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- > abiotic and biotic water components;
- > ecosystem-level studies;
- > systematics and aquatic ecology;
- > paleolimnology and environmental histories;
- > laboratory experiments and modeling



Dear colleagues,

The risks of climate change associated with freshwater resources increase significantly with growing concentrations of greenhouse gases. In the 21st century, along with rising temperatures, there will be an increase in the proportion of the global population suffering from scarce water resources. According to long-term estimates, the climate change during the 21st century will lead to a considerable reduction in the number of renewable resources of the surface waters, hence, strengthening the competition for water between regions. The main scientific trend of the past decades is the concept of the adaptation mechanism of aquatic ecosystems under the instability of the current global climate and the increasing anthropogenic load. Thus, most experts from Intergovernmental Panel on Climate Change (IPCC) believe that due to the climate change during the 21st century and beyond much of the freshwater species will face the high risk of extinction especially resulted from the interaction of the climate change with other stress factors, such as habitat change, overexploitation, pollution and invasive species.

In this regard, we consider extremely relevant to widely cover the issues concerning the functioning of freshwater ecosystems. The journal «Limnology and Freshwater Biology» will provide an opportunity to quickly publish the original scientific papers on the research of biotic and abiotic components of water bodies. Although most of the articles published in the journal deal with the study of lakes, we can accept the works related to the study of ecosystems of rivers, wet-

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The scope of the journal comprises:

- hydrological, hydrochemical and geochemical aspects of aquatic ecosystems;
- molecular and classical biology of ecosystem research;
- systematics and ecology of aquatic ecosystems;
- natural and anthropogenic impacts on water systems and resources;
- paleolimnology and history of the environmental development;
- field and laboratory studies, and modelling.

The journal is published every two months. We invite you to take part in this journal, both as authors of various materials and reviewers.

Director of Limnological Institute SB RAS,
D.Sc. Andrey P. Fedotov

Hydrochemical studies in Lake Baikal: history and nowadays

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ABSTRACT. This work is about the history and the main stages of the hydrochemical studies in Lake Baikal for the last 90 years. The first studies of Baikal water chemistry, including its deep-water zone, had been carried out at the late 1920s under the guidance of G. Yu. Vereshchagin. At the same time, the guide that was used during many years by chemical hydrologists for water chemistry analyses not only on Baikal had been developed. The long-term hydrochemical research of 1950s-1960s gave data on background concentrations of chemical components both in the lake and in its tributaries. The nowadays data evidence that the content of major ions in the deep-water zone of Lake Baikal is constant. It is found that the temporal and space changes of nutrients concentrations in the pelagic zone of the lake depend mainly on phytoplankton growth, whereas the current increase of nutrients concentrations in the littoral is mainly induced by human activities and the development of tourism.

It is found that the Selenga River, the largest tributary of the lake, undergoes the highest anthropogenic load. The pollutants, which enter the river in Mongolia, affect the content of major ions, nutrients and organic matter on the territory of Russia up to the mouth; more pollutants are added by Russian local wastewater sources. Amid the ongoing low water levels and increased anthropogenic load, the annual average concentrations of mineral phosphorous in the Selenga River show a trend to decline, whilst the content of phosphorous organic compounds increases. The efflux of mineral and total phosphorous by the Selenga is governed by changes of the water runoff. Changes in chemical composition of low-mineralized rivers of South Baikal (Khara-Murin and Pereemnaya) whose water catchment areas were affected for many years by emission of the Baikal Pulp and Paper Mill and by transfer of pollutants from the industrial complexes of Pribaikalye are registered.

Keywords: Lake Baikal, chemical composition, major ions, nitrogen, phosphorus, tributaries, biogenic elements

1. Hydrochemical studies in Lake Baikal and its tributaries in 1926-2000.

In terms of water chemistry, Lake Baikal is one of the most well studied lakes of the world. The hydrochemical studies of the lake and its tributaries began in 1925 with the organization of the Baikal expedition of the USSR Academy of Sciences. In 1926, A.G. Frank-Kamenetsky first obtained the data on the chemical composition of Baikal deep water from a depth of 1000 m. In 1927, G.Yu. Vereshchagin, director of Baikal Limnological Station (BLS) of the USSR Academy of Sciences, characterized the water of the deep zone in South Baikal as low mineralized, with content of major ions, such as bicarbonate, calcium, sulfate and magnesium, less than 100 mg/L. Other components, such as oxygen, free carbon dioxide, silicon, and organic matter, changed in the abyssal zone (Vereshchagin, 1927). At the same time, T.B. Forsh started studying the lake tributaries and obtained the most significant

results. The chemical composition of the tributaries was compared with geological structure of their basins; there was an increase in mineralization of river waters during wintertime. G.Yu. Vereshchagin and I.P. Sidorychev carried out more detailed investigations of the Selenga River, in which they paid a special attention to its gas regime (Vereshchagin and Sidorychev, 1929). In 1930, G.Yu. Vereshchagin headed the development, publication and translation into foreign languages of a manual on standard chemical methods of field water analyses, which for many years served as a handbook for chemical hydrologists (Vereshchagin, 1933). Unfortunately, many materials on the Baikal water chemistry remained unprocessed and then were lost.

The next stage in the hydrochemical studies began in 1948, when a new director of BLS chemical hydrologist V.A. Tolmachyov initiated studies of water chemistry at a longitudinal section across the lake, monitoring observations near the Listvyanka settlement, studies of the largest bays and tributaries of Lake Baikal, as well as the Angara River. The studies carried

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out on the instructions of government bodies ahead of designing the Irkutsk Hydropower Station (Tolmachyov, 1957).

In the late 1940s – early 1950s, the chemical hydrologists I.V. Glazunov and K.K. Votintsev started working in BLS. The publications by I.V. Glazunov were notable for versatility, singularity of reasoning, thorough collection and processing of materials. He collected data on water chemistry of the Listvennichny Bay, the Angara River head and the main tributaries of the lake. He first found an inflow of the Selenga waters distributed in 25-50 m layer in the area of the Listvennichny Bay, summarized results of investigations of the hydrological regime of the Angara River, and quantified the chemical flow from Lake Baikal (Glazunov, 1963). One of the first publications by K.K. Votintsev was the study of the Baikal ice phase. Based on the materials collected during 1948-1955, he wrote the monograph “Water chemistry of Lake Baikal” (Votintsev, 1961), where he examined in details the chemical composition of water, vertical distribution, seasonal and daily fluctuations of specific components, gave a general hydrochemical characteristic of open Baikal and isolated areas of the lake (bays, coves). He also briefly described the composition of bottom sediments and interstitial waters, discussed hydrochemical zonal distribution of the lake water masses, examined the water chemistry of the largest tributaries and the chemical composition of precipitations, and quantified the chemical balance of Lake Baikal. Great attention was paid to the dynamics of nutrients, silicon migration pathways, cycles of organic matter and oxygen. This book was for decades and remains nowadays a handbook for those who study Baikal.

In the late 1950s – early 1960s, A.I. Meshcheryakova, N.V. Verbolova, T.P. Kozhevnikova, and L.A. Gorbunova continued hydrochemical investigations. Their hands sampled tons of water and carried out thousands of analyses that became a basis for characterization of the hydrochemical regime of the whole lake up to 300- m depth and the abyssal zone of South Baikal up to the depth 1400 m (Votintsev et al., 1963). They processed and summarized all materials on water chemistry of Baikal tributaries available at BLS. This work resulted in publishing the monograph “Water chemistry of rivers of the Baikal catchment area” (Votintsev et al., 1965). This monograph characterizes the water chemistry of 250 tributaries of the lake and of a few rivers of its catchment area. It describes in details the hydrochemical regime of 18 main tributaries that were regularly monitored for many years. All the tributaries were divided into 5 groups and referred to 5 types according to their water composition and hydrochemical regime. Until now, this book has not lost its importance being the integral reference book on the water chemistry of surface waters in the region. These materials were used as a basis for hydrochemical mapping of the Baikal catchment area and for the map of hydrochemical facies (Atlas of the Irkutsk Region, 1962). The design and construction of the Baikalsk Pulp and Paper Mill triggered systematic investigations of the southern end of Lake Baikal and its tributaries in

the area in 1960-1963. The construction of the mill also initiated the studies of possible impact of its effluents on the composition of Baikal water. This became one of the main research directions for many years, and when the mill began operating, they took form of regular observations.

