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Sea-ice eukaryotes of the Gulf of Finland, Baltic Sea, and evidence for herbivory on weakly shade-adapted ice algae

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Abstract

To determine community composition and physiological status of early spring sea-ice organisms, we collected sea-ice, slush and under-ice water samples from the Baltic Sea. We combined light microscopy, HPLC pigment analysis and pyrosequencing, and related the biomass and physiological status of sea-ice algae with the protistan community composition in a new way in the area. In terms of biomass, centric diatoms including a distinct *Melosira arctica* bloom in the upper intermediate section of the fast ice, dinoflagellates, euglenoids and the cyanobacterium *Aphanizomenon* sp. predominated in the sea-ice sections and unidentified flagellates in the slush. Based on pigment analyses, the ice-algal communities showed no adjusted photosynthetic pigment pools throughout the sea ice, and the bottom-ice communities were not shade-adapted. The sea ice included more characteristic phototrophic taxa (49%) than did slush (18%) and under-ice water (37%). Cercozoans and ciliates were the richest taxon groups, and the differences among the communities arose mainly from the various phagotrophic protistan taxa inhabiting the communities. The presence of pheophytin *a* coincided with an elevated ciliate biomass and read abundance in the drift ice and with a high *Eurytemora affinis* read abundance in the pack ice, indicating that ciliates and *Eurytemora affinis* were grazing on algae.

Keywords: 18S rRNA gene; Accessory pigments; Herbivory; Photoacclimation; Sea ice

Introduction

Sea ice is composed of solid ice and saline water called brine (Petrich and Eicken 2010). Brine lies and flows in pockets and interconnected channels within the sea ice, offering habitats for small-sized organisms. The diameter of the brine pockets and channels varies from 1 μm to several centimetres (Eicken et al. 1995), depending on the temperature and salinity of the parent water (Palosuo 1961; Petrich and Eicken 2010). The habitable space within the ice is substantially smaller at the low temperatures (≤10 °C) occurring during winter than at the near-zero temperatures of spring. In addition, the volume of the brine-channel system is considerably reduced in low-salinity seas such as the Baltic Sea

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(salinity range 3–10), compared with truly marine seas (salinity > 24). Due to the small size of the brine channels, the Baltic eukaryotic community consists mainly of protists, and the only notable metazoans present are rotifers and copepod nauplii (Kaartokallio 2004; Meiners et al. 2002; Norrman and Andersson 1994).

Knowledge of the taxonomy and ecology of Baltic Sea ice-algal communities has accumulated since the first studies were conducted (Hällfors and Niemi 1974; Häyren 1929; Hickel 1969; Huttunen and Niemi 1986; Niemi 1973), and it has been estimated that these algal communities contribute about 10% of the primary production during the ice-covered season (Haecky and Andersson 1999). Usually, the predominant autotrophic eukaryotes are diatoms (Haecky et al. 1998; Meiners et al. 2002; Norrman and Andersson 1994), but in contrast to Arctic sea ice, the dinoflagellate and green algal biomass is considerable in Baltic Sea ice (Kaartokallio et al. 2007; Piiparinen et al. 2010; Rintala et al. 2010). Another peculiarity of Baltic Sea ice is that the surface-layer algal biomass may significantly contribute to the overall algal biomass (Meiners et al. 2002; Piiparinen and Kuosa 2011; Piiparinen et al. 2010).

The heterotrophic compartment of the eukaryotic community in Baltic Sea ice is less well known; previous studies have not included detailed identification of heterotrophic protists, with the exception of publications by Vørs (1992), Ikävalko and Thomsen (1996, 1997) and Ikävalko (1998). The lack of detailed species identification is not due to indolence on the part of these early investigators, but rather that many species cannot be identified with light microscopy (LM) (e.g. Lowe et al. 2011). The same issue also holds for the smaller (<10 μm) autotrophic flagellated eukaryotes. These challenges to identification may be disentangled, using elaborate electron microscopy techniques (e.g. Vørs 1992), but also more indirectly by analysing pigments, using high-performance liquid chromatography (HPLC) (Bidigare et al. 2005) or more cost-effectively and thoroughly by molecular methods (Logares et al. 2012).

