

Conference Abstract

Consistency between high throughput sequencing and microscopy-based morphological characterization of phytoplankton communities

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Abstract

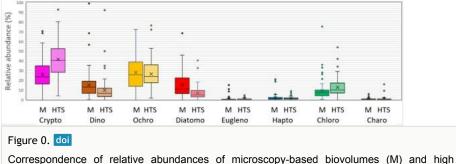
Thus far, the phytoplankton community composition analyses, used e.g. monitoring and assessment of the ecological status of water bodies, are based on time-consuming and expertise-demanding light microscopy analyses. Currently, DNA-based molecular tools are being developed to replace microscopy-based analyses. Although most of the DNA is expected to be found in living cells, long-lasting DNA in damaged cells or soluble extracellular or relic DNA can make up a large proportion of the total DNA in water samples. DNA-based phytoplankton analysis has been shown to be affected by the huge variation in the copy number of the rRNA gene between phytoplankton species (Mäki et al. 2017). On the contrary, RNA is present only in living organisms and reflects active protein synthesis. Therefore, RNA-based methods can provide a more reliable estimate of community composition.

The large copy number variation of ribosomal genes and the absence of universal primers for simultaneous amplification of cyanobacterial and eukaryotic phytoplankton genes hinder the application of DNA-based molecular methods. We applied a directional random-primed high throughput sequencing (HTS) approach (Mäki and Tiirola 2018) to analyse 16S and 18S rRNA community structures of 83 boreal lakes in Finland, Northern Europe.

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With the method it was possible to simultaneously amplify all groups of aquatic microorganisms in addition to cyanobacteria and eukaryotic phytoplankton.

A comparison between microscopy and HTS showed that the relative phylum (Fig. 0) and class level abundances of eukaryotic phytoplankton and order level abundances of cyanobacteria were consistent between the methods, despite that the HTS method overestimated the relative abundance of cyanobacteria. However, correspondence was low at genus and species level, mainly due to the lack of reference library sequences. HTS revealed more genera and was able to differentiate cryptic genera lacking morphological characteristics, but microscopy revealed a longer list of species (Vuorio et al. 2020).



throughput sequencing -based 18S rRNA gene sequences (HTS) of eukaryotic phytoplankton phyla in water samples of 83 lakes.

The RNA-based method applied showed potential, but it is not yet able to replace microscopy, mainly due to the lack of full length 16S and 18S sequences in the reference libraries. The main advantage of the method is that it is not limited to phytoplankton, but can be applied to simultaneous investigation of the total composition of microbes, including all bacteria, protists, ciliates, rotifers and zooplankton.

Keywords

RNA, DNA, phytoplankton, HTS, microscopy

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