Since 1962, synchronous limnological surveys on 12 transversal profiles across the entire Baikal water area were carried out for several years. They were carried out simultaneously on six vessels, and this allowed getting an “instant picture” of the whole state of Baikal. The results were included to the report of the National Program of the USSR, which was performed within the framework of the International Biological Program. At the same time, the data on annual integrated monitoring at the network of regular stations in the Selenga area, the most important fishing area of Baikal, were summarized (Votintsev and Glazunov, 1963). In 1971-1974, these studies were continued (Bogdanov, 1972) and included the Selenga shallows, as well as the sors (lagoons) Proval, Istok and Posolsk, where changes in hydrochemical composition associated with the rise of water level in Baikal after the construction of the Irkutsk Hydropower Station Dam were recorded. The construction of the Baikal-Amur Main Line (BAM) and its influence on the water chemistry of North Baikal gave rise to investigations of the northern end of Lake Baikal and its tributaries. The comprehensive long-term studies at the Baikal portion of BAM allowed developing recommendations for reducing negative effects of the construction activities on the water quality in the basin of the Upper Angara River, which is the main tributary of North Baikal (Bogdanov, 1978).

In the early 1960s, new fields of research appeared in the water chemistry: chemistry of interstitial water, exchange processes at the water-bottom interface, processes of early diagenesis in sediments, and biogeochemical migration of individual chemical elements in the Baikal ecosystem, which were headed by I.B. Mizandrontsev. He and his team studied the composition of bottom sediments, their physical and chemical properties, processes of consolidation and transformation, as well as diagenetic transformations of iron and manganese. They gave a theoretical description of the matter exchange between the water and bottom in the oxygen-depleted hypolimnion of some water bodies. They made a theoretical analysis of the influence of sedimentation conditions on the physicochemical environment and functioning of the benthic organisms (Mizandrontsev, 1990).

In 1965, E.N. Tarasova headed the studies of organic matter: the direct measurements of organic carbon, COD, organic nitrogen and phosphorous concentrations were introduced both in lake water and in the suspensions of the water column (Tarasova, 1975). Developing studies of balance and cycling of organic matter in Baikal K.K. Votintsev quantified the role of organic matter in the biological productivity of the lake. In this regard, he proposed a general quantitative scheme for the bioenergetic transformation of organic matter in food chain of the ecosystem in the pelagic zone of the lake (Votintsev, 1971; Votintsev et al.,

1975). Since 1976, the studies have been resumed on the chemical composition of precipitation and eolian particles falling onto the surface of the lake (Votintsev and Khodzher, 1981). These studies allowed clarifying the role of the atmospheric component in the chemical balance, assessing the changes since 1950s, indicating local atmospheric pollution, and detecting its sources (Khodzher, 1988). In 1975-76, the draft of the National Baikal Water Quality Standard was developed. Mathematical processing of more than 700 complete analyses of the lake water yielded average values and confidence intervals for individual chemical components and confirmed their homogenous distribution both throughout the lake water area and by depths (Votintsev and Mizandrontsev, 1981).

The study of the formation of the hydrochemical regime of the Angara River and the Angaro-Yenisei basin was one of the important research areas of the Laboratory of Hydrochemistry and Atmosphere Chemistry. The most extensive studies concerning forecast and formation of the hydrochemical regime in the Bratsk, Ust-Ilimsk, Sayano-Shushensk and Kureisk reservoirs were carried out in 1980-1990. The studies at the Nizhaya Tunguska and the Lower Yenisei Rivers served as a basis for predicting the water quality of the intended Turukhansk Reservoir. The data obtained during these studies in cooperation with other institutes of the Siberian Branch of the Academy of Sciences of the USSR for the first time in Soviet limnology prevented the construction of this hydroelectric power plant.

From the beginning of the 90s, a new stage of hydrochemical research began, a distinctive feature of which was the development and implementation of new methods of analysis, careful quality control of the data obtained, and close cooperation with foreign scientists. Thus, in the late 80s and early 90s, within two international expeditions there were studies of the major ions and trace elements in the pelagic zone of the lake from the surface to the bottom along all three basins. The results of these studies were published in the journal *Limnology and Oceanography* (Falkner et al., 1991; 1997), and they once again confirmed the conclusions of the previous long-term studies that the concentrations of major ions in different basins and at different depths of open Baikal are the same. M.A. Grachev summarized the results of international studies at Lake Baikal performed in the 80s and 90s were in the monograph "The current state of ecological system of Lake Baikal" (Grachev, 2002), which provides an overview of the main data on the chemical composition of Baikal water that had been obtained by Russian and foreign researchers.

2. Hydrochemical investigations of Lake Baikal and its tributaries in 2000-2017.

Since the beginning of the 2000s, the main hydrochemical studies have been expanded both by objects and by territory studied and are currently being carried out in the following areas: Water chemistry of Lake Baikal and its tributaries under climate change and anthropogenic load; The chemical composition of the

atmosphere in the Baikal Natural Territory, including analysis of the composition of gas impurities, aerosols, precipitation and snow cover; Geochemical processes at the water-air and water-bottom interfaces, chemical composition of Baikal pore waters; Hydrochemical regime and water quality forecast of Siberian reservoirs.

The measurements carried out at present using the methods developed in the Institute have high accuracy: up to 15% for biogenic elements and 5-7% for major ions (Baram et al., 1999; Vereshchagin et al., 2000). Comparison of the 1940 – 1960 data with modern ones showed that the results of analyses performed by classical methods of the early 20th century are comparable with the data obtained today. Over the past 18 years, Russian and international qualification comparative tests monitor the results of annual chemical water analyses. According to the international qualification comparative tests, the deviations of the analysis results from the true values in general do not exceed 10-15%, which indicates the reliability of the data on the hydrochemistry of natural waters (Khodzher et al., 2004).

Currently, the study of the chemical composition of Baikal waters and its tributaries remains the urgent task due to the climate change and the increasing anthropogenic load on the lake ecosystem. Due to the long time of water exchange in Baikal (more than 300 years), the content of major ions in its water column remains constant, without variations by depths and basins. The regular expeditions around Baikal carried out at 20 deep-water stations and 7 cross sections across the lake confirm this feature. Seasonal sampling is performed in the mouths of large tributaries and the littoral zone of the lake. During each expedition, 350-400 water samples are taken. Gas composition and nutrients are analyzed onboard, and macro and microelements are analyzed in the laboratory (Khodzher et al., 2017). Table 1 shows the content of major ions in the pelagic zone of Lake Baikal. It indicates that the water composition in the abyssal zone of Lake Baikal currently remains constant. Statistical processing of the data showed that the variations in alkalinity and concentration of sodium, calcium, magnesium, and sulfate ions in Baikal water do not exceed $\pm 5\%$. The statistical error is significantly more, up to 10%, in determining K^+ and Cl^- , which concentrations are low (Grachev, 2004).

In contrast to the major ions, the content of oxygen and biogenic elements in the Baikal water has an obvious vertical stratification and seasonal dynamics due to biological processes and the dynamics of the lake water masses. During 2014-2018, more than 1500 individual water samples at 20 deep-water stations from 10-14 depth horizons at each station were taken and analyzed for silicon and various forms of phosphorus and nitrogen. In spring, the maximum concentration gradient of mineral phosphorus is observed in the upper 200 m layer in the southern and central basin, and in the upper 100 m layer in the northern part of the lake due to its later ice-breakup (Fig. 1). The content of mineral phosphorus increases with depth and in the near-bottom area is 15-16 $\mu\text{g/L}$ in the south and north basins, and 18-20 $\mu\text{g/L}$ in the central basin of the lake. Interannual variations of the average concentration of

Table 1. Concentrations and the sum of major ions in the pelagic area of Lake Baikal measured in different years, mg/L

Authors	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	Σ _i
Vereshchagin, 1947	15.2	4.1	3.9	2.3	63.6	0.7	5.0	94.8
Votintsev, 1961	15.2	3.1	3.8	2.0	66.5	0.6	5.2	96.4
Falkner et al., 1991	16.1	3.1	3.6	0.9	66.7	0.4	5.5	96.3
Grachev et al., 2004	16.4	3.0	3.3	1.0	66.6	0.4	5.2	95.9
LIN SB RAS data, 2015-2016	16.0	3.1	3.4	1.0	64.9	0.5	5.4	94.3

mineral phosphorus throughout the water column, except for the upper 100 m layer, are insignificant and do not exceed 8% of the average value in each basin. In the upper 100 m layer of the southern basin, these variations are higher (up to 30%) due to the different development intensity of spring phytoplankton.

