

# Fungal community structures in Lake Biwa and surrounding shallow lagoon lakes using a metagenomics analysis

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## Chapter 1

Fungi are considered to comprise one of the most diverse group of organisms in the world, fungi collected from an environment are ephemeral and cryptic that it is difficult to culture and identify them using microscopic analysis. Fungi may therefore account for a significant proportion of all unidentified microbial species—especially in aquatic systems. Zoosporic fungi can be the dominant fungi in freshwater lakes, especially the pelagic zone in deep lakes, and a role of zoosporic fungi on material cycling in an aquatic ecosystem is neglected. However, limited information especially diversity on zoosporic fungi has been shown from aquatic ecosystems. Recently, the development of molecular techniques, particularly a high-throughput sequencing, has extended our capacity to identify the diversity of fungal communities.

Lake Biwa is the largest lake in Japan, it is also the world's twentieth oldest lake, having existed for almost 4 million years. It has extensive pelagic areas and various types of sediment, littoral and lagoon lakes. The remarkable geographical features of Lake Biwa include lagoons lakes that are connected by channels that significantly contribute to maintaining the abundance and diversity of native species throughout the lake basin. Information on the lake's fungal community, especially its diversity, is still limited. In order to clarify the diversity, distribution, seasonal variation and also the difference between deep and shallow lakes of fungal communities, especially the zoosporic fungi communities, a metagenomic analysis based on high-throughput sequencing targeting ITS2 and LSU region was done.

## Chapter 2

Only limited information is available on fungal community structure, especially in large lakes such as Lake Biwa. In this study, whole fungal communities were determined seasonally and spatially using a high-throughput sequencing technique. Water samples were collected from the epilimnion, 0-20 m depth, with a Van Dorn sampler at a pelagic site and from the surface at a littoral site in the north basin of Lake Biwa. All pelagic depth samples were combined into one sample. All samples from both sites were sieved with a 20- $\mu$ m mesh, and residuals on the mesh were used for following analysis. Sampling occurred on 24 April, 22 May, 10 July, and 16 September 2015. DNA was extracted from filtered samples. Metabarcoding analysis targeting fungi-specific internal transcribed spacer 2 regions was performed using an Illumina MiSeq platform.

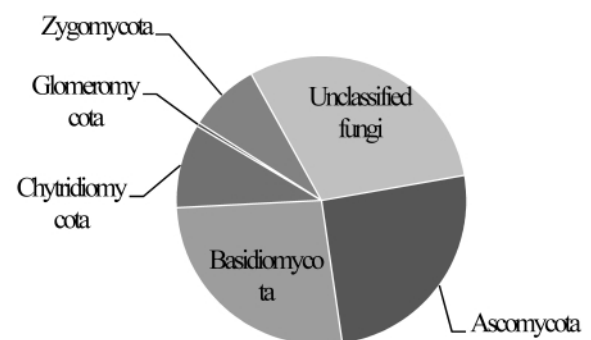


Figure 1. The phylum composition of 479 fungal OTUs identified in the present study.

Epilimnetic fungal communities showed high diversity, with 479 operational taxonomic units (OTUs). The OTUs included 122 belonging to the phylum Ascomycota, 127 to Basidiomycota, 38 to Zygomycota, 45 to Chytridiomycota, 2 to Glomeromycota, and 145 were unclassified fungi. Fungal

community structures varied seasonally and spatially. Few of the fungal OTUs overlapped between seasons and sites, and specific communities of fungi were detected on each sampling occasion. Results indicated that spatio-temporal variations in fungal communities were high and may be influenced by both internal factors and external factors, such as terrestrial inputs.

