

EFFECTS OF THERMAL STRATIFICATION ON BACTERIAL COMMUNITY COMPOSITION THROUGHOUT WATER COLUMN IN A DEEP LAKE

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Abstract. This study aimed to assess the effects of stratification on bacterial community structures throughout a water column in the alkaline Lake Iznik. For this purpose, the samples were collected from the deepest point of the lake from three depths representing the thermal layers namely, epilimnion (0.5 m), metalimnion (20 m), and hypolimnion (40 m) in August 2020. 16S rRNA gene-targeted amplicon sequencing approach was used to assess the bacterial community compositions and sequencing was carried out in Illumina® MiSeq™ platform. The temperature (from 25.76 to 9.37°C), EC (from 1035 to 398 µS/cm), and pH (from 9.1 to 7.7) were gradually decreased throughout the water column. While a remarked increase was observed in phosphorous (from 25.75 to 119.72 µg/L) and nitrate (from 94.68 to 167.71 µg/L) levels along with the water column. DO level significantly decreased to 1.04 mg/L in the hypolimnion. The bacterial community in the epilimnion was predominated by Actinobacteria which represented 52% of the total reads and the abundance went down through the water column and the minimum level was recorded in the hypolimnion (28%). Proteobacteria (18%) was the second abundant phylum in the epilimnion followed by Bacteroidetes (12%) and Verrucomicrobia (9%). Proteobacteria became dominant in the hypolimnion and 46% of the bacterial community was represented by Proteobacteria species. Among the classified bacterial families, the majority of the total reads were assigned to Sporichthyaceae, Ilumatobacteraceae, Burkholderiaceae, and Microbacteriaceae in all three samples. The diversity and evenness of the bacterial communities were increased through the water column. This work contributes to existing knowledge of bacterial diversity of stratified lakes by providing high throughput data from a unique alkaline environment.

Keywords: alkaline lake, bacterial community, freshwater microbiome, high throughput sequencing, water quality

1 INTRODUCTION

Freshwater lakes are crucial ecosystems in both ecological and economic terms; they provide habitat for a complex biological community while also serving as a source for humans. Within these ecosystems, bacteria play an important role in environmental services and influence water quality (Ozbayram et al., 2020). In temperate regions, reservoirs are generally stratified during summer months, affecting water quality, nutrient concentrations, and ecosystem functions. Stratification results in higher light intensity, temperatures, and dissolved oxygen concentrations in surface waters (epilimnion) than that of deep waters (hypolimnion). The top and bottom water layers are separated by the metalimnion interface region (Yu et al., 2014). The variation in the environmental conditions affects the diversity of aquatic microbial communities (Shilei et al., 2020). While the stratified aquatic systems provide diverse habitats for microorganisms, the stratification regime also has impacts on the community dynamics and contributes to community alterations. Due to the specific mixing and stratification dynamics, deep lakes are appealing environments for studying the effect of habitat heterogeneity on microbial community succession (Shilei et al., 2020; Yu et al., 2014).

The alkaline lake Iznik is one of Turkey's biggest lakes, with a surface area of 308 km² and a maximum depth of 65 meters (Akcaalan et al., 2014). It has significant ecological, recreational, social, and economic value. There are more than 24 small-to-moderate scale industrial plants around the town Orhangazi in the west which forms the biggest and organized industrial region (Ünlü et al., 2010) resulting in pressure on the lake. Additionally, agricultural development and anthropogenic wastes threaten the ecosystem. Last decades

intensifying land use, increasing pollution in the streams draining to the lake and excess water withdrawals affected the lake's water quality in which the symptoms of eutrophication became noticeable. It is a mesotrophic lake that stratifies from June to September and is well mixed from October to April. Surprisingly, the effects of this feature on the bacterial community composition have not been closely examined in the lake.

This study set out to investigate the bacterial succession throughout the water column under the stratification conditions in Lake Iznik. To achieve this aim, high throughput 16S rRNA amplicon sequencing method was used to assess the diversity.

2 METHODS

2.1 Sampling and Physico-chemical characterization

The sampling was performed in the stratification period that occurred in August 2020. The samples were collected from the deepest point of the lake from the three thermal layers namely, epilimnion (0.5 m), metalimnion (20 m), and hypolimnion (40 m).

Total phosphorus, nitrate, and nitrite concentrations were determined following the procedure described in American Public Health Association (APHA, AWWA, WEF, 1989). Total nitrogen (TN) was measured with an Elementar analyzer (Elementar, Germany). Chlorophyll *a* (chl-*a*) was determined according to the ISO standard method (ISO 10260, 1992). A portable multiparameter (650 MDS, YSI, USA) was used to measure pH, temperature, and dissolved oxygen levels.