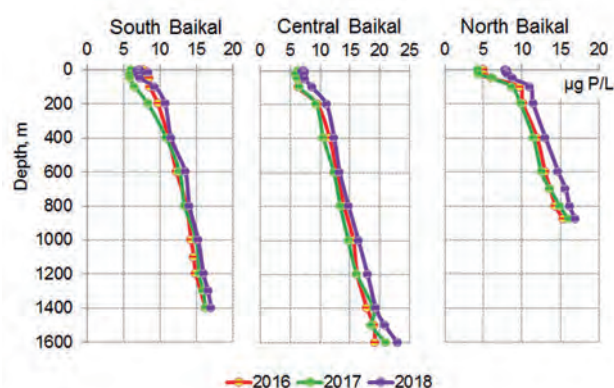
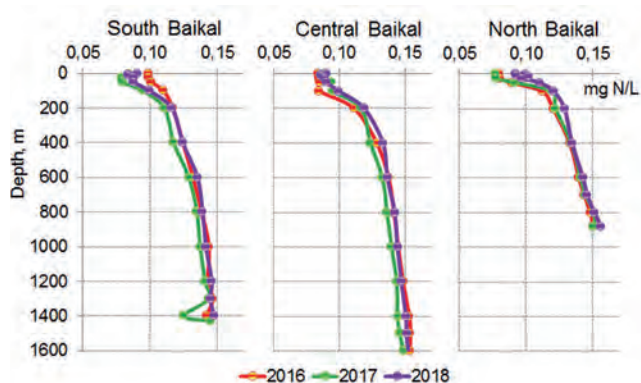
The distribution of total phosphorus is similar to mineral. The content of organic phosphorus in the deep-water area of the lake is 2-3%, and in the upper 100 m layer it reaches 20-30%.

Mineral forms of nitrogen in Baikal are mainly represented by nitrate. The seasonal dynamics and vertical distribution of nitrate are similar to those of mineral phosphorus, with minimum values in the surface layer and maximum values in the near-bottom waters (Fig. 2). The concentration of nitrates in the near-bottom water reaches 0.16 mg N/L; in the upper water layer it varies from 0.01 to 0.07 mg N/L depending on seasons. Interannual variations of the nitrate concentrations throughout the water column are also insignificant and do not exceed 8%.

Small amounts of ammonium and nitrite nitrogen are found only in the upper water layers, mainly, at the end of the algae vegetation period, as well as in the bottom horizons. The littoral zone of the lake usually contains ammonium and nitrite nitrogen, but the content of these forms of nitrogen is insignificant.

At present, there is a problem of pollution in the Baikal littoral zone driven by discharge of insufficiently treated wastewaters from towns and settlements located on the coast, as well as increase in tourist load and vessels, which negatively affect the ecosystem of the lake. Climate change aggravate negative consequences affecting the water regime of the lake and its catchment area, duration of the ice period, temperature of the upper water layers, and productivity processes. Over the past decade, detailed seasonal studies of the dynamics of biogenic elements and gas composition in the Baikal littoral zone were conducted in the area of South Baikal (Golobokova et al., 2009; Domysheva et al., 2010; 2012; 2013; Sakirko et al., 2015).

The most detailed seasonal studies in the littoral zone were conducted near Bolshiye Koty (western shore of South Baikal). Figure 3 shows the results of measurements of the surface and near-bottom water performed each 3 hours within 24 hours in separate cycles during 2013-2015. The content of biogenic elements clearly shows the annual variation with a maximum in the winter and a minimum in the summer.

**Fig. 1.** Mean mineral phosphorus concentration in the individual basins of Lake Baikal in May-June 2014-2016.**Fig. 2.** Mean nitrate concentration in the individual basins of Lake Baikal in May-June 2014-2016.

During intensive development of phytoplankton, there were significant diurnal changes in pH values and saturation of water with oxygen.

In addition, in June and September 2014-2016 the investigations were carried out in the littoral zone of more than 30 permanent stations around the lake. Analysis of the results indicated that in June the concentrations of biogenic elements and organic matter in the littoral water were significantly higher than in September due to the intra-annual dynamics of the components concentrations in Baikal water and the coastal runoff in spring and summer, when the lake level rises. During the observations, the phosphate concentrations varied from 2 to 11 µg P/L in June, and in September

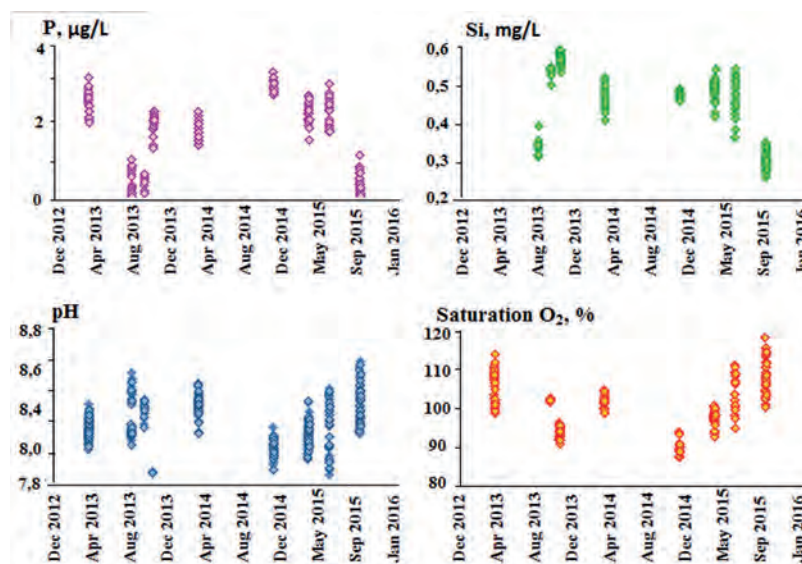


Fig. 3. Concentrations of mineral phosphorus, silicon, pH value and oxygen saturation in the littoral zone of South Baikal (Bolshye Koty), 2013-2015.

they did not exceed 7 µg P/L. The total content of mineral nitrogen ($\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$) varied from 0.01 to 0.3 mg N/L. To determine reference concentrations of biogenic elements in the littoral water of Baikal, 63 water samples at 23 stations located outside the zone of influence of permanent settlements and towns were collected and analyzed. The results showed that the phosphate concentrations were in the range 0-6 µg P/L in 90% of the analyzed samples; the content of mineral nitrogen did not exceed 0.05 mg N/L in 89% of the samples (Fig. 4).

The determination of the N and P reference concentrations allowed assessing the situation in the littoral zone under anthropogenic pressure. Despite the high phosphate concentrations in some seasons, their average values did not exceed reference values during the period of observations in these areas. At the same time, the average concentrations of mineral nitrogen can be 2.5-3 times higher than reference values (Fig. 5).

Notably, in the coastal water near the settlements of Khuzhir, Maksimikha, Sakhyurta, Severobaikalsk, Kultuk and others, there were rather high concentrations of ammonium nitrogen (up to 0.04 mg N/L). In certain periods, ammonium nitrogen dominated nitrates, which is not typical of well-aerated Baikal waters with a high pH value.

One of the most important abiotic factors that determine the chemical composition of Baikal water is the river runoff. The waters of most rivers as well as those of the lake by relative content of major ions correspond to the bicarbonate class of the calcium group. The main regularities and the first most complete data on the formation of chemical water composition of the tributaries were obtained in 1940-1960 (Bochkarev, 1956; Votintsev et al., 1965). These data characterize the natural regional chemical composition of river waters and can serve as a reference in assessing its changes caused by the development of industry and agriculture in river basins. In the late 1990s, the concentration of sulfate and its runoff into the lake with the waters of the Selenga River, the main tributary of the lake,

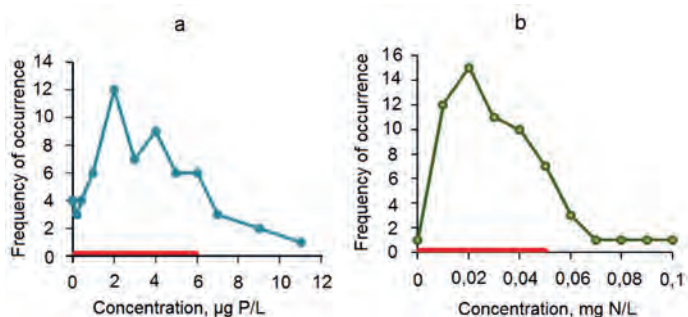


Fig. 4. Variation curves reflecting the distribution of phosphate (a) and mineral nitrogen (b) concentrations in the littoral water samples.

