Vertical Distribution of Bacteria in Various Lakes of East Java, Indonesia

Sitoresmi Prabaningtyas*, Agung Witjoro, Endang Suarsini, Dhiyaudin Aridowi, Nafizatuzamrudah, Afifah Nur Aini, Adji Purnomo, Yuniar Indra Permana, Meirinda, Dina Aribah, Yanis

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Jl. Semarang 5 Malang 65145, Indonesia

*Corresponding author’s email: sitoresmi.prabaningtyas.fmipa@um.ac.id

Abstract: Ecosystems are composed of a large number of diverse organisms that make up very complex interactions. Several studies have proven the influence of abiotic factors on the distribution of bacteria in the environment. Bacteria play an important role in biogeochemical cycles and support the productivity of ecosystems. The important role of bacteria in the environment causes the need for research on the vertical distribution of bacteria. The purpose of this research was to determine the vertical distribution of bacteria in some lakes in eastern Java. The samples were taken from 3 lakes in East Java namely, Ranu Grati, Ranu Regulo, and Ranu Pane. The bacterial samples were taken at 5 stations in each lake. The samples, vertically taken from 4 points, the upper, middle and lower surface according to the depth of the Secchi disc and the bottom of the lake, were isolated and identified. It was found 14 species of bacteria from Ranu Pane, 24 species of bacteria from Ranu Regulo, and 36 species of bacteria from Ranu Grati. The most dominant and abundant bacteria in Ranu Pane, Ranu Regulo, and Ranu Grati were coded with A, B, G3 respectively. The bacteria in all the lakes were spread over all stations and were vertically scattered at all points. The abiotic factors measured have a positive effect on the vertical distribution of bacteria.

Keywords: Vertical distribution, bacteria, lake, abiotic.

1. Introduction
The presence of bacteria in the environment is greatly influenced by abiotic factors [1]. In a lacustrine (lakes) ecosystem, microorganisms play a pivotal role in driving the biogeochemical processes [2]. First, the photosynthetic and chemolithotrophic microorganisms are the main contributors to the primary productivity. Second, the chemoorganotrophic and chemoheterotrophic microorganisms are actively involved in the decomposition of organic matters. Third, appreciable amounts of microorganisms are the foodstuff for filter-feeding organisms and form the foundation of the aquatic food web [3,4]. Therefore, it is of great interest to study the bacterial communities in Ranu Grati, Ranu Pane, and Ranu Regulo lakes.

Lakes are ecosystems that are composed of a large number of diverse organisms that make up very complex interactions. Interactions can occur in the lake ecosystem between microbes and environment [5]. Bacteria play an important role in biogeochemical cycles and support the productivity of ecosystems [6]. However, the profiles of the distribution bacteria in Ranu Pane, Ranu Regulo, and
Ranu Grati lakes remain unknown. The important role of bacteria in the environment causes the need for research on the relationship of vertical distribution of bacteria with abiotic factors. Thus, it is of great interest to study the microbial communities in Ranu Grati, Ranu Pane, and Ranu Regulo lakes.

In this study, the vertical distribution of bacterial was investigated in 2017. There are about 25 lakes in East Java. The famous lakes in east Java among them are Ranu Pane, and Ranu Regulo located at the border areas of Pasuruan, Lumajang, and Malang at 2100 MASL and Ranu Grati Lake, is located at Pasuruan, with an altitude of about 91m above sea level. The area of Ranu Pane is approximately 1 hectare and the area of Ranu Regulo is about 0.75 hectares. Ranu Grati area is around 198 hectares. The physicochemical parameters of the lake water were examined as well. In addition, the correlation of vertical distribution bacteria with abiotic factors of the lake and the structure of the bacterial communities in the lake were discussed.

2. Experimental Methods

2.1. Study site and sampling stations
Ranu Pane, Ranu Regulo, and Ranu Grati are eutrophic lakes, with the depths about (0.5 - 7 m), (0.5 - 50 m) and (7 - 150 m) respectively. Five sampling stations in the Ranu Pane, Ranu Regulo, and Grati lakes were selected. The five sites were distributed over the lake at the deeps varies between 0.5 m and 91 m.

