

# Ecology of bacterioplankton inhabiting oxygenated hypolimnia of deep freshwater lakes

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In marine and freshwater systems, there are  $10^4$ – $10^7$  cells mL<sup>-1</sup> of bacterioplankton in the water column, playing central roles in the microbial food web and biogeochemical cycling. In oligo-mesotrophic deep freshwater lakes, such as Lake Biwa, Baikal and The Laurentian Great Lakes, the hypolimnion is a dark, cold, aerobic water layer below the thermocline that accounts for a majority of the lake volume. Analogous to the deep ocean, the oxygenated hypolimnion is a realm for important biogeochemical processes, such as accumulation and decomposition of semilabile organic matter, nitrification, and methane oxidation. However, our knowledge for the diversity and ecology of the bacterioplankton in the oxygenated hypolimnion is still limited and even poorer than that in the deep ocean. To fill this gap, the present study comprehensively investigated the bacterioplankton community composition in the oxygenated hypolimnia of deep freshwater lakes.

To obtain data from lakes with varying depth, surface area, water temperature, trophic state, pH, lake origin and water retention time, 13 Japanese lakes and 7 European perialpine lakes have been sampled so far. The waters were collected vertically at the deepest point in each lake and the bacterial community composition was determined by 16S rRNA gene amplicon sequencing and catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH). The result revealed that the oxygenated hypolimnion is inhabited by hypolimnion-specific bacterioplankton lineages that were phylogenetically different by phylum-level from commonly-known freshwater bacterioplankton lineages dominating in epilimnia. While most of the hypolimnion-specific lineages were uncultured and thus their eco-physiology was not well understood, the fact that they commonly predominate in global lakes suggests their importance in the microbial food web and biogeochemical cycling in deep freshwater lakes.

Among the hypolimnion-specific lineages, the CL500-11 lineage (phylum Chloroflexi) is particularly abundant, accounting for more than 10% of bacterioplankton cells in the hypolimnion in many lakes. CARD-FISH analysis revealed their relatively large cell size (1–2  $\mu$ m), suggesting their contribution in biovolume is even greater. Together with the information collected from literatures and sequence databases, the results demonstrated that CL500-11 inhabit most freshwater lakes with the oxygenated hypolimnion, although their low abundances were observed in lakes with low pH, shallow depth, or short water retention time (<1 year). The results as well as metagenomic and geochemical insights from published studies allowed to hypothesize possible characteristics of substrates supporting CL500-11 growth, most likely nitrogen-rich autochthonous dissolved organic matter (DOM) ubiquitously available in the hypolimnion that may turnover within an annual timescale; for instance, DOM derived from the epilimnion transferred by winter vertical mixing, or DOM secondary produced in the hypolimnion, such as cellular debris from other microbes.

In conclusion, this study revealed that the microbial food web and biogeochemical processes in the oxygenated hypolimnia of deep freshwater lakes are driven by a specific bacterioplankton communities that have eluded previous studies only focusing on the surface waters. Future works should further investigate the ecophysiology of the individual lineages by a metagenomic-based approach or by establishment of their isolated cultures.

Keywords: Limnology, Microbial Ecology