Pigment analyses have been routinely used in phytoplankton research (Jeffrey et al. 2011) and to some extent in sea-ice research (Alou-Font et al. 2013; Kudoh et al. 2003), but not yet in research on Baltic Sea ice algae. Identifying algal taxa based on pigment data is not straightforward, since many pigments are found in several algal groups (e.g. fucoxanthin in diatoms, haptophytes and chrysophytes), and at the very best, taxa can be identified to genus level (Zapata et al. 2004) but usually to class level (Jeffrey et al. 2011). In addition, the downward-attenuating light conditions in the sea-ice column strongly affect cellular pigment composition (Alou-Font et al. 2013) and algae acclimate to changing light climates by adjusting their pigment pool. In the case of light-harvesting chlorophylls and carotenoids, this regulation occurs on a time scale of hours, and in photoprotective xanthophyll-cycle pigments from 1 to several hours (Claustre et al. 1994; Moline 1998). Hence, the ratios of various accessory pigments to chlorophyll *a* (chl-*a*) and photosynthetic carotenoids (PSCs) to photoprotective carotenoids (PPCs) are widely used indicators of photoacclimation in algae (e.g. Alou-Font et al. 2013; Arrigo et al. 2014). In addition, during the senescence and death of the cells, the chl-*a* synthesized by algae undergoes degradation to a variety of chl-*a* derivatives, e.g. pheophytin *a*, and thus the presence of pheophytin *a* can be used as an indicator of cell senescence and grazing (Louda et al. 1998; Prins et al. 1991; Strom 1993).

DNA-based approaches have proven to be useful, e.g. for detecting ciliates and flagellates that are difficult to distinguish under LM, and have revealed that heterotrophic protistan taxon richness is higher in sea ice than observed by microscopy (Comeau et al. 2013; Majaneva et al. 2012). As in pigment analysis, DNA sequencing has its own limitations; e.g. taxa are not identified to species level, but the 18S ribosomal RNA (rRNA) gene is used as a proxy for species. The level to which individual taxa can be identified is variable and may be restrained by imperfect reference databases and lineage-specific evolutionary rates in the 18S rRNA gene (Caron et al. 2009). The number of 18S rRNA gene copies per cell also varies from one to tens of thousands among different eukaryotes (e.g. Zhu et al. 2005), resulting in values that represent not the cellular abundance but the number of 18S rRNA gene copies in the sample. At the same time, no other method can identify all eukaryotic micro-organisms, including cryptic species (Lowe et al. 2011), with the same precision and efficiency as sequencing. Consequently, molecular methods are sovereign tools in differentiating protistan communities (e.g. Comeau et al. 2013).

Here, our aim was to relate the biomass and physiological status of sea-ice algae to the protistan community composition in the Gulf of Finland, Baltic Sea. First, we determined the pigment composition of the sea-ice samples, using HPLC to measure the response of the algae to downward-attenuating light conditions. Secondly, we enumerated the dominant taxa and their biomass, using LM. Thirdly, we pyrosequenced the partial 18S rRNA genes of eukaryotes to identify the eukaryotic taxa present in the samples.

Material and Methods

Sampling

We collected 20 samples (15 sea-ice, 3 slush and 2 under-ice water samples) from three research vessel (R/V) Aranda sea-ice cruise stations (Gulf of Finland, Baltic Sea, 8–19 March, 2010): a drift-ice station on 9 March (59°55.67′ 26°01.08′), a heavily packed fast-ice station on 11 March (60°14.30′ 26°37.56′) and a level fast-ice station on 13 March (60°19.66′ 26°51.73′; Supplementary Fig. S1 in the online version at DOI: 10.1016/j.ejop.2016.10.005).

We collected the ice samples with a motorized Cold Regions Research and Engineering Laboratory (CRREL)-type ice-coring auger (9 cm internal diameter, Kovacs Enterprises LLC, Roseburg, OR, USA). We obtained