficiencies of 94, 92 and 51 % for GC contents of 40, 44 and 53 %, respectively (McDowell et al. 1998). Likewise, a methodological study based on 454 amplicon sequencing of prokaryotic mock communities found decreased abundance (attributed to lowered amplification) of sequences with high GC content, working with GC contents of 51–65 % (Pinto and Raskin 2012).

Rather than a GC content derived amplification bias as an explanation for the discrepancy between molecular and pigment-based studies, differences in cell size between picoplanktonic mamiellophytes and haptophytes may provide at least a partial explanation: picomamiellophytes are typically small (down to 1 μm) compared to picohaptophytes (2–3 μm, Vaulot et al. 2008), giving the latter a volume up to 27 times larger. By comparison, there may be only a small difference in copy number, for example, the mamiellophytes *Ostreococcus tauri* (Derelle et al. 2006) and *M. pusilla* and the haptophyte, *Emiliania huxleyi,* (Zhu et al. 2005) have 18S rDNA copy numbers of 4, 4 and 3, respectively, despite *E. huxleyi* being the larger cell of the three. The result is a higher chlorophyll *a* content per 18S rDNA copy for picoplanktonic haptophytes when compared to mamiellophytes. This is consistent with a previously reported underrepresentation of haptophytes sequences in clone libraries compared to their biovolume

from artificially constructed samples (Amacher et al. 2011). This uncoupling of cell volume and 18S rDNA copy number may help explain the 19′-hex-fucoxanthin paradox. Thus, although mamiellophytes may be numerically dominant in a community, haptophytes can still be more abundant in terms of picophytoplanktonic biomass.

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