2.2. Bacterial samples collection
Bacterial samples were collected from water at 5 stations in the three lakes (Figure 1). It was vertically taken from 4 points i.e. the surface, middle, lower surface (according to the depth of Secchi disc) and the bottom of the lake. The bacterial samples were isolated and identified.

![Figure 1](image_url)

**Figure 1.** The conditions of each sampling site in Ranu Grati (A), Ranu Pane (B) and Ranu Regulo (C). The codes of the station taken as samples were (1, 2, 3, 4, 5)

2.3. Bacteria Collection
For collecting the bacteria, 100 ml water from each point of each station was taken using sterile “water sample bottle”, and then transferred to a sterile bottle. The water samples from all station were transported in an ice box and stored at about 4°C with ice gel [7].

2.4. Analysis of physicochemical parameters of water
Water transparency was determined in situ using the Disc method. The salinity, concentrations of DO (dissolved oxygen), temperature, and pH were measured using Refractometer ATAGO S-28E, DO meter Lutron 5510, pHmeter Lutron 5509 respectively [8]. The next analysis was conducted in the laboratory. The concentration of total nitrogen was analyzed using the Total Kjeldahl method. The content of dissolved inorganic phosphorus (DIP) was determined using the phosphorus molybdenum blue colorimetric method [9,10].
2.5. Data analysis
Principal coordinate analysis (PCoA) plots were generated based on the weighted Unifrac pairwise distance matrices and on the Bray-Curtis coefficient matrix in order to evaluate vertical distribution in the microbial community over the points of the samplings. Canonical correspondence analysis (CCA) was also carried out to determine the correlation between physicochemical parameters and the relative abundance of bacteria in the microbial community. Multivariate analyses were done using Paleontological Statistics software (PAST, version 2.15). The heat map representing the mean relative abundance of microbial genera was generated by a hierarchical cluster analysis of different genera mean prevalence Paleontological Statistics software (PAST, version 2.15). Moreover, the species richness indices (Chao1 and ACE) and Shannon’s diversity index were computed with the software Paleontological Statistics software (PAST, version 2.15).

3. Results and Discussion
The water salinity of the three lakes was around 0.00% – 0.4% and the pH was around 6.83–9.40 at the surface layer. Dissolved Oxygen (DO) of the lake water at the surface layer was between 4.2 and 11.6 mg/L. The transparency of the lake water was between 50 to 130 cm (Table 1, 2 and 3).

Table 1. The Physicochemical Water Parameters of Ranu Regulo Lake

| Point | DO (mg/l) | pH   | Salinity (%) | Transparency (cm) |
|-------|-----------|------|--------------|------------------|
| 1     | 8.9       | 9.38 | 0.3          | 75               |
| 2     | 8         | 8.65 | 0.2          | 75               |
| 3     | 8.4       | 8.65 | 0.2          | 80               |
| 4     | 11.3      | 9.3  | 0            | 75               |
| 5     | 11.6      | 9.4  | 0.2          | 75               |

Table 2. The Physicochemical Water Parameters of Ranu Pane Lake

| Point | DO (mg/l) | pH   | Salinity | Transparency (cm) |
|-------|-----------|------|----------|------------------|
| 1     | 4.2       | 7.24 | 0.4      | 80               |
| 2     | 5         | 6.83 | 0.3      | 82               |
| 3     | 6         | 6.95 | 0.2      | 100              |
| 4     | 11.6      | 7.3  | 0.4      | 50               |
| 5     | 6.7       | 6.88 | 0.4      | 50               |

