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A laboratory experiment for Meromixis in an integrated sample of soda lake Doroninskoye (Transbaikalia)

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ABSTRACT

Under laboratory conditions, in an aquarium containing an integrated sample of water from Lake Doroninskoye, we modeled meromixis, in which a brightly colored pink layer formed in the anaerobic zone. The presence of bacterial morphotypes, which are analogous to purple sulfur bacteria and non-sulfur purple bacteria, was observed to be abundant, specifically up to 10^8 cells/mL. Meromixis formation and stability depend on light, level of penetration into the water column, and anaerobic zone illuminance. Our results demonstrate the effectiveness of laboratory modeling experiments and provide a method for investigating the complex process of meromixis.

Keywords: soda lake, meromixis, chemocline, aerobic and anaerobic zone, microbial community

INTRODUCTION

In many soda and salt lakes not entire water column is involved in the process of annual mixing. Such phenomenon is well known as meromixis [1]. The boundary between mixo- and monimolimnion (the chemocline) is very often populated by the anoxygenic phototrophic microorganisms which make this water layer a brightly colored pink [2, 3]. While the meromixis was detected in Lake Doroninskoye (Transbailkalia) pink layer of phototrophic microorganisms was not visualized in this lake. In contrast such a layer is observed in two other Siberian meromictic lakes – Shira and Shunet [4].

Strongly continental climate in the permafrost zone (N 51°25´; E 112°28´), alkaline pH values (9.72) and relatively high water and sediments salinity (32.3 g/L) [5] create specific conditions for the growth of photo- and chemolithotrophic organisms in Lake Doroninskoye. It appeared as a low level of illumination in comparison with other stratified Siberian meromictic lakes which decreased in meromixis zone from 3 to 1 lx (less than 0.001% of light intensity).

Microbial communities in extreme environments are usually simplified and have limited species diversity [6], but in natural condition a lot of environmental factors have an effect on ecosystem functioning. Microcosms are a convenient model for one or limited parameters variation in order to study the formation of the structure, especially microbial communities. A laboratory experiment was established for modeling the illumination condition, meromixis, and investigation of ecology of organisms inhabited water of Lake Doroninskoye.

MATERIALS AND METHODS

Experiments were performed in an aquarium (19.8 L) under room temperature (19.3–22.0°C) and natural illumination of October without additional lighting. We established a microcosm using an integral sample of water (16.8 L) and sand-silt sediment (about 3.0 L from the depth of 1.0 m and 0.6 L – from 3.75 m) from the lake. Physico-chemical water characteristics (temperature, pH, Eh, conductivity, and oxygen content) were determined during observation of visible parameters (deposition of suspended materials, changing of water colors, appearance of water stratification and layer with aquatic organisms) with an automatic portable laboratory (WTW, Germany). Analyses of biological communities were performed with methods similar for examining communities in their natural environments: total bacterial number (TBN) was estimated with microscopic method, bacterial diversity was studied via cloning of bacteria-specific amplicons [7, 8].

Table 1. Bacterial diversity revealed in microcosm and Lake Doroninskoye via cloning in comparison with microscopic analysis and bacteria cultivation according to Gorlenko et al. [9].

Sample name	Lake Doroninskoye	Microcosm
Water, aerobic zone	**Oxygenic phototrophs**	
	Cyanobacteria	
	Phormidium sp.* *Nodularia* sp.* *Synechococcus* sp.* *Synechocystis* sp.* *Symploca* sp.	ND**
	Diatoms	
	*Nitzschia**	ND
	Anoxygenic phototrophs	
	Photoheterotrophic bacteria	
	Nd	*Rhodobaca* sp.
	Aerobic bacteriochlorophyl a-containing bacteria	
	Roseinatronobacter sp.* *Roseinatronobacter monicus*	*Roseinatronobacter* sp.
	Sulfur-oxidizing chemoautotrophs	
	Thioalkalimicrobium sp. *Thioalkalimicrobium microaerophilum*	ND
	Chemoorganotrophs	
	*Halomonas campisalis** *Halomonas* sp. *Spirochaeta alkalica* *Nitriliruptor alkaliphilus* *Marinobacterium* sp. *Shewanella aquimarina* *Hydrocarboniphaga* sp.	*Halomonas* sp.
Water, chemocline	**Oxygenic phototrophs**	
	Cyanobacteria	
	Synechococcus sp.	ND
	Anoxygenic phototrophs	
	Photoautotrophic bacteria	
	*Ectothiorhodospira magna**	*Ectothiorhodospira magna*
	Photoheterotrophic bacteria	
	Dehalococcoides sp. *Rhodobaca barguzinensis** *Thiocapsa imhoffii*	*Rhodobaca sp.*
	Aerobic bacteriochlorophyl a-containing bacteria	
	Roseinatronobacter monicus	ND
	Sulfur-oxidizing chemoautotrophs	
	Thioalcalivibrio sp.*	*Thioalcalivibrio* sp.
	Chemoorganotrophs	
	Nitrincola sp. Candidatus *Limnoluna rubra* *Nitriliruptor alkaliphilus*	ND
Sediments, anaerobic zone	**Anoxygenic phototrophs**	
	Photoautotrophic bacteria	
	*Ectothiorhodospira variabilis** *Ectothiorhodospira magna**	*Ectothiorhodospira magna*
	Photolithoautotrophic bacteria	
	*Thioalkalicoccus limnaeus**	ND