2.2 DNA extraction and Amplicon Sequencing

The water samples were filtered from a 0.22 µm filter and the total DNAs were isolated from the filter papers using the NucleoSpin® Soil Kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. The concentrations of the isolated DNAs were determined by NanoDrop 1000 (Thermo Fisher Scientific, Inc., DE, USA). The DNAs were stored at -20°C until further analysis.

16S rRNA gene-targeted amplicon sequencing approach was used to assess the bacterial community compositions. In brief, the V4 region of the 16S rRNA gene was targeted and an amplicon library was prepared using the primers 341f (5'-CCTACGGGNGGCWGCAG-3) and 805r (5'-GACTACHVGGGTATCTAATCC-3'). Following the purification and quantification of the amplicon libraries, the sequencing was carried out in the Illumina® MiSeq™ platform (USA) with 300 bp paired-end chemistry. CASAVA data analysis software was used for demultiplexing and clipping of sequence adapters from raw sequences (Illumina®, USA). The data was analyzed in QIIME2 v2020.2 (Bolyen et al., 2019) following the amplicon sequencing workflow. The fragments with any mismatches to the barcodes or primers were filtered and primers were removed (Martin, 2011). Following joining the paired-end reads and quality filtering, the sequences were denoised (Amir et al., 2017). Taxonomy was assigned to each amplicon sequence variant (ASV) using the 'feature-classifier classify-sklearn' plugin against the pre-trained Naive Bayes classifier specific for 16S rRNA (Comeau, Douglas, & Langille, 2017). The alpha diversity indices, Shannon, Faith's PD, and Pielou's evenness, were calculated based on the final ASV table. The bacterial community structures were visualized by Krona graphs (Ondov, Bergman, & Phillippy, 2011; Ozbayram, Koker, Akcaalan, Ince, & Albay, 2020).

3 RESULTS & DISCUSSION

Physicochemical characteristics of water quality and chlorophyll-*a* concentrations are summarized in Table 1. The water temperature ranged from 25.76 to 9.37°C along with the water column. Because of the stratification, the water temperature difference between the surface and bottom layers was about 16 °C. Thermal stratification influences many physicochemical and biological processes in water bodies (Zhang et al., 2016). There was a considerable difference in DO concentration between the epilimnion and hypolimnion. It was measured as 1.04 mg/L in the hypolimnion. DIN concentrations were minimum at the surface layer where dissolved oxygen was sufficient. Due to DO depletion, there was an accumulation of DIN in hypolimnion (Noori et al., 2018). A remarked increase was observed in phosphorous (from 25.75 to 119.72 µg/L) along with the water column. The alkalinity, pH, and electrical conductivity levels were high and in accord with the previous studies carried out in the lake (Oğuz et al., 2020; Roeser et al., 2016). In summer, with increased temperature transparency, chlorophyll-*a* concentration was high in the epilimnion. However, the metalimnetic and hypolimnetic chlorophyll-*a* decreased.

Table 1. Environmental parameters

Parameter		Epilimnion	Metalimnion	Hypolimnion
Temperature	°C	25.76	11.52	9.37
EC	uS/cm	1035	768	398
pH		9.1	8.07	7.70
Dissolved Oxygen	mg/L	9.26	4.89	1.04
SRP	μg/L	1.09	11.84	71.14
TP	μg/L	25.75	46.10	119.72
DIN	mg/L	0.14	0.23	0.25
chl- <i>a</i>	μg/L	11.84	2.13	2.22
Alkalinity	mg/L CaCO ₃	352	340	325

The bacterial community pattern of the epilimnion is shown in Figure 1. More than half of the reads were assigned to the phylum Actinobacteria. Proteobacteria was the second dominant phylum followed by Bacteroidetes and Verrucomicrobia. At the family level, by far the greatest number of sequences belonged to Sporichthyaceae representing 33% of the total reads. Ilumatobacteraceae was the second abundant family followed by Burkholderiaceae.



Figure 1. Bacterial community profile of epilimnion

Figure 2 shows the bacterial community structure of metalimnion. The community was dominated by Actinobacteria species however the abundance went down compared to the epilimnion. On the other hand, Proteobacteria and Bacteroidetes members enriched in metalimnion and together they represented 46% of the sequences. Similar to the epilimnion, the highest share of the sequences were assigned to Sporichthyaceae. While 8% of the bacterial community was represented by Burkholderiaceae species, 7% of the reads were assigned to Ilumatobacteraceae. Furthermore, a higher abundance of Cyclobacteriaceae (5%) was detected in metalimnion compared to the epilimnion.