### Chapter 3

Fungal community structures in Lake Biwa may be influenced by autochthonous sources, such as bottom sediments, surrounding rivers and shallow lagoon lakes. Shallow lagoon lakes may also show specific fungal community structures that differ from those from Lake Biwa due to the specific ecosystems. In this Chapter, fungal community structures were determined from six shallow lagoon lakes, i.e. Lakes Yanagihira-ko, Jinjyo-numa, Kohokunodanuma, Hasu-ike, Katada-naiko and Hamabun-numa, in August, October 2014, February, and May 2015, comparing with those from epilimnion in the north basin of Lake Biwa in May to December 2016 based on LSU region of rDNA. In Lake Biwa, the fungal community structures in the epilimnion was compared with those in the deep water just above the bottom and in the sediment in September

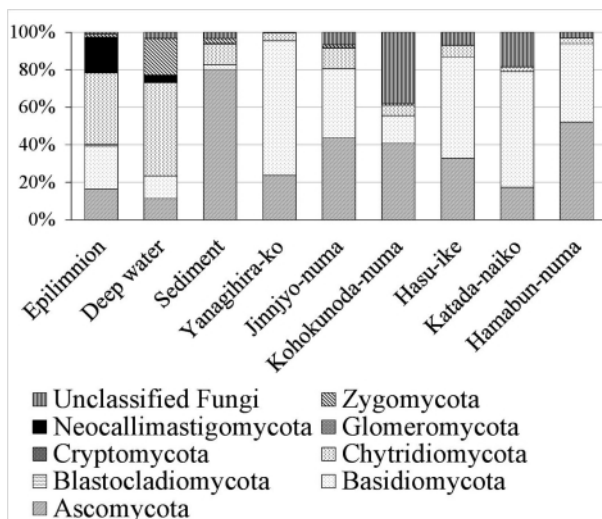


Figure 2. Phylum composition in number sequences with a high-throughput sequencing analysis for fungal assemblages collected in Lake Biwa and six lagoon lakes.

2016, in order to clarify the vertical distribution of fungal communities. Although the year of investigations was different between the lagoon lakes and Lake Biwa, more OTUs were detected from the lagoon lakes than Lake Biwa. Seasonal variation of OTUs in number was stable in Lake Biwa while larger in the lagoon lakes. Zoosporic fungi, mainly in the phylum Chytridiomycota and Neocallimasti- gomycota, were dominant both in number of sequences and OTUs in Lake Biwa, while Dikarya, i.e. Ascomycota and Basidiomycota, mostly dominated in the lagoon lakes. More classes were detected from Lake Biwa than those from the lagoon lakes. The most abundant genera in lagoon lakes were similar among the lakes, while being quite different from those in Lake Biwa.

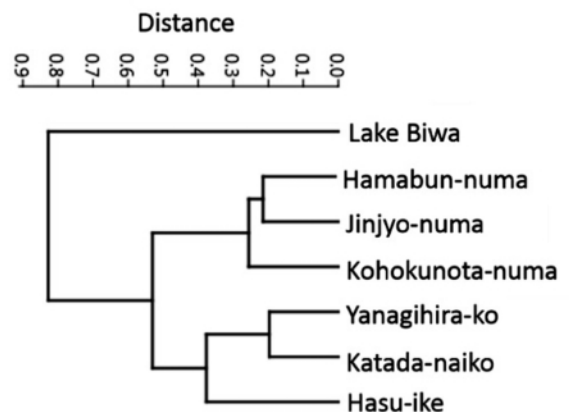


Figure 3. Cluster analysis based on Gower's General Similarity Coefficient for the most 10 abundant genera from epilimnion in Lake Biwa and lagoon lakes.

In September 2016, yeasts and zoosporic fungi were the most dominant fungi in the epilimnion of Lake Biwa, while filamentous fungi dominated in the sediment. The dominant genera in each habitat were different. Less overlap between fungal OTUs (22) from the epilimnetic water and the bottom sediment, while high overlap between those (50 OTUs) from the bottom water and the sediment. These results indicated that different habitats can harbor specific fungal communities in Lake Biwa.