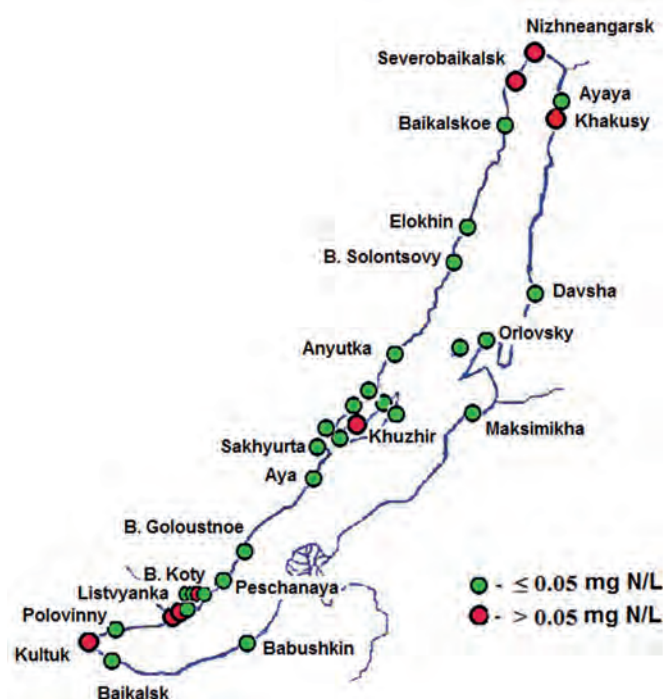


Fig. 5. Mean mineral nitrogen concentrations in the coastal water of Lake Baikal, 2014-2016, mg/L

already twice increased compared to Votintsev et al. (1965), nitrate nitrogen – by 57%, mineral phosphorus – by 42% (Sorokovikova et al., 2000a; 2000b). In the past two decades, the chemical composition and hydrochemical regime of the rivers in the Baikal catchment area are formed under climate change, prolonged low water level and increased anthropogenic stress. The Selenga River experiences the highest technogenic load due to intensification of economic activity both in the Russian and Mongolian parts of the river. In the 1990s, the sulfate concentrations at the Naushki settlement bordering with Mongolia remained at the reference levels of 7.5-10.5 mg/L (Sorokovikova et al., 1995); by 2010 they increased to 10.6-16.4 mg /L (Tomberg et al., 2010; Sorokovikova et al., 2013), and in 2018 they were 17.1–21.7 mg /L. The increase in anthropogenic load under the low water conditions also caused an increase in concentrations of biogenic elements in the water of the Selenga River and their runoff into the lake.

In 2010-2016, the average runoff of total phosphorus into the lake increased and was 1770 tons/year. At the same time, the minimum runoff (670 tons) occurred in an abnormally low water level in 2015, whereas the maximum one (3200 tons) – in 2013 (Fig. 6). The average runoff of mineral phosphorus during this period reduced to 206 tons/year, i.e. 34% of the corresponding value of the 1990s (Sorokovikova et al., 2000b). Obviously, the structure of phosphorus intake into the lake changed: the runoff of its mineral compounds decreased, while the runoff of its organic compounds increased. The excess of the runoff of organic phosphorus over mineral phosphorus in the low-water year was 769 tons, in the high-water year – 2886 tons, whereas in 1983-1984 the excess was at the level of 120 tons (Tarasova and Meshcheryakova, 1992).

The concentrations of the major ions and their total content in the Barguzin and Upper Angara rivers and their tributaries changed slightly in the perennial aspect, except for sulfate concentration, which increased in the Barguzin River (Drucker et al., 1997; Sorokovikova et al., 2015). The seasonal dynamics of concentrations of biogenic elements in the rivers not subjected to pollution by sewage water is mainly determined by changes in water runoff and the intensity of plankton development. The smallest concentrations of biogenic elements were determined in the water of the Upper Angara River due to the low development of its basin.

The rivers of South Baikal (Snezhnaya, Utulik, Khara-Murin, Solzan, Pereyomnaya) are characterized by low mineralization (14-115 mg/L) due to the significant heights of their basins and higher moisture. For fifty years, the catchment areas of these rivers were under influence of emissions from the Baikalsk Pulp and Paper Mill and the regional transfer of pollutants from the Angaro-Cheremkhovsky industrial complex, which affected the chemical composition of precipitation and, consequently, the composition of river waters (Sorokovikova et al., 2004). Acidic precipitation (4.7-5.3 pH) with a high content of nitrogen and sulfur compounds made the extremely low mineralized waters of

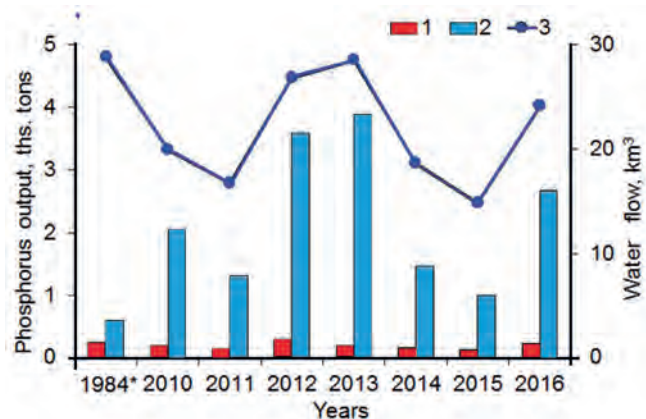


Fig. 6. Changes in the mineral (1) and total (2) phosphorus runoff by the Selenga River in years with different water content (3)

the Khara-Murin and Pereemnaya rivers less resistant to acidification. The waters of these rivers correspond to the sulfate class of calcium group for a significant part of the year. The waters of the Solzan, Utulik and Snezhnaya rivers have a higher buffer capacity; their composition remains stable and corresponds to the bicarbonate class of calcium group (Obolkin et al., 2016; Tomberg et al., 2016).

Increasing economic and tourist activities in Listvyanka (the western shore of South Baikal), particularly, the construction of hotels and the discharge of untreated wastewater, led to an increase in the nitrogen and phosphorus content in the estuaries of the Krestovka River, as well as in Kamenushka, Malaya Cheremshanka and Bolshaya Cheremshanka streams flowing into Lake Baikal (Chebunina et al., 2018). In recent years, Listvyanka has been experiencing an “environmental crisis” – the death of endemic plant and animal species and their replacement by widespread species, one of the reasons for which may be an increase in biogenic elements in the Baikal littoral zone (Kravtsova et al., 2012; 2014; Khanaev et al., 2018).

3. Conclusion

Long-term hydrochemical studies conducted in the Limnological Institute and analysis of recent years (2014-2017) showed that the Baikal deep waters are low mineralized and belong to the bicarbonate class of calcium group. The content of the major ions (calcium, magnesium, sodium, potassium, bicarbonates, sulfates, and chlorides) is constant throughout the water column of the abyssal zone of the lake. There is small number of biogenic elements (nitrogen, phosphorus, and silica), and their concentrations increase with depth. The temporal and spatial dynamics of biogenic elements in the trophogenic layer is due to the intensity of phytoplankton development.

Though the pelagic zone of the lake remains stable, the problems of local pollution of the coastal waters of the aquatic area with biogenic elements, organic matter coming along with the tributaries from settlements, tourist complexes located on the coast of the lake, and the number of ships constantly increasing remain the main thread for the ecosystem of the lake.

Changes in the littoral zone of the lake increase under climate warming.

In the 1970-1980s, the development of industry and agriculture in the basin of the main tributaries of the lake caused an increase in anthropogenic load and decrease in the water quality. In recent years, there is a high content of major ions in the waters of the Selenga River due to a decrease in the river water level and an increase in the groundwater supply. Concentrations and the runoff of mineral phosphorus by the river into the lake decreased, whilst the intake of total phosphorus, organic matter and phytoplankton biomass increased. The content of total phosphorus in certain periods reaches the values characteristic of eutrophic waters. The influence of small tributaries on the chemical composition of the chemical composition in the lake water is insignificant and is found only in the estuaries.

Hydrochemical studies of the Baikal water and its tributaries performed in the monitoring regime with the modern analytical equipment and by high-precision analytical methods provide reliable results on the state of the lake ecosystem. This work is extremely important and will be continued in order to develop a strategy to protect the lake from pollution.

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Heat balance of Lake Baikal and the relationship of its ice-thermal and water regime with global atmospheric circulation in the Northern Hemisphere during the modern period

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ABSTRACT. The article presents a summary of the most important results obtained during the studies of the heat balance at Lake Baikal since the 1930s. The systematic regime measurements of water temperature initiated in Limnological Institute since 1970 indicated the features of the spatial structure and temporal changes in the elements of the Baikal heat balance. We have determined the magnitude of the annual evaporation from the surface of Lake Baikal by the independent method considering the data on temperature of the water column. We have investigated one of the main renewal processes of deep and near-bottom Baikal waters associated with thermobaric effect. As a result of this effect, the water renewal and aeration in the near-bottom layer annually occurs under certain hydrological conditions. We have determined the vertical flows of mass, heat and gas exchange of the upper and deep layers in Lake Baikal.

We have studied the effect of changes in the characteristics (indices) of the atmospheric circulation in the Northern Hemisphere on the variability of the characteristics of climate and ice-thermal regime at Baikal, i.e. air temperature and water surface temperature, ice thickness, as well as dates of ice phenomena during the modern period. Anomalies of the atmospheric circulation are most obvious in the ice processes. We have found the relationship between the water temperature of the warm (May-September) period and the indicators of the zonal circulation (positive relationship), as well as indices of the formations blocking the western transfer (negative relationship). There is an obvious effect on the water surface temperature of the winter circulation indices. We have shown that these changes are associated with the fluctuations in the intensity of the zonal circulation and the baric formations blocking them. There is the effect of circulation on the moisture characteristics (precipitation and runoff), but it is complicated by large differences in climate within the Baikal catchment area.

