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Applicability of massively parallel sequencing on monitoring harmful algae at Varna Bay in the Black Sea

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ABSTRACT

In this study the plankton diversity in 13 environmental samples from Varna Bay (in the western Black Sea) was analyzed using massively parallel sequencing (MPS). This preliminary study was undertaken to assess the potential of this technology for future implementation in monitoring programs in the Black Sea. Amplicon sequences of the 18S rRNA gene (V4-5 regions) were obtained using the Illumina MiSeq 250PE platform. A total of 1137 operational taxonomic units (OTUs) were obtained among which 242 OTUs with >0.990 BLAST top hit similarity (21.3% of all detected OTUs) closely related to sequences belonging to −protists. A large portion (175 OTUs = 72.3%) was identified at the species levels, including species typical for the Bulgarian Black Sea plankton community, as well as many that haven't been reported earlier in the Bulgarian Black Sea coast (124 OTUs = 51.2%). Dinoflagellates were represented by the highest species number (77 OTUs comprising 31.8% of protist species), with dominant genera *Gyrodinium* and *Heterocapsa*. The present survey revealed the presence of 12 species listed as harmful, some of which have been previously overlooked, such as *Cochlodinium polykrikoides*, *Karenia bicuneiformis*, and *Karlodinium veneficum*. Species identification was possible for 10.3–36.0% of the detected OTUs in the six major supergroups. The frequency in Rhizaria was significantly lower than that in other major groups ($p < 0.05$–0.01), implying difficulties in the classification from morphology-based observations. The metagenetic data had an insufficient resolution of the 18S rRNA gene for species identification in many genera. These issues may hamper the implementation of MPS-based surveys for plankton monitoring, especially for detecting harmful algal blooms (HAB). The sequencing technology is steadily improving and it is expected that sequence length and quality issues will be resolved in the near future. The ongoing efforts to register taxonomic information and quality controls in the international nucleotide sequence databases (INSDs) will be essential for improving taxonomic identification power.

1. Introduction

The Black Sea is an unique marine environment. Challenging physiological conditions for marine species are the result of a high degree of isolation from the world ocean, an extensive drainage basin, strong vertical stratification, low salinity, and the presence of H_2S below depths of 150–200 m, leading to low species diversity as compared to other basins within the world ocean (Zaitsev and Mamaev, 1997). Having been strongly affected by eutrophication in the past (Mee, 1992; Zaitsev, 1993), the Black Sea ecological state requires rigorous monitoring.

Phytoplankton is a sensitive indicator of ecosystem perturbations (Beaugrand, 2005; Hays et al., 2005) and is used as a Biological Quality Element (BQE) for assessment of the ecological status of marine ecosystems (the Water Framework Directive, WFD, 2000/60/EC, and the Marine Strategy Framework Directive, MSFD, 2008/56/EC), including the Black Sea. Among the over 1600 phytoplankton species that live in the Black Sea (Black Sea

Abbreviations: HAB, harmful algal blooms; INSD, international nucleotide sequence databases; MPS, massively parallel sequencing; OTUs, operational taxonomic units.

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phytoplankton check-list in Moncheva and Parr, 2015), about 49 have been reported as toxic or harmful in other locations, and new invasive species are continuously reported (Zaitsev and Ozturk, 2001; Moncheva and Kamburska, 2002; Yasakova, 2011). Furthermore, phytoplankton blooms are considered a key issue affecting the health of the Black Sea ecosystem (Moncheva et al., 1995, 2001; Velikova et al., 1999; Nesterova et al., 2008 and references therein)., Our knowledge of marine phytoplankton biodiversity is limited by methodological constraints of traditional species identification techniques (Finlay, 2004; Cerme&o et al., 2013; Rodrıguez-Ramos et al., 2014; Massana et al., 2015), which could lead to underestimation of microbial species diversity in the examined environments. During the last decade, molecular surveys have become instrumental in decoding microbial diversity for improving our understanding of marine protists (Logares et al., 2014; Massana et al., 2015). The Roche 454 pyrosequencing and Illumina MiSeq have become popular methods for conducting studies on microbial diversity and species richness in marine and freshwater ecosystems, owing to the recent development of massively parallel sequencing (MPS) technologies. The significant advantage of applying MPS-based technology for monitoring biodiversity is the great potential for more precise species identification based on genetic information, especially species that are indistinguishable by traditional morphology-based microscopic observation. This new technology delivers high-throughput performance and allows the detection of several hundreds of operational taxonomic units (OTUs) from marine and freshwater ecosystems (Cheung et al., 2010; Medinger et al., 2010; Nolte et al., 2010; Edgcomb et al., 2011; Monchy et al., 2012; Chaput et al., 2015; Egge et al., 2015a, 2015b; Majaneva et al., 2015; Tanabe et al., 2015; Nagai et al., 2016a, 2016b, 2016c).