Notice: *- Gorlenko et al. 2010; **ND – no data

RESULTS AND DISCUSSION

A subdivision of water into distinct layers occurred in the aquarium 2.5 months after the establishment of the microcosm. Physico-chemical characteristics are presented on Fig. 1. An upper aerobic water layer was light green in color and had an Eh of +40 mV. The chemocline zone was observed as a well-defined thin opaque layer up to 0.5 cm in thickness. Just beneath the chemocline zone, a purple layer with dark-green accretions was formed under anaerobic conditions with an Eh of –140 mV. Known that in natural environment of majority of meromictic lakes, phototrophic anaerobic purple bacteria utilized hydrogen sulphide in anoxygenic photosynthesis and took part in formation and visualization of meromixis. Dominance of these bacteria found at normal light intensity in chemocline zone suggested optimal conditions for their growth in microcosm.

Microscopy experiments showed that total bacterial number (TBN) in the upper aerobic water layer during the meromixis period was approximately 10^5 cells/mL, which is insignificantly lower than the values observed under natural conditions in Lake Doroninskoye, varying from 1.9×10^6 to 6.7×10^6 cells/mL depending on the season. Dominant morphotypes were cocci with diameters of 0.6–0.8 × 1.6–1.9 µm and bacilli with diameters of 0.5–0.8 × 0.8–2.2 µm, which are similar morphologically to lithoheterothrophic *Halomonas* sp. and aerobic bacteria *Roseinatronobacter* sp. previously isolated from natural waters [7].

In the chemocline, bacterial abundance under both laboratory and natural conditions in Lake Doroninskoye was approximately comparable at 1×10^6 and 6.2×10^6 cells/mL, respectively. Notably, the dominant morphotypes were vibrio and short spirilla that are phenotypically close to *Thioalkalivibrio* sp. and *Ectothiorhodospira* sp. bacteria previously isolated from the chemocline of Lake Doroninskoye [7].

The peak of bacterial abundance (10^8 cells/mL) and the peak of morphotypes diversity were observed at the anaerobic pink layer. It is known that in meromictic lakes, water color is determined by pigments of phototrophic bacteria, which form a dominant community in aerobic and anaerobic zones [9]. Under the natural conditions in Lake Doroninskoye in the aerobic zone, TBN is up to 9.7×10^6 cells/mL and there are no visually observed pink colors typical for purple sulfur bacteria. Most likely it is due to the low transparency of the water and insufficient light intensity. In the laboratory microcosm we created elevated in comparison with the chemocline in Lake Doroninskoye light conditions. As a result under high light intensity in the chemocline of laboratory microcosm we observed an increase in the abundance of purple sulfur bacteria. These bacteria become the dominant species in the microbial community of the anaerobic zone. We conclude that under these conditions, the bacteria could conduct specific biogeochemical reactions that normally occur in natural conditions such as in the meromictic lakes Shira and Shunet and establish the population. For example, in Lake Shunet, total bacterial number of microorganisms without taking into account purple sulfur bacteria (PSB) in the “purple” water layer of chemocline zone varied from 2×10^8 to 4×10^8 cells/mL depending on the season; the abundance of purple sulfur bacteria was 4×10^7 – 1.8×10^8 cells/mL [4].

Cloning of bacteria-specific amplicons has shown that the composition of the main bacterial taxa in aerobic and anaerobic zones of the microcosm was similar to that in natural waters, and the phylotypes *Nitrincola, Roseanotronobacter, Rhodobaca, Thioalkalimicrobium,* and *Nitriliruptor* were detected along the entire water column (Table 1).

We observed in the microcosm well developed community of the invertebrates. The development of the zooplankton and ciliates confirm active functioning (metabolism and growth) of the microbial community, as microorganisms are producers of organic matter, and they are trophically associated to zooplankton and ciliates. Dense protozoa aggregates were detected in the chemocline. The ability of zooplankton communities to utilize the organic matter which is produced in the chemocline of meromictic lakes was widely discussed [10]. We also detected rotifers (*Brachionus angularis*) in the aerobic layer which were inconsistently detected in the anaerobic layer in small amounts. The dynamics of rotifer abundance in the different layers showed a well-expressed daily rhythm depending on illumination (Fig. 2).