Keywords: Baikal, water-atmosphere heat fluxes, evaporation, intrusions and renewal of nearbottom waters, atmosphere circulation, hydrological processes

1. Introduction

In 1934, Russian Academy of Sciences convened the first meeting on limnology. G.Yu. Vereshchagin and L.L. Rossolimo much contributed to its organization. The meeting dealt with the problems and objectives of limnological research, as well as methodology issues. G.Yu. Vereshchagin identified the research areas concerning Baikal (Vereshchagin, 1937) and headed their development at the Baikal Limnological Station (BLS):

- 1) the origin and history of Baikal, as well as of its flora and fauna;
- 2) specific lacustrine processes characteristic of deep lakes;

- 3) the relationship of the lake water masses with the environment;
- 4) limnological forecasts.

Work in areas 1) and 2), had not only theoretical, but also practical importance, since their results could be used to explain the rational development of natural (water, biological, recreational, etc.) resources of Baikal and Siberia. Such work required a significant expansion of the material and human potential of the research team, which could not be implemented in the prewar period. This became possible only in the 60-70s of the past century, when the demand for the rapid development of the natural Baikal resources, primarily the hydropower resources of the Angara River, increased significantly. During this period, the Baikal

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Limnological Station (which has become Limnological Institute since 1961) significantly boosted the personnel and technology. The Institute received almost all-weather vessel with modern navigation and scientific equipment for physicochemical and biological investigations throughout the water column.

2. Heat balance of Lake Baikal

The first stage in this research area was a comprehensive study of the heat balance in Baikal (Verbolov et al., 1965). Data were obtained on the average thermal interaction of the lake with the atmosphere for the long-term period and in certain years (Verbolov et al., 1965). For the complete closure of the heat balance equation, one of the main components, the annual heat budget of the entire water mass of Baikal, was determined experimentally (Shimaraev, 1996). It was based on the long-term systematic observations of the water temperature in the pelagic zone of the lake in 1950-1996. Considering these data, the average value of annual evaporation from the surface of Lake Baikal (400 mm), was first determined by the independent heat balance method.

Temperature monitoring in all parts of the lake identified the main processes of vertical heat exchange, as well as patterns of structure and dynamics of the temperature field at different depths (Shimaraev, 1977; 1996; Shimaraev and Granin, 1991; Shimaraev et al., 1994).

The analysis of the stratification conditions and the mechanism of free convection has implied the division of the Baikal water column into two main zones by the processes of heat transfer. In the upper zone, the water temperature (T) twice a year passes the value T_{md} (3.96 °C), which is accompanied by the change in T stratification (in spring – from reverse to direct, and in autumn – from direct to reverse) and development of free temperature convection (Shimaraev et al., 1994). Convection in combination with wind mixing in the spring provides active heating, and in the autumn – cooling of the waters in the upper zone. In the spring, convective-wind mixing is limited to the position of the mesothermal maximum temperature (T_{mm}), average 220-250 m, with a temperature $T_{mm} = T_{md}$, that was formed by convective-wind mixing at the end of the previous year. Such depth determines the dimensions of the upper zone of Baikal, which normally exceeds average and maximum depths of most deep freshwater dimictic lakes in the world.

3. Intrusions

Deep zone of Lake Baikal is close to warm tropic lakes or seas by the constancy of direct temperature stratification ($T > T_{md}$), positive or negative coefficient of thermal expansion and weak stability. Thermal convection in this zone can occur as a forced convection, when cold water with $T < T_{md}$ ascends from the upper zone (Shimaraev and Granin, 1991; Weiss et al., 1991). This ascending is possible with vertical circulations in the field of currents (upwelling), with water compac-

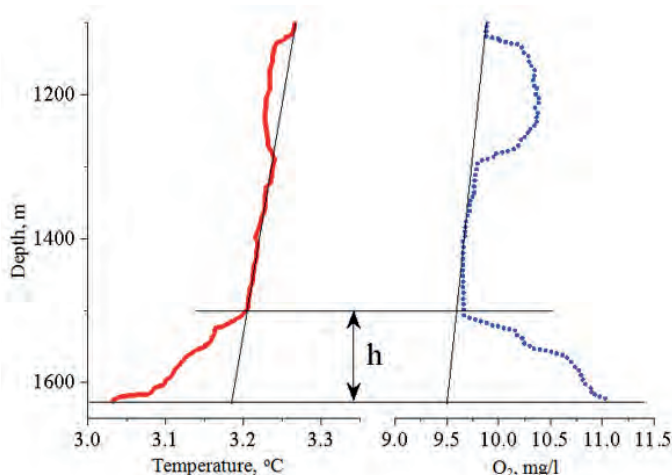


Fig. 1. Vertical distribution of temperature T and concentration of dissolved oxygen O_2 in near-bottom cold layer h (1500-1626 m) in 7 km from Izhimey Cape of Central Baikal on June 6, 2012; secondary intrusion is recorded by decrease in T and increase in O_2 in a layer of 1130-1300 m.

tion at the front of the spring thermal bar and strong wind forcing. The ascending leads to violation of the stratification stability (thermobaric instability) at the upper boundary of the zone as well as occurrence of intrusions of cold water lowering to the horizon with the equal temperature (density) and cooling the waters in deep and near-bottom zones of Lake Baikal. Deep intrusions are associated with the renewal of deep waters and formation of a cold near-bottom layer with a thickness of up to 100-222 m or more (Fig. 1).

The actual data on annual heat budget of the entire water mass of Lake Baikal allowed applying the method of heat balance to independently determine the annual evaporation rate from the surface of Baikal. It was equal to 400 mm, which corresponds to calculations using semi-empirical formulas that consider the stratification character of the atmosphere in the near-bottom layer.

4. Ice-thermal and water regime of Baikal and the atmospheric circulation in the Northern Hemisphere in 1950-2017.

The problem of the effect of climate on lakes is one of the pressing problems in limnology. For Baikal, this effect is revealed as a change in the abiotic components of the ecosystem, which can affect the biota of the lake (Shimaraev, 1977; Afanasyeva, Shimaraev, 2006; Izmet'seva et al., 2015). Structure of modern climate changes includes a 'secular' trend of warming and intrasecular fluctuations exceeding this trend in scale that are caused by the atmospheric circulation. We have studied the effect of changes in the global atmospheric circulation in the Northern Hemisphere on the ice-thermal and water regime of Baikal from the middle of the 20th century to the present.

Regression equations of air temperature (T_a) with circulation indices describe 38-66 % of the changes in annual T_a , and only 17 % – in winter, spring, summer, and autumn T_a (Sizova, 2017). The main role in chang-

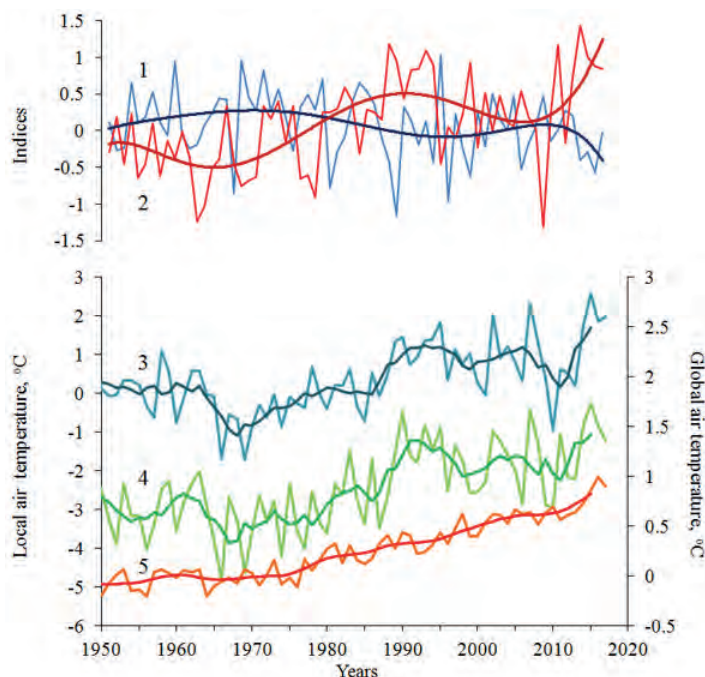


Fig. 2. Changes in SCAND (1) and NAO (2) indices in 1950-2017, as well as their polynomials and air temperatures at weather stations Babushkin (3) and Nizhneangarsk (4), as well as in the Northern Hemisphere (5), and their smoothed values for 5 years.

ing T_a belongs to the zonal transfer indices (AO and NAO), as well as the transfer blocking indices (SCAND, Sh). The effect of other circulation indicators, as a rule, is secondary in importance. The nature of the change in circulation indices and T_a indicates the end of the intasecular T_a cycle in 2010-2011, which has begun as early as in 1970 (Fig. 2).