Application of MPS-based surveys to study protist biodiversity in the Black Sea is limited (Coolen et al., 2013; Massana et al., 2015) and molecular eukaryotic diversity remains poorly explored (Coolen and Shtereva, 2009). In this study, the phytoplankton diversity in 13 field samples from Varna Bay (in the western Black Sea) was analyzed using an MPS-based technique. This preliminary study was performed to evaluate the potential of this technology for future implementation in monitoring programs in the Black Sea.

2. Materials and methods

2.1. Sampling and DNA extraction

Seawater samples were collected between December 2013 and September 2015 in Varna Bay (43.184174°, 27.898766°) of the Bulgarian Black Sea coast (Fig. 1). Samples were taken from the surface water layer using a plastic bucket, and 13 samples were analyzed in this study (Table 1). To trap the plankton cells from the samples, 350–1000 mL until the filter clogs of the seawater was filtered through 3-μm cellulose nitrate membrane filters (Whatman, Dassel, Germany, Thermo Fisher Scientific). The filters were stored in liquid nitrogen until use. For DNA extraction, the frozen filters were thawed and incubated in a DNA extraction buffer with 10 mM Tris.HCl (pH 8.0); 1 mM EDTA (pH 8.0), sodium dodecyl sulfate (final concentration 0.5%), and proteinase K (final concentration, 100 $\mu g\,mL^{-1}$) at 37 °C for 60 min. After that 5 M NaCl and CTAB/NaCl were added to lysates and incubated at 65 °C for 10 min. Nucleic acids were extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with isopropanol. The residual CTAB was removed with 70% ethanol. The precipitated DNA was dissolved in 50 μL of Milli-Q water and used as the template DNA.

2.2. Paired-end library preparation and MiSeq sequencing

To carry out metagenetic analysis using the MiSeq 250PE platform (Illumina, USA), a set of primer pairs for the 18S-rRNA

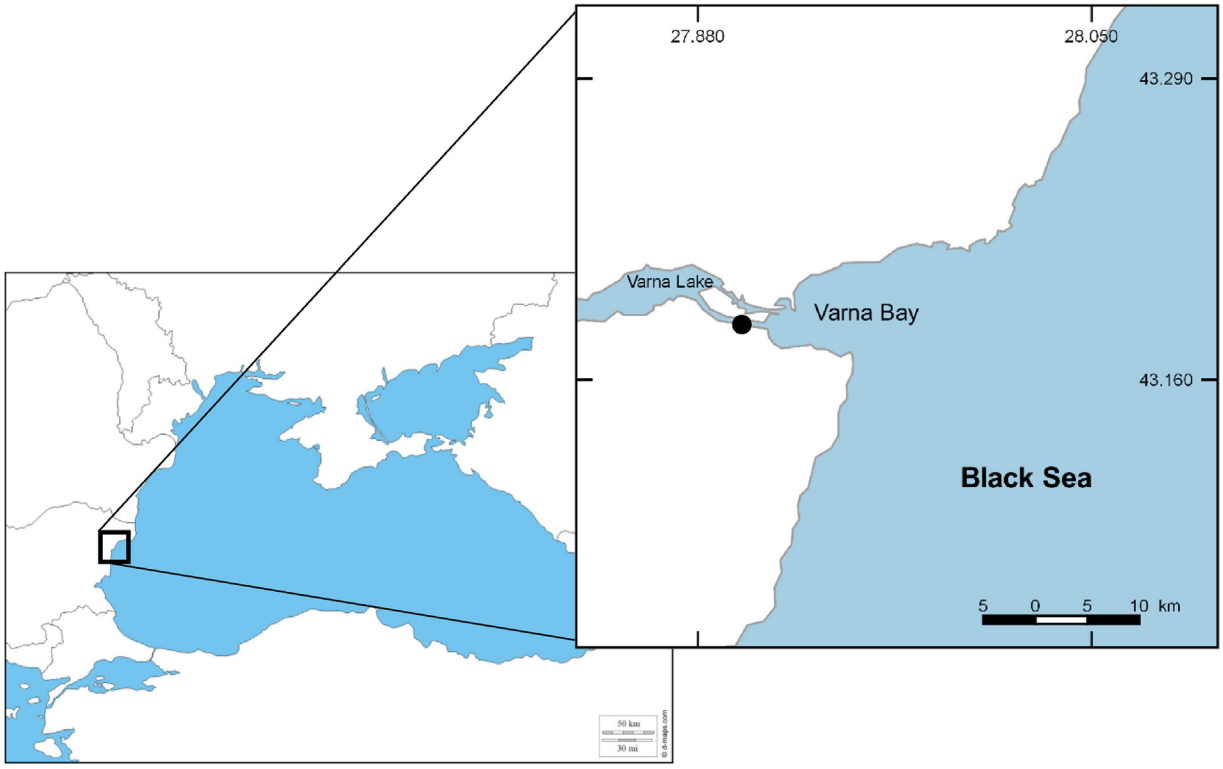

Fig. 1. Sampling location in Varna bay, Black Sea, Bulgaria.