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Parallel assessment of marine autotrophic picoplankton using flow cytometry and chemotaxonomy

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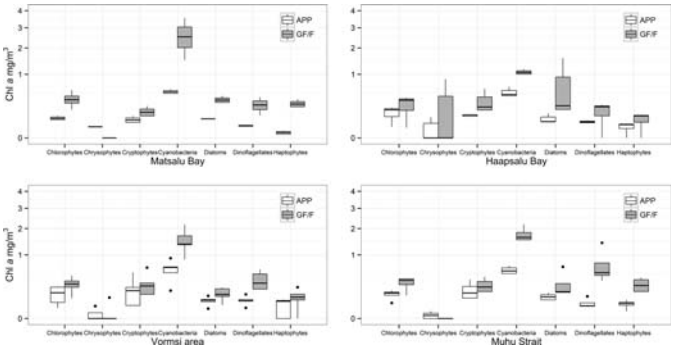
HIGHLIGHTS

- Autotrophic picoplankton was analyzed with CHEMTAX and flow cytometry.
- The study covers area of the West-Estonian Archipelago Sea (Baltic Sea).
- Picoplankton is a significant primary producer in eutrophic coastal areas.
- Picoeukaryotes play an important role in the coastal areas.
- The spatial variability of picoplankton can be considerable even in small regions.

GRAPHICAL ABSTRACT



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ABSTRACT

Autotrophic picoplankton (0.2–2 μm) can be a significant contributor to primary production and hence play an important role in carbon flow. The phytoplankton community structure in the Baltic Sea is very region specific and the understanding of the composition and dynamics of pico-size phytoplankton is generally poor. The main objective of this study was to determine the contribution of picoeukaryotic algae and their taxonomic composition in late summer phytoplankton community of the West-Estonian Archipelago Sea. We found that about 20% of total chlorophyll *a* (Chl *a*) in this area belongs to autotrophic picoplankton. With increasing total Chl *a*, the Chl *a* of autotrophic picoplankton increased while its contribution in total Chl *a* decreased. Picoeukaryotes play an important role in the coastal area of the Baltic Sea where they constituted around 50% of the total autotrophic picoplankton biomass. The most abundant groups of picoeukaryotic algae were cryptophytes (16%), chlorophytes (13%) and diatoms (9%). Picocyanobacteria were clearly dominated by phycoerythrin containing *Synechococcus*. The parallel use of different assessment methods (CHEMTAX and flow cytometry) revealed the share of eukaryotic and prokaryotic part of autotrophic picoplankton.

1. Introduction

Autotrophic picoplankton (APP) is the smallest fraction of phytoplankton, ranging in size from 0.2 to 2 μm (Sieburth et al., 1978). APP consists of the prokaryotic (picocyanobacteria) and eukaryotic component. The importance of APP was underestimated for a long time but it is now evident that APP can be a considerable contributor to primary production and hence play an important role in carbon flow (Kuosa, 1991; Fogg, 1995; Morán, 2007; Gaulke et al., 2010). APP dominates the autotrophic phytoplankton biomass in most of the world's oceans (Stockner, 1988; Fogg, 1995; Marañón et al., 2001). In oligotrophic

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lakes and oceans the input of APP to the total primary production may reach even 50–90% (Caron et al., 1985; Goericke and Montoya, 1998). In eutrophic conditions the relative contribution of APP to total phytoplankton biomass generally declines (Bell and Kalff, 2001). Picocyanobacteria also constitute an important part of the microbial loop where dissolved organic matter is returned into the food web (Azam et al., 1983; Kuosa, 1991; Worden et al., 2004; Pomeroy et al., 2007).

Being one of the world's largest brackish water basins, the Baltic Sea is exposed to strong anthropogenic pressure. It is one of the most polluted seas worldwide and the long retention time (approximately 25 years) exacerbates many problems that are brought about by organic pollutants and eutrophication. Human-induced climate change is also expected to have a significant impact on the Baltic Sea and the first warming trend is already apparent (Andersson et al., 2015). The large range of salinity (from <1 to ~25 psu) is an important characteristic of the Baltic Sea determining the distribution of phytoplankton species. The projected increase in precipitation resulting in bigger freshwater inflow may decrease the salinity in many areas of the Baltic Sea (Graham, 2004; Kjellström and Ruosteenoja, 2007). Such events have a direct effect on the phytoplankton community leading to changes in the foodweb and hence, the production at higher trophic levels.

The overall phytoplankton community in the Baltic Sea is region specific with no common trends found across all sub-regions (Klais et al., 2011; Wasmund et al., 2011; Klais et al., 2013). The trends in the different regions of the Baltic Sea may even be opposite (Wasmund et al., 2011). Therefore the regional scale studies contribute to revealing the patterns and dynamics of phytoplankton in changing environmental conditions in the Baltic Sea.

During extensive studies of the Baltic Sea, relatively good knowledge has been accumulated on larger phytoplankton. However, the understanding about the composition and dynamics of pico-size phytoplankton is still poor and the focus there tends to be on pico-cyanobacteria. Since the stability of aquatic ecosystems is unquestionably affected by APP, the planktonic microbial communities merit particular attention to improve the understanding of how communities and ecosystems respond to the changing climate (Sánchez-Baracaldo et al., 2008).