The effect of circulation on T_a during the cold season determines its leading role in changing the characteristics of the ice regime (Livingstone, 1999; Todd and Mackay, 2003). During the intensification of the zonal transfer and increase in its indices (1970-1995), the T_a values increase, freezing dates gradually delay, the ice breaks up earlier, and the maximum ice thickness decreases (Fig. 3). Reverse trends in the changes in characteristics of the ice regime occur during weakening of the zonal transfer and strengthening of the blocking mechanisms of SCAND and Sh circulation, in 1950-1970 and 1995-2009 (Sizova, 2017).

The relationship between the date of the ice-breaking at Lake Baikal and the surface water temperature (T_w) explains the effect of the mean values of the circulation indices in December-March to the mean value of the Baikal water temperature in May-September (Shimaraev, 2007). The relationship of T_w with the zonal transfer indices NAO, AO, EA, and EAWR is positive ($r = 0.26 \div 0.54$), and with the indices SCAND and Sh is negative ($r = -0.27 \div -0.39$) in all parts of the lake. The multiple regression equations describe 31 % of the T_w variability in Southern Baikal, 48 % – in Central Baikal, and 22 % in Northern Baikal, with $S \pm 0.7-0.9^\circ\text{C}$. AO (62 %), make the predominant contribution in the southern part, NAO (61 %) – in the central part, and SCAND (70 %) – in the northern part of the lake.

Precipitation is associated with SCAND ($r = -0.32$) in the spring, with POL and PNA ($r = -0.27 \div -0.31$) – in the autumn, with AO – from May to October ($r = 0.26$), as well as with AO ($r = 0.30$) and SCAND ($r = -0.29$) – in general, during a year. The regression equations for these periods describe 14-22 % of the precipitation variability.

The circulation processes predominantly influence on the total water flow in Lake Baikal and runoff of the main tributaries from June to October ($R^2 = 0.32$, $S \pm 8.7 \text{ km}^3$). The EAWR indices influence the changes in the total annual inflow of rivers and the annual runoff of the Selenga River, WP indices – the annual runoff of the Barguzin River, and EA indices – the annual runoff of the Upper Angara River. Atmospheric circulation processes describe approximately 28 % of the annual runoff of the Selenga River, 41 % of the annual runoff of the Barguzin River, and 16 % of the

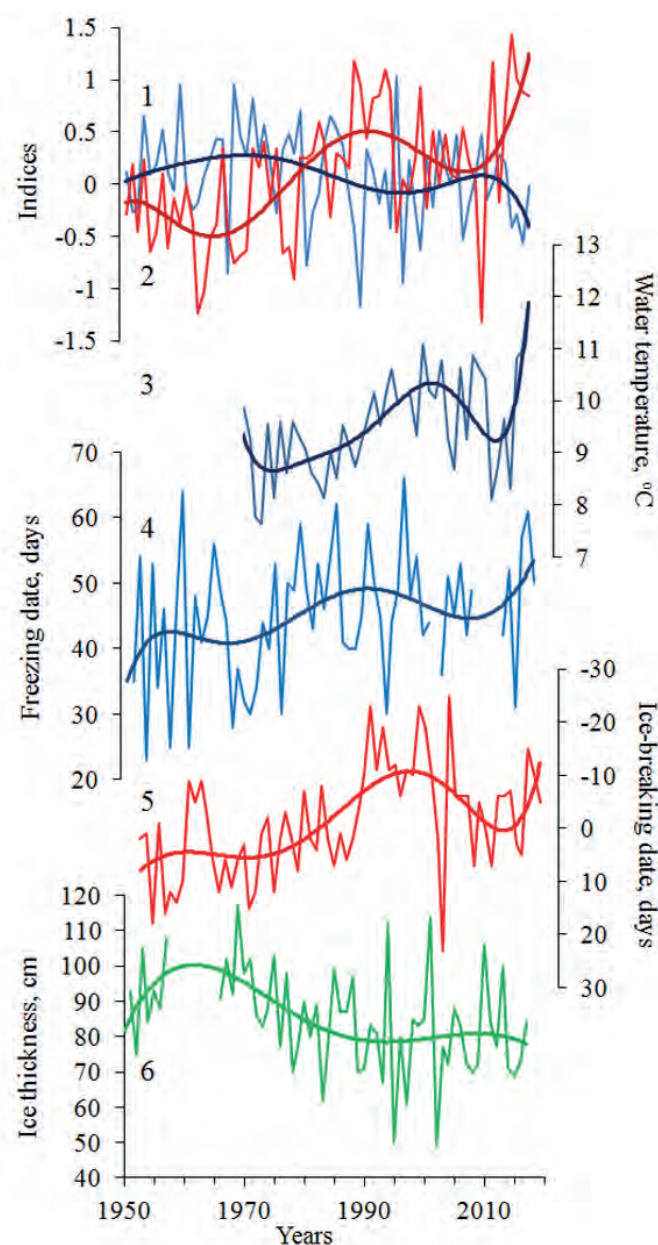


Fig. 3. Changes in SCAND (1) and NAO (2) indices in 1950-2017, as well as water surface temperature (3), dates of freezing (4) and ice-breaking (5), ice thickness (6), and their polynomials.

annual runoff of the Upper Angara River.

The role of meteorological factors for the rivers of the basin is ambiguous. Change in precipitation explains 51 % of the fluctuations in the total annual inflow into the lake, 12 % of the annual runoff of the Upper Angara River and 34 % – of the Selenga River, as well as 62 % of the fluctuations of the inflow of the Barguzin River. The effect of the air temperature on the total inflow from the entire basin and the runoff of the Barguzin River is very small, and its contribution in the variability of the inflow is statistically insignificant. In case of the Selenga River, this contribution increases up to 8 %, and in case of the Upper Angara River, it reaches 87 % (Sizova, 2017).

The reason for the differences in trends of the change in the inflow of the main Baikal tributaries is the different reaction of their water content to the warming due to differences of natural conditions in their basins.

The heterogeneity of heat-transfer properties of the underlying surface (represented by water or soils) causes a different reaction of the ice-thermal and water regime in separate parts of Lake Baikal and its basin to the climate change (Sinyukovich et al., 2013; Sinyukovich and Chernyshov, 2017; Shimaraev et al., 2018). The differences in the water regime on the territories of the Baikal watershed most clearly indicate it. Thus, in the basin of the Selenga River predominated by the territories with moisture deficit (more than 80% of the area) the warming leads to the increase in the deficit due to the high loss of moisture by evaporation, which causes a decrease in the Selenga water runoff. On the contrary, the warming of the Upper Angara and the Barguzin rivers, which basins are located within the mountain frame of the Baikal Basin having a moderate moistening, with permafrost can involve the additional moisture to the nourishment of the rivers. This is especially obvious for the Upper Angara River.

Less obvious effects include poor relationship between the thickness of the ice cover and the atmospheric circulation in Northern Baikal due to the increase in the thickness of snow on ice, which reduces the thermal conductivity of the ice-snow cover.

During the summer heating of the lake, a more active vertical exchange due to upwelling (Troitskaya et al., 2015) in Southern Baikal results from the relatively weak heating of the upper water layers compared to Central and Northern Baikal.

Intracellular fluctuations of the elements of the ice-thermal and water regime caused by the atmospheric circulation should be taken into account in the long-term prognostic estimates of the changes in the ecosystem of Lake Baikal.

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Modern changes in the ecosystem of Lake Onego with climate warming

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ABSTRACT. We have shown the changes in the ecosystem of Lake Onego¹ with climate warming over the past 30 years. Due to climate warming in winter, the river runoff increases, as well as the flow of allochthonic substances into the lake. With the increased runoff of the Shuya River, the main tributary of the Petrozavodskaya Guba (Bay), there is an additional influx of iron and phosphorus to the lake combined with the humus substances. Deep-water benthic communities in the lake become depressed. Therefore, in the past three decades the role of climate warming in the aquatic ecosystem has increased in comparison with anthropogenic impacts, which differs significantly from the changes in previous decades.