Figure 2. Bacterial community profile of metalimnion

Actinobacteria abundance decreased throughout the water column and the lowest abundance was observed in the hypolimnion (Figure 3). Contrarily, Proteobacteria species enriched along the water column and became the dominant in the hypolimnion, accounted for 46% of the total sequences. Similar to the upper layers, Bacteroidetes was the third abundant phylum in the bacterial community. 25% of the bacterial community was represented by the families, Sporichthyaceae, Burkholderiaceae, and Ilumatobacteraceae. Alteromonadaceae and Flavobacteriaspecies enriched in the hypolimnion and 5% and 4% of the total reads were assigned to these families, respectively.

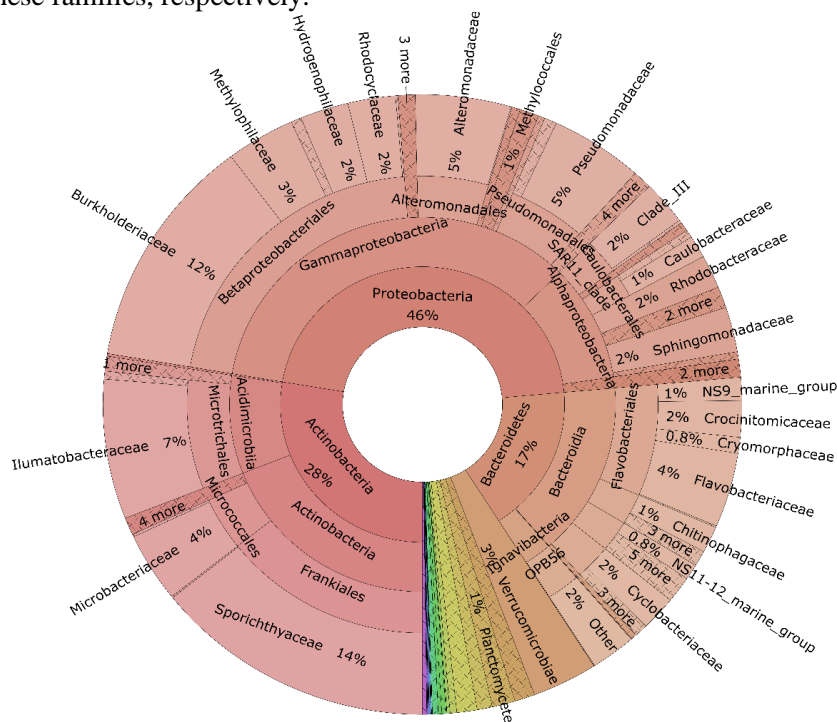


Figure 3. Bacterial community profile of hypolimnion

The results of this study are in line with similar studies in which the lake's bacterial community is mainly composed of Actinobacteria, Proteobacteria, and Bacteroidetes species (Ozbyram et al., 2020; Yang,

Huang, & Zhang, 2015; Yu et al., 2014). However, Cyanobacteria abundances were less than 1% in our study in all layers. Studies also showed significant community shifts between the different layers during the stratification period. Similar to our results, Yu et al. (2014) also observed a decreasing pattern of Actinobacteria abundance along with the water column with decreasing dissolved oxygen levels. In another study carried out in oligotrophic Lake Michigan by Newton & McLellan (2015), Sporichthyaceae found as the predominant family representing more than 25% of the bacterial community.

The Shannon and Pielou's evenness indices showed that the diversity and evenness of the bacterial communities were increased through the water column and the highest values were calculated for the sample collected from the hypolimnion (Table 2). The results are in agreement with those obtained by Yang et al. (2015) in which the authors also calculated higher diversity indices in the hypolimnion. A possible explanation for these results may be due to the microbial mutual effects between the sediment and water, and nutrient release from the sediment.

Table 2. The alpha diversity of the bacterial communities

	Number of OTUs	Shannon	Pielou's evenness	Faith's PD
Epilimnion	160	5.54	0.76	25.07
Metalimnion	345	6.66	0.79	41.21
Hypolimnion	434	7.43	0.85	45.56

4 CONCLUSIONS

Overall, this study evaluated the effects of thermal stratification on bacterial community profile and abundance along with the water column by high-throughput sequencing technology. The results indicated a more diverse and evenly distributed bacterial community in the hypolimnion. Moreover, the bacterial communities were composed of common freshwater phyla and the abundances differed between the water layers. This study contributes to existing knowledge of bacterial diversity of stratified lakes by providing high throughput data from a unique alkaline environment.

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