Until recently, picocyanobacteria have received more attention than the eukaryotic picoplankton since they have been easier to identify. While the understanding of the significance of picoeukaryotes has improved considerably, most of the data available is still at bulk biomass level. Since epifluorescence microscopy and flow cytometry are deficient in taxonomic resolution, newer molecular and chemotaxonomic methods are starting to fill the knowledge gap in the phylogenetic diversity of picoeukaryotes (Ansotegui et al., 2003; Lovejoy et al., 2006; Not et al., 2007; Massana, 2011; Hugerth et al., 2014).

An interesting hypothesis involving picocyanobacteria in the Baltic Sea proposes that diazotrophic cyanobacterial blooms in the Baltic Sea are controlled by picocyanobacteria that exhaust the formers' nutrient pools (Stal et al., 2003). For further understanding of the controlling mechanisms of cyanobacterial blooms, the information about APP might be more important than we realize now.

This study aims to (1) describe the structure and abundance of late summer APP community in the West-Estonian Archipelago Sea (further denoted by its Estonian name Väinameri for brevity); (2) determine the taxonomic composition of picoeukaryotes and their contribution to the total APP; (3) analyse the agreement between pigment-based chemotaxonomy and flow cytometry in determining the composition of APP.

2. Methods

2.1. Study site and sampling

Sampling stations ($n = 14$) were situated in the Väinameri area (Fig. 1) remaining between the continental Estonia and its western islands in the north-eastern Baltic Sea. This sea area is generally shallow (average depth < 10 m) and the seafloor consists mostly of soft sediments such as mud and sand. Some of the sampling points were located in shallow (average depth 1.5–2 m) and moderately eutrophied bays of Haapsalu and Matsalu which wedge far into the land. Salinity of the region is variable depending on the season and location with higher values occurring in the bay mouth (~5–6.5 psu) and dropping further inside the bays (2.5–4 psu in Haapsalu and down to 0.5 psu in Matsalu) (Kotta et al., 2008). The phytoplankton biomass peaks in late summer when the chlorophyll *a* (Chl *a*) values can reach 17 μg Chl *a* L^{-1} in Haapsalu Bay and 6 μg Chl *a* L^{-1} in Matsalu Bay. The phytoplankton community in Haapsalu Bay has no clear dominant group during summer and different diatom or cyanobacterial species can dominate. In Matsalu bay the abundance of cyanobacteria grows towards the bay mouth with most abundant species being *Pseudanabaena limnetica*, *Merismopedia punctata*, *Aphanizomenon flos-aquae*, and *Nodularia spumigena* (Jaanus, 2003; Kotta et al., 2008).

Sampling took place in July 2013. The surface water samples were collected from the research vessel and kept in a fridge until further onshore processing a few hours later. Secchi depth was measured in all sampling points. Subsamples for spectrophotometric measurements, flow cytometry, and high-performance liquid chromatography (HPLC) were taken from the water sample in the laboratory as soon as possible after the initial water collection.

2.2. Pigment extraction and HPLC analysis

For HPLC measurements of pigment content and composition, seawater samples were vacuum filtered through 47-mm Whatman GF/F (0.7 μm pore size) filters. For the picoplanktonic fraction seawater was vacuum filtered sequentially through the following 47-mm diameter filters with decreasing pore size: 8 μm, 2 μm (both Whatman Nuclepore) and 0.2 μm (Whatman nylon membrane filters). Further we denote the GF/F results as the "conventional" pigment content as this type of filter is used in most standard protocols for pigment analysis (e.g. Chavez et al., 1995; Aminot and Rey, 2002), opposed to the "total" pigment content, i.e. the sum of the results of fractionation filtering that comprises also the 0.2–0.7 μm fraction.

All filters were placed in 5-mL plastic vials, frozen immediately and stored at −70 °C until analysis. Pigments were extracted with acetone containing an internal standard (2 mL) and sonicated (Branson 1210) for 5 min. Samples were kept in dark at −20 °C for 24 h. Extracts were filtered through 0.45 μm syringe filters (Millex LCR, Millipore) before HPLC analysis.

Reversed-phase HPLC (Shimadzu Prominence, Japan) was used with a photodiode-array (PDA) and fluorescence (ex 440 nm, em 660 nm) detectors for the separation of the phytoplankton pigments. The latter assured correct measurements of Chl *a* even at low concentrations. 0.5 M ammonium acetate (ion-pairing reagent) was added in a volume ratio of 2:3 to each sample before the injection. The autosampler was cooled to +5 °C to prevent chemical decomposition of the pigments (Reuss and Conley, 2005). Pigment separations were carried out in a reversed-phase mode by using two Waters Spherisorb ODS2 3 μm columns (150 mm × 4.6 mm I.D.) in-line with a pre-column (10 mm × 5 mm I.D.). A binary gradient elution method was used (isocratic holds 0–2 and 30–43 min) with a constant flow rate (0.8 mL/min) throughout the elution. Absorbance was detected from 350 to 700 nm. Data analysis was done with the 'LC solution ver. 1.22' (Shimadzu) software. For peak identification and quantification external standards (DHI, Denmark) were used. In respect of accuracy the HPLC Chl *a* data was validated with both spectrophotometric Chl *a* and microscopy biomass data.

2.3. Chemotaxonomic analysis

HPLC pigment data was further used to estimate the algal class abundance with matrix factorization program CHEMTAX (version 1.95).