Keywords: Anthropogenic impact, climate changes, Lake Onego, eutrophication, warming, ecosystem, benthic communities

1. Introduction

Assessing the contribution of anthropogenic and climatic factors to the changes in the ecosystem of lakes is fundamental for understanding the causes and consequences of these changes and making management decisions. Lake Onego is a relatively young water body of a glacial-tectonic origin, in which the formation of flora and fauna continues at present time; there are relict forms of crustacean. Studies have shown that the changes in the hydrological regime of Lake Onego due to the regulation of the water level in the lake in the late 50s of the 20th century by the Upper Svir Hydroelectric Station are only a few tens of centimetres, and they had a little effect on the ecosystem of the lake. At the same time, increase in biogenic load and pollutants discharge caused eutrophication and pollution of the lake (The largest lakes-reservoirs..., 2015; Ladoga and Onego, 2010). The transformation process of the lake ecosystem is a sequence of stages during which the ecosystem was transformed in different ways depending on the external influences and intra-aquatic processes. The increase in the trophicity of the most contaminated bays of Lake Onego, such as Petrozavodskaya and Kondopogskaya, was accompanied by a decrease in the stability of phytoplankton and its restructuring aimed at change in balance of the ration of large and small cell species towards increase in the latter (*r-strategists*) having more opportunities for adaptation to changing environmental conditions. In 2000, with a decrease in

anthropogenic load the stability of phytoplanktonic community increased (The largest lakes-reservoirs..., 2015). The zooplankton community in the central oligotrophic areas of Onego is in a dynamic equilibrium and stable. However, with a sharp decrease in anthropogenic load after 1991 ecosystem of the lake is restored very slowly (Rukhovets et al., 2011). In the past 20 years, new scattered sources of pollution of Onego have appeared. They are trout farms concentrated in the north-western bays, where more than 30% of all trout in Karelia are grown. Decrease in the total load of phosphorus and organic matters from these farms is possible by transferring them to the sea, as in the Scandinavian countries.

The spontaneous introduction of an alien species, Baikal amphipod *Gmelinoides fasciatus* Stebbing, in the littoral zone of the lake can be regarded as an indirect anthropogenic impact on the Onego ecosystem. In areas with higher aquatic vegetation, it determines up to 80% of the number and biomass of macrozoobenthos. This species acts as a new factor of mineralization in the water body destroying up to 300 tonnes of organic matter per year. The perch nutrition includes the new species; thereby it becomes included in food chains (Sidorova and Kalinkina, 2015).

The current state of biocoenoses in Onego generally reflects the consistent changes under the influence of anthropogenic factors, from the initial increase in productivity to their complete destruction in the upper part of Kondopogskaya Bay. In recent decades, the in-

¹ Hereinafter we will also use the short name Onego

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fluence of climatic factors on the lake ecosystem and its catchment area has been increasing.

2. Materials and methods

We used the data on observations at meteorological stations of Russian hydrometeorological service for a period of instrumental measurements from 1900 to 2017, measurements of water balance elements (river runoff, precipitation and evaporation) and water temperature from 1955 to 2016, as well as information about changes in the water level and characteristics of the ice cover from 1900 to 2017 at hydrometeorological stations located in the catchment area of the lake.

A probabilistic analysis of these data on hydrometeorological observations was carried out to study the pattern of variability in the time scales from decades to intra-annual fluctuations. Trends and quasi-cyclical fluctuations were identified.

It was also used data on the content of the total iron and phosphorus in water of the Shuya River in different seasons of 1992–2018 obtained by researchers from Northern Water Problems Institute of Karelian Research Centre of the Russian Academy of Sciences (Analytical..., 2018). To study dynamics of the state of biological parameters, in particular, macrobenthos of Onego, the database (Polyakova, 2012) was used, as well as data on regular observations on the network of stations in Onego over a period of more than 40 years (Kalinkina and Belkina, 2018). Macrozoobenthos was sampled according to standard methods (Guidelines..., 2005).

3. Modern ecosystem changes with climate warming

According to the analysis of hydrometeorological observations in the Onego catchment area for 1900–2017, there is a positive trend in the change of the average annual air temperature, which is 0.20–0.34°C per 10 years. Almost in all years, since 1988, the average annual air temperature exceeded the climatological normal calculated for 1961–1990 (Filatov et al., 2014). With climate warming, the duration of the ice-free period at Onego increased on average from 215 days per year at the end of the 19th century to 227 days per year at the beginning of the 21st century. In the intra-annual course, the change in the average air temperature per month occurs unevenly for different seasons of the year. Notably, there is warming in winter (March) of up to +0.45 ... +0.6°C per ten years. In 1951–2017, there was an increase in the annual air temperature by 1.2°C, total evaporation – by 70–80 mm and total precipitation – by 60–90 mm. Despite the significant interannual variability of the total river inflow into Onego, there are no trends over 60 years (1951–2010), since the increase in the total evaporation offsets the annual total precipitation. There is an evidence that the duration of the ‘biological summer’ for 60 years has increased more than by 15 days (Nazarova, 2015). At the same time, the share of spring-spawning species of fish tended to increase relative to autumn-spawning ones (Georgiev

and Nazarova, 2015). Climate changes have affected the state of the catchment area: there is an increase in the number of thaws and in the proportion of liquid precipitation over the solid one, as well as a decrease in soil freezing (Nazarova, 2015).

4. Results and discussion

Almost 10 years ago, based on the data on mathematical modelling (Ladoga and Onego..., 2010) and field observations N.A. Petrova with colleagues (see in Rukhovets et al., 2011) noted that anthropogenic factors mainly contribute to the changes in the ecosystems of Ladoga and Onego, and climate plays a secondary role. Changes in the phytoplankton development caused by warming are visible only in autumn, while the heterogeneous development of zooplankton is more obvious, but it also does not exceed the fluctuations resulted from changes in the biogenic load.

With climate warming and increased runoff in winter for the past 30 years, the iron runoff to Onego with waters of the Shuya River has increased by 35%, and phosphorus – by 25% due to winter and spring months (Kalinkina et al., 2018). During the winter months, the water runoff increased, and there was its intra-annual redistribution, which corresponds to the general tendency to the change in the water runoff in the north-western regions of Russia (Dzhamalov et al., 2017). In winter and spring months, the concentration of iron and phosphorus in the river waters increased, and the consistency of changes in the iron and phosphorus concentrations indicates their terrigenous nature (Kalinkina et al., 2018). The change in runoff into the Petrozavodsk Bay caused an increase in the content of iron and phosphorus in its waters. In the same period, there was the iron accumulation in bottom sediments of the Petrozavodsk Bay and a 4-fold decrease in the biomass of macrozoobenthos, *Oligochaeta* and *Amphipoda* (Kalinkina and Belkina, 2018) (Fig. 1).

The changes identified in the Onego ecosystem in terms of the geochemical characteristics of the region (Tekanova et al., 2018) decrease its productivity and resistance to external influences.

The process of transformation of the lake ecosystem is a sequence of stages during which the external factors of influence changed. The first stage of

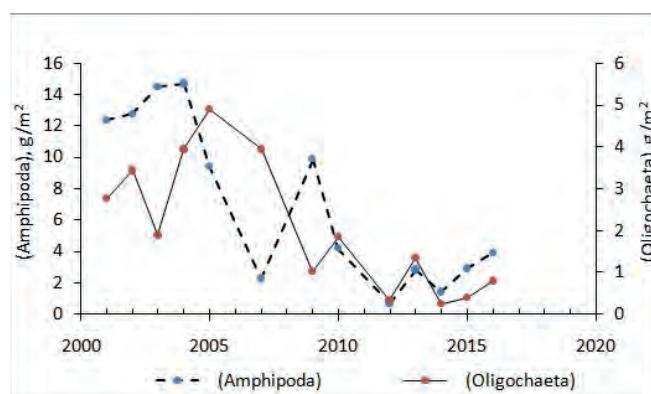


Fig.1. Biomass dynamics of the main macrozoobenthos groups in the Petrozavodsk Bay of Onego Lake (2001–2016).

anthropogenic changes in the water body was due to timber rafting and a significant deterioration of the water quality in the mouth areas of large tributaries. The intensive work of the main industrial centres on the Onego Bays (Petrozavodskaya and Kondopogskaya) and the centralized wastewaters discharge into the lake outlined the next stage of the ecosystem transformation, which is local anthropogenic eutrophication. At different times and in different parts of the lake, anthropogenic eutrophication had different types: heterotrophic or autotrophic (Timakova et al., 2011). The economic recession in 1990s led to a decrease in the ecological stress in the areas adjacent to the industrial centres, Petrozavodskaya and Kondopogskaya Bays. However, the role of the intra-aquatic processes increased, and there was an obvious influx of nutrients and slowing down the restoration of the ecosystem. In recent years, the most obvious changes in the ecosystem have been due to climate warming.

5. Conclusion

In the late 1990s and early 2000s, the role of climate warming in the changes of the Lake Onego ecosystem increased. Currently, an increase in the surface water temperature and change in the vegetation period reflect the impact of climate warming. Due to climate warming and increase in the runoff from the catchment area in winter time, formation of the hydrochemical regime change leading to an increase in colour of water, content of iron and phosphorus in humus complexes, as well as the abundance and structure of aquatic communities.

