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Method paper

Winter picoplankton diversity in an oligotrophic marginal sea

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ABSTRACT

Marine picoplankton, unicellular organisms with cell sizes up to 3 µm in diameter, numerically dominate marine ecosystems, encompassing Archaea, Bacteria, Eukarya (protists and fungi) as well as viruses. Autotrophic and heterotrophic picoplankton abundance and community composition with a focus on picoeukaryotes (PEs) were investigated in the winter of 2016 at three stations along a coast-to-offshore transect in the southern Adriatic Sea. Abundances were estimated by flow cytometry, while community composition by Illumina High-Throughput Sequencing (HTS) of 16S and 18S rRNA genes. The photosynthetic picoplankton diversity was also investigated by High-Performance Liquid Chromatography (HPLC) of liposoluble pigments. Heterotrophic bacteria and cyanobacteria (*Prochlorococcus* and *Synechococcus*) accounted for up to 7×10^5; 2.3×10^4 and 2.5×10^4 cells mL^{-1}, respectively, while photosynthetic picoeukaryotes peaked with 3×10^3 cells mL^{-1}. Prokaryotes, as revealed by HTS were dominated by *Alphaproteobacteria* (mainly SAR11, 44.91% of total 16S sequence reads), followed by *Gammaproteobacteria* (*Oceanospirillales* and *Pseudomonadales*, 14.96%), *Bacteroidetes* (mainly *Flavobacteriales*, 13%), *Cyanobacteria* (*Prochlorococcus* and *Synechococcus*, 9.52%), *Marinimicrobia* (SAR406, 7.97%), *Deltaproteobacteria* (SAR324, 3.83%), *Actinobacteria* (2.24%) and *Chloroflexi* (SAR202, 1.90%). Photosynthetic pigment concentrations were very low (12.12 μgL^{-1} at the most) and taxonomic pigments could be attributed to *Prochlorococcus*, *Synechococcus*, Prymnesiophyceae, Bacillariophyceae, Chrysophyceae, and Prasinophyceae. HTS data revealed that PEs were dominated by heterotrophs, such as Syndiniophyceae, parasitic dinoflagellates (79.67% of total 18S sequence reads), Dinophyceae (8.7%) and the radiolarians Collodaria belonging to Sphaerozoidae (22.1%) and Spumellaria (5.0%). On the other hand, photoautotrophs, including Chlorophyta (Mamiellophyceae, Prasinophyceae, Trebouxiophyceae, and Ulvophyceae), Stramenopiles (Bacillariophyta, Chrysophyceae, Dictyochophyceae, Pelagophyceae), photoautotrophic Cryptophyta and some Haptophyta (Prymnesiophyceae), did not exceed 5% of total sequence reads. This study provides the first snapshot of the PEs diversity in oligotrophic euphotic waters of the southern Adriatic Sea, hence setting the stage for large-scale surveying and characterization of the eukaryotic diversity in the entire basin.

1. Introduction

Marine picoplankton, unicellular organisms with cell sizes up to 3 µm in Equivalent Spherical Diameter (ESD) dominate most marine ecosystems both numerically and in terms of carbon biomass, encompassing Archaea, Bacteria, Eukarya (protists and fungi) as well as viruses (Pomeroy et al., 2007). Picoplankton is heterotrophic (mainly Archaea and Bacteria) and photosynthetic, the latter including both cyanobacteria (*Prochlorococcus* and *Synechococcus)* and picoeukaryotes (PEs), mainly dominated by prymnesiophytes (Jardillier et al., 2010). PEs include photoautotrophic, mixotrophic and heterotrophic protists, and are an important and mostly overlooked component of marine ecosystems, which under certain conditions can represent the main drivers of plankton ecosystems (as producers, decomposers, parasites, symbionts, etc.) (Li, 1994; Biegala et al., 2003; Not et al., 2004).

Recent global expeditions such as Malaspina, *Tara* Oceans and Biosope (Grob et al., 2007; Claustre et al., 2008; Shi et al., 2009; Pernice et al., 2015; Duarte, 2015; De Vargas et al., 2015; Bork et al., 2015) have explored the world's oceans using genomic approaches, and have shed light on the huge and still unknown diversity of marine microbes and of their adaptation to environmental conditions and climate zones. These studies showed that among the nanoplankton and microplankton, diatoms and dinoflagellates dominate communities, with three groups: Alveolata, Rhizaria, and Excavata (De Vargas et al.,

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