During evening, they were present in the upper aerobic layer, whereas during day they migrated in cycles into the anaerobic zone. The movement of rotifers in opposite directions suggests active migration rather than passive transfer due to water flow. Taking into account the high abundance of microorganisms in the anaerobic layer (TBN was 10^3 times higher than in the aerobic layer), we can hypothesize that such migrations are related to organism feeding.

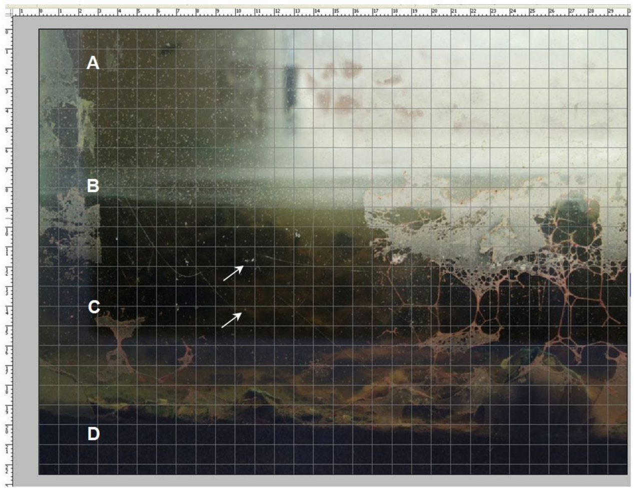

Figure 1. Vertical stratification during meromixis in a microcosm with integral samples of water and sediments from Lake Doroninskoye (Transbaikalia, Russia)
Aquarium size: height 24.7 cm, length 40.0 cm, width 20.0 cm
Exposure temperature: 19–22°C
A - Upper aerobic layer (0–6.5 cm): T 19.5°C, pH 9.8; Eh +40 mV; oxygen content 2.11 mg O_2/L, salinity 26 g/L
B - Chemocline (6.5–7.0 cm): T 19.5°C, pH 9.8; Eh 0 mV; oxygen content 2.13 mg O_2/L, salinity 26 g/L
C - Low anaerobic layer of water column (7.0–20.0 cm): T 19.5°C, pH 9.9; Eh –140 mV, oxygen content 0 mg O_2/L, salinity of 28 g/L
D - The lower layer is anaerobic sediments (20.0–24.7 cm): T 19.3°C, pH 10.1; Eh –200 mV, oxygen content of 0 mg O_2/L, salinity of 31 g/L
White arrows - Migration of zooplankton in the anaerobic zone

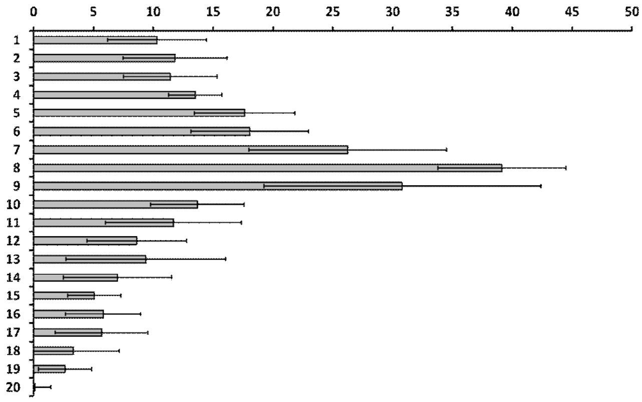

Figure 2. The dynamics of rotifer abundance in the different layers of aquarium. Photos were done with camera Fuji Finepix S9500. To estimate the vertical distribution of rotifer the raw image with size of 91.4 × 68.6 cm was adjusted to size of 30.0 × 22.5 cm. Grid lines were drawn through 1 cm (as shown at Fig. 1), the upper left corner was accepted as the starting point. The square identification was done according to their position respectively the width and height of the image. The white point with steep contours were identified as an individual rotifer.

CONCLUSION

The results of this study showed that in laboratory microcosm the meromictic conditions could be established. High light intensity in the anaerobic zone below the chemocline lead to the formation of a brightly colored pink layer of dominant morphotypes which were similar to purple sulfur and non-sulfur bacteria detected but were not dominant in the lake. The experiment revealed the adaptation potential of natural microbial community in Lake Doroninskoye, in which microbial species diversity and microorganisms with a specific set of biochemical characteristics are presented. These characteristics provide benefits such as flexibility in adapting to different environments under varying conditions. The active migration of the zooplankton to the anaerobic layer have been also observed. The microcosm experiment has been clearly demonstrated that animals perform individual migrations to the anoxic layers. Feeding on the abundant microbial community is the most plausible explanations of these migrations. Thus, the laboratory microcosms could be used to model and investigate the oxic-anoxic interfaces, the mechanisms underlying the formation of chemocline communities and interactions between different organisms in such systems. Our experiment has been demonstrated that the light penetration into the water column and anaerobic zone illuminance control the structure of microbial community in the chemocline.

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