Table 3. The Physicochemical Water Parameters of Ranu Grati Lake

| Point | DO (mg/l) | pH   | Salinity | Transparency (cm) |
|-------|-----------|------|----------|------------------|
| 1     | 7.8       | 8.82 | 0        | 130              |
| 2     | 7.1       | 7.1  | 0        | 100              |
| 3     | 8.4       | 8.4  | 0        | 100              |
| 4     | 7.2       | 8.8  | 0        | 110              |
| 5     | 8.5       | 8.82 | 0        | 110              |

Among the three lakes observed, the water of Ranu Grati Lake had the salinity of 0‰. Based on the morphology of bacterial colonies, it was found 14 species of bacteria from Ranu Pane, 24 species of bacteria from Ranu Regulo, and 36 species of bacteria from Ranu Grati. The most dominant and abundant bacteria in Ranu Pane, Ranu Regulo, and Ranu Grati were coded with A, B, G3 respectively. Based on the characteristics of bacterial physiology the species G3 is Enterobacter gergoviae. The Vertical Distribution of bacteria in Ranu Pani was scattered in all point (Figure 2).
Figure 2. The Vertical Distribution of Bacteria in Ranu Pani. A, B, C and so on are the codes of bacterial species. P1.1, P2.2 and so on are codes of sampling stations.

Figure 3. The Vertical Distribution of Bacteria in Ranu Regulo. A, B, C and so on are codes of bacterial species. R1.1, R2.2 and so on are codes of sampling stations.

The diversity profile of bacteria in Ranu Grati, Ranu Pane, and Ranu Regulo was computed with the software Paleontological Statistics software (PAST, version 2.15). The results showed that bacteria profile were scattered in another depth of samples (Figure 1, 2, and 3). The bacteria in the lakes were spread over all stations and were vertically scattered at all points. The abiotic factors measured have a positive effect on the vertical distribution of bacteria.

The bacteria were distributed evenly across the sampling point. Abiotic factors measured did not show any significant difference. Certain species exists only at a certain point. Bacteria have a tendency to grow at a particular station and depth. The differences in this tendency are influenced by the type of bacteria, the type of nutrients and the possible range of environments for these types of bacteria. The physicochemical variables measured in this research were transparency, salinity, OD, pH, and turbidity.
Figure 4. Vertical Distribution of Bacteria in Ranu Grati. 1, 2, 3 and so on are codes of bacterial species. R1.1, R2.2 and so on are codes of sampling stations

In this research, these physicochemical variables were likely among the dominant factors driving (directly or indirectly) the changes in bacterial community structure. The distribution of bacteria was influenced by its growth which was controlled by various biological and biochemical processes (including the reproduction rate, diffusion of chemical species, microbial species interaction, gene mutation, evolutionary adaptation, and species formation). The rise in the temperature will elevate the rate of biological processes in microorganisms and enhance the growth of bloom-forming Cyanobacteria from the littoral zones [10]. The bacteria in the lake were spread over all stations and vertically scattered at all points.

The composition of bacterial communities appeared to be relatively homogeneous among depth samples, as indicated by UPGMA dendrogram (see Fig. 3). The nutrient availability is considered to be a primary factor that can change the microbial communities in lakes. In our study, the types of lakes seemed to have little effect on the whole bacterial communities in the lakes. Although Ranu Pane, Ranu Regulo, and Ranu Grati lakes was a closed lake, its lake water kept being an active material exchanging and energy transformation with the environment through atmospheric circulation, rainwater precipitation, etc. Consequently, the lake water became an open system and its bacterial communities were determined by the environment. In contrast, the bottom sediments could not freely communicate with the environment and therefore were separated as exclusive niches to preserve the unique properties (including microorganisms) of this lake. The bacteria inhabiting in the sediments of lakes, especially those unidentified, might be a reserve of ancient bacteria which were of geological value.
4. Conclusion
Based on the results and discussion, we conclude that the bacteria in Ranu Pane, Ranu Regulo, and Ranu Grati lakes are spread over all stations and vertically scattered at all points. The physicochemical variables were likely among the dominant factors driving directly or indirectly the changes in bacterial community structure. Furthermore, the abiotic factors measured have a positive effect on the vertical distribution of bacteria.

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