## Chapter 4

Zoosporic fungi especially the chytrids can be the dominant fungi in the pelagic of the deep lakes. In this chapter, community structure of zoosporic fungi was determined using a high-throughput sequencing analysis, and sequence number of the zoosporic fungi was compared with number of microbial components, i.e. heterotrophic nanoflagellate (HNF), autotrophic nanoflagellates (ANF), bacteria and cyanobacteria, and large phytoplankton infected by fungal sporangium, which were counted with traditional microscopic analysis, from May to December 2016 at a pelagic site in the north basin of Lake Biwa.

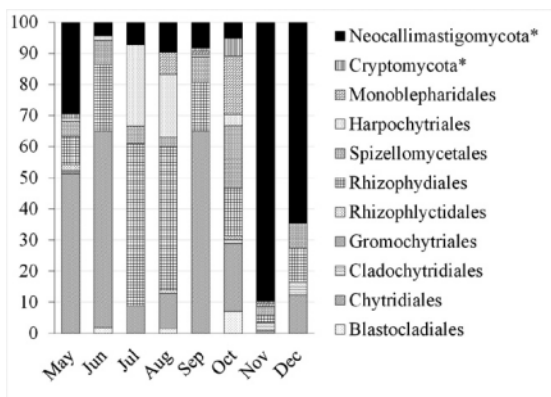


Figure 4. Composition of order in number of read of zoosporic fungi i.e. Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota from 6 May to 12 December 2016 at Stn 3 in north basin of Lake Biwa.

Metagenomic analysis targeted to fungal specific LSU regions of ribosomal DNA was made. All of the microbial components and zoosporic fungi community showed the apparent seasonal changes. Zoosporic fungal communities showed high diversity with 107 operational taxonomic units (OTUs). Four zoosporic fungal phyla, Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota, were detected. Chytridiomycota was the most diverse and abundant phylum among them. Some chytrids, i.e. *Staurastromyces oculus*, *Zygorhizidium planktonicum* and *Pendulichytrium sphaericum*, that were obligate parasite on phytoplankton, occurred throughout the study period. Less fungal OTUs overlapped between seasons,

and specific communities were detected from each sampling occasion, as shown in whole fungal community (Chapter 2).

HNF abundances were tightly related to

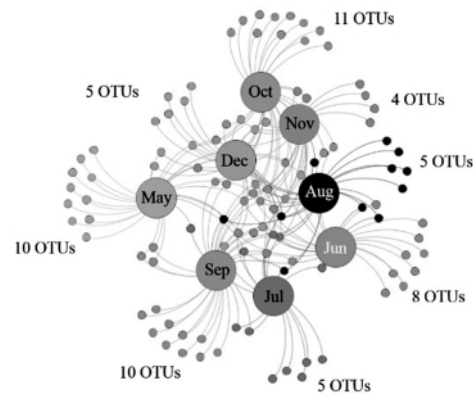


Figure 5. Gephi network diagram illustrating i.e. Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota and highlighting the number of unique OTUs.

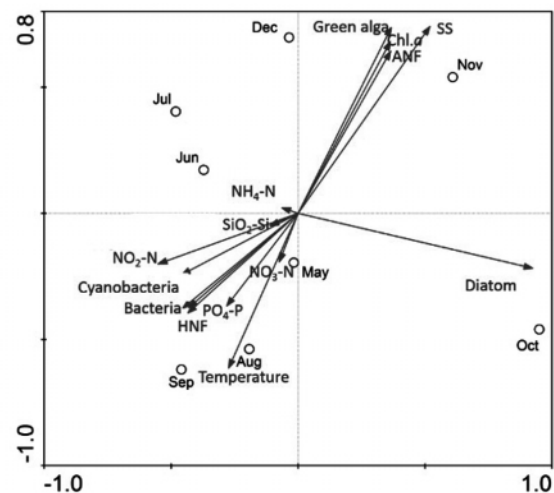


Figure 6. Redundancy analysis between zoosporic fungi i.e. Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota and environment factors from 6 May to 12 December 2016 at Stn 3 in north basin of Lake Biwa.

number of sequences of chytrid reads support that HNF may include chytrid zoospores for the traditional microscopic methods.