

## From sediment to bloom: spatial patterns of algal resting stages and harmful algal bloom (HAB) risk in the Baltic Sea

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Besides Cyanobacteria, harmful algal blooms (HABs) of Eukaryotes are becoming increasingly prevalent in the Baltic Sea, altering phytoplankton diversity and negatively impacting higher trophic levels. Many HAB species form resting stages which may seed future blooms. To assess the bloom potential of HABs in the Baltic Sea and identify risk areas, we analysed spatial distribution patterns of resting stages in the sediment. Using a modified DNA extraction protocol, sediment samples from the German Bight and the Gulf of Finland enabled selective 18S rRNA gene metabarcoding of resting stages. These data highlighted strong spatial differences between western and eastern Baltic Sea sediments, but also regional differentiation potentially due to freshwater inflow. German sediments showed higher Shannon and Simpson diversities, with a presence of up to 40 % green algae sequences. Finnish sites were dominated by diatom and dinoflagellate resting stages, while the latter showed the highest relative presence offshore. HAB species were more frequently sequenced at German stations, indicating invasion of toxic algae into the Baltic Sea. To explore dispersal patterns of algal cells, we use numerical particle tracking simulations that will help identify risk areas of HAB development and promote a better understanding of HAB dynamics in the Baltic Sea.

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## Size does matter: tiny microbes slipping out 0.2 µm pores may alter standard biodiversity assessment

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In the last decades, advances in sequencing technologies have enabled a better understanding of prokaryotic dynamics in the Ocean by providing unprecedented details about their biodiversity. In amplicon sequencing studies of planktonic prokaryotes, while we are still far from a global consensus protocol regarding target regions (e.g. choice of primers) and approach (long vs. short reads), biomass collection using 0.2 µm pore membranes for DNA extraction is an undisputed standard procedure. In this study, we challenged this dogma by analyzing the 0.2 µm filtrate of samples collected monthly over two years at a coastal station in the northern Adriatic Sea. Surprisingly, about 2.5% of the total free-living community escaped the standard 0.2 µm pores. The biodiversity of small microbes was limited, with the most abundant

eight Amplicon Sequence Variants accounting for 75% of the total, on average. These were made up of well-known small bacteria such as SAR11 clade I and II and C. Actinomarina as well as by members of the mostly uncultured NS5 group of the Flavobacteriaceae and Marine Group II archaea. Overall, our results suggest caution in the use of 0.2 µm pore membranes for the study of these taxa and for rare biosphere-oriented investigations.

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## Comamonadaceae and Flavobacterium as bioindicators of River Run-Off in European Coastal Waters

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River run-off influences marine coastal ecosystems by discharging nutrients, sediments, contaminants, and microorganisms into the sea. Understanding the consequences of this advection is crucial for ecosystem monitoring and management. Environmental DNA (eDNA) can contribute to better describing microbial communities, and therefore it was used here to trace freshwater-origin bacteria diluted in marine waters, in order to define bioindicators of river run-offs. As part of the EU-OBAMA-NEXT project, this study combined data from the English Channel, Bay of Biscay, and Mediterranean Sea, encompassing river-influenced (mixed) to marine areas. Bacterial communities were analysed by sequencing V4-V5 16S rDNA amplicons. Indicator Species Analysis (IndVal), Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC), and Threshold Indicator Taxa ANalysis (TITAN) enabled the identification of key taxa. Comamonadaceae and Flavobacterium showed a strong statistical correlation with freshwater influence at all sites, beyond local ecosystem variability and geographical boundaries. These findings highlight the tolerance of these bacterial groups in tolerating salinity gradients and demonstrate the effectiveness of eDNA for high-resolution marine microbial ecology studies. The results support the development of a standardized European framework for monitoring freshwater inputs and improving the management and protection of coastal ecosystems.

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