

# **Ecology and diversity of freshwater picocyanobacteria in Japanese lakes**

Ji Cai

## **Abstract**

### **Introduction**

Picocyanobacteria are photosynthetic prokaryotes defined by cell sizes less than 2  $\mu\text{m}$ . They are numerous and ubiquitous in a variety of lakes and oceans, playing important roles in primary production and aquatic food web. Freshwater picocyanobacteria appear as both single cells and colonies, with the former being the dominant phytoplankton in oligotrophic lakes and the latter being more common in meso-eutrophic lakes. The two morphotypes are closely related that single cells could form colonies and vice versa, the processes in which grazers involve. However, because the colonies usually constitute a minor part of total phytoplankton biomass, they have been overlooked in earlier studies so that the ecology of colonial picocyanobacteria remain largely unknown. On the other hand, although freshwater picocyanobacteria are considered to possess greater diversity compared to the marine counterparts, their phylogeny remain poorly understood as most studies have been conducted in marine environments. Therefore, in order to gain a better understanding of the ecology of freshwater picocyanobacteria, this thesis conducted a series of studies to investigate the ecological differences between single cells and colonies, and to explore the phylogenetic diversity of picocyanobacterial communities in Lake Biwa and other freshwater lakes in Japan.

## **Materials and methods**

Vertical water samples were collected twice a month for two years in Lake Biwa and also from eight lakes in Japan during the stratification period. Firstly, seasonal variation of single-celled, colonial picocyanobacteria and their potential grazers – heterotrophic nanoflagellates, ciliates, rotifers, cladocerans and copepods in abundance were enumerated by microscopic observation. These time series data collected from Lake Biwa were analyzed using empirical dynamic modeling, to explore how grazers could affect the dynamics of picocyanobacteria. Secondly, seasonal picocyanobacteria community analysis was conducted in Lake Biwa by clone library using primers for cyanobacterial 16S rRNA. Thirdly, the phylogenetic diversity of picocyanobacteria from 9 Japanese lakes including Lake Biwa was analyzed. To improve the phylogenetic resolution, long-read amplicon sequencing was used with primers for cyanobacterial 16S rRNA and adjacent ITS region.

## **Results**

The two morphotypes of picocyanobacteria in Lake Biwa showed different spatial and temporal distribution. Single cells were highly abundant in the epilimnion during the summer-autumn stratification, while colonies mainly distributed in the metalimnion and only abundant at the beginning of summer. Furthermore, time series analyses showed that grazers played important and differing roles in controlling the abundances of single cells and/or colonies.

Analysis of the phylogenetic structure showed that the picocyanobacterial community varied with seasons and even years, with different operational taxonomic

units (OTUs) dominated in each season. Importantly, a winter bloom of single cells was novelly recorded, during which the psychrotolerant lineage dominated.

Ubiquity and predominance of the psychrotolerant lineage was also observed in more than half of the investigated Japanese lakes, and the rest are dominated by other picocyanobacterial lineages detected from worldwide freshwaters. Moreover, analysis based on the variable ITS region revealed that the degree of sequence divergence varied largely among lineages. Especially, the psychrotolerant lineage exhibited an apparent pattern in phylogeography differentiation that the sequences from oligotrophic lakes tightly clustered together and separated from those in more nutrient-rich lakes.

## **Discussion**

The study of picocyanobacteria in Lake Biwa show that the seasonal dynamics of single cells and colonies are influenced by a variety of grazers. These results indicated that single-celled and colonial picocyanobacteria may play differing ecological roles in the aquatic food web. On the other hand, the proliferation of psychrotolerant lineage in Lake Biwa showed the necessity to shed more light on the winter communities, as studies on picocyanobacteria have been mostly conducted during warm seasons when they are abundant. Lastly, by investigating picocyanobacterial communities in Japanese lakes with various environmental conditions, this study revealed the enormous diversity of freshwater picocyanobacterial lineages. Especially, the usage of ITS region provided novel insights into intra-lineage microdiversity. The present thesis highlighted the advantage of frequent sampling in lakes and the application of long-read amplicon sequencing, which would improve understanding of the ecology and phylogeny of freshwater picocyanobacteria.