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Microbiological studies of Lake Baikal in Limnological institute: past and present



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ABSTRACT. The article deals with the history of studies of microbial communities in Lake Baikal performed by the researchers of Limnological Institute SB RAS. We provide the information about the studies of freshwater organisms in the world and the formation of aquatic microbiology in Russia. Initial data on bacteria from the lake were obtained in the 1920s of the past century. The studies were carried out at Baikal Biological Station and Baikal Limnological Station of the USSR Academy of Sciences. The first studies and large discoveries at Lake Baikal were made by microbiologists who came at different times from research institutes of Moscow and Leningrad. When Baikal Limnological Station was reorganized into Limnological Institute of the USSR Academy of Sciences, the studies of microorganisms in Lake Baikal became systematic and complex, and they were mostly performed by the researchers from the Laboratory of Aquatic Microbiology. The long-term regular observations have provided significant results on the species composition, abundance, seasonal and interannual dynamics, horizontal and spatial distribution of bacteria in the water column and bottom sediments of Lake Baikal, as well as their functional role. In the past decades, the introduction of molecular-biological, electron microscopic and analytical methods allowed determination of the genetic, taxonomic and morphological diversity of bacteria and viruses. Since 2009, main research area of Laboratory of Aquatic Microbiology is the study of biofilm microbiocenoses, including the determination of the taxonomic composition, structural organization, formation features, as well as biotechnological potential of bacterial and viral communities of the surface microlayer of water and biofilms formed on biogenic and abiogenic substrates. The 90-year period of microbiological research of a unique ecosystem of Lake Baikal appeared to be successful and rich in discoveries.

Keywords: bacteria, cyanobacteria, virus, Baikal, biofilm, plankton

Microorganisms are the most abundant biological entities on the Earth. Microscopic dimensions serve as a common feature of taxonomically different organisms: bacteria, archaea, fungi, algae, protozoa and viruses. The most significant characteristics of microorganisms are their high metabolic activity, wide variety of enzyme systems and flexible ways of regulating metabolism, which leads to rapid reproduction and growth rates, as well as active adaptation to changing environmental conditions. Due to their unique competitiveness, microorganisms settle everywhere, in all ecological niches from the polar regions to deserts. Water is one of the most favourable habitats for them; in aquatic ecosystems microorganisms reach high abundance and play a key role. Microorganisms are crucial and necessary parts of the cycle of chemical elements in waterbodies, providing the continuous generation and destruction of organic matter as a result of interconnected functions.

The water microbiocenoses consist of producers

(microalgae, cyanobacteria, photo- and chemoautotrophic bacteria), destructors (most bacteria and fungi) and abundance regulators: consumers (protozoa) and bacteriophages. Phototrophic and heterotrophic bacteria, as a link in the microbial loop, serve as an important component of the food chains in waterbodies, since they transform a significant proportion of organic matter through a complex of trophic relationships between algae, bacteria and protozoa, before it enters the conventional food chain (Sommaruga, 1995). The traditional study objects in aquatic microbiology are prokaryotes, bacteria and archaea, as well as viruses, which are organisms with an extracellular structure, and eukaryotes, represented by very small fungi.

Microscopic studies of aquatic microorganisms began in the middle of the 19th century, before the era of culturing bacteria on solid media according to the method proposed by R. Koch in 1883 (Collins, 1963). The first bacteria observed under a microscope were large, filamentous forms with characteristic morphol-

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ogy, such as *Beggiatoa*, *Thioploca*, etc. Since 1884, the quality of lake and river water as a source of drinking water, pathways of entry and fate of allochthonous bacteria in lakes have become subjects of study. During this period, P. Frankland laid the fundamental and practical foundations of sanitary microbiology (quoted after Taylor, 1940; Collins, 1963). Cultivation of bacteria on nutrient media in the 90s of the 19th century indicated their ubiquitous distribution in aquatic ecosystems and allowed the description of typical aquatic heterotrophic bacteria. Early studies in aquatic bacteriology identified seasonal fluctuations in the abundance and vertical and spatial distribution of cultured bacteria of Lake Zurich. Later, similar studies were carried out in a number of American and European lakes (quoted after Henrichi, 1938; Collins, 1963).

The key to understanding the global role of bacteria in the biogeochemical cycles of elements was the discovery in 1887 of chemosynthesis by the great Russian scientist S.N. Vinogradsky (1856-1953). His follower, V.L. Omelyansky (1867-1928), was the first to develop Vinogradsky's ideas on the biospheric role of bacteria. Thanks to this Russian microbiologist, the ideas of S.N. Vinogradsky contributed significantly to the formation of the school of Russian microbiologists. V. L. Omelyansky showed the involvement of freshwater bacteria in the carbon cycle and made a mark on the history of science as the founder of aquatic microbiology (Omelyansky, 1905; 1917).

The formation of aquatic microbiology as an independent scientific discipline in Russia and the USSR was also associated with the names of prominent Russian microbiologists G.A. Nadson and B.L. Isachenko. G.A. Nadson (1867-1940) was the first director of the Institute of Microbiology of the USSR Academy of Sciences and was distinguished by a wide range of interests. He studied the role of bacteria in the cycle of biogenic elements and the transformation of substances in aquatic environments. His Master's thesis was dedicated to cyanobacteria (Nadson, 1895). G.A. Nadson is widely known as the founder of radiation genetics; he can also be considered as the initiator of studies on epilithic bacteria, since he was the first to show that boring bacteria secrete acid that dissolves lime substrates, thereby participating in the calcium cycle (Nadson, 1900-1902). B.L. Isachenko (1871-1948), a follower of G.A. Nadson, was a famous polar explorer who made a major contribution to the study of marine microorganisms.

In the 1940s of the past century, Soviet scientists suggested new and effective methods of studying microorganisms that largely determined the development of international microbiology. The introduction of direct method of enumeration in bacteria, primarily by membrane filtration (Razumov, 1932), became a breakthrough in the evaluation of the number of aquatic bacteria. Quantitative assessment showed a much higher number of bacteria in freshwater ecosystems (10^5 - 10^6 cells per ml) than plate counting method. N.G. Kholodny developed the method of glass fouling, which allowed not only determination of the bacterial

number but also observation of the diversity of their morphotypes in the natural habitat, as well as their ability to form associations and interact with each other (Cholodny, 1929; Kholodny, 1935). For the first time, the formation of structured microbial associations, i.e. biofilms, was shown as a natural state of existence of bacteria in the aquatic environment. In 1934, G.G. Winberg (1905-1987) was the first in the world to propose a method for determining the rate of production and destruction of organic matter in waterbodies. Subsequently, it allowed the productivity and trophic role of bacteria to be evaluated (Winberg, 1936). In the international literature, R. Lindeman is credited with discovering the theory of biological productivity and the role of bacteria in freshwater ecosystems. In the 1940s, he placed "microbial ooze" in the centre of a diagram depicting the trophic dynamics in the ecosystem of Cedar Creek Bog (Lindeman, 1942).

In microbiology and science in general, schools and traditions play a very important role. The history of studying microorganisms in Lake Baikal is inseparably linked with the history of microbiology in Russia. The merits of the Russian school in the study of aquatic microorganisms are generally recognized, and the international prestige of Russian microbiology was rather high for a long time, until the 1970s. The first studies and large discoveries at Lake Baikal were performed by scientists, who came at various times from research institutions of Moscow and Leningrad. In the unique setting of the largest and deepest lake on the Earth, they applied their knowledge and ideas, many of which have not lost their scientific value, and the problems, which they set, have remained relevant to this day.

In Soviet times (1925-1950), a course was set up to organize a network of stationary microbiological research laboratories directly on the banks of various waterbodies. At Baikal, in 1916 the Baikal Biological Station was established on the instructions of the Academy of Sciences and under the initiative of V.Ch. Dorogostaisky. In 1921, the Station was transferred to the Irkutsk State University. In 1928, the Baikal Expedition of the Academy of Sciences was established. Later, it was reorganized into the Baikal Limnological Station of the Academy of Sciences of the USSR. Its staff made a great contribution to the formation and development of microbiological research at Lake Baikal.

The first studies of the bacterial community in Lake Baikal, which were carried out by B.A. Blankov under the direction of V.N. Yasnitsky in 1927 near the settlement Bolshiye Koty, indicated the number of plankton bacteria cultured on protein media (Yasnitsky et al., 1927). In subsequent years, the researchers identified the number and biomass of bacterioplankton in the entire lake, its interannual dynamics and vertical distribution, found peaks in seasonal development and the connection with the development level and composition of phytoplankton, as well as with water temperature, determined the time of bacterial generation, and initiated the study of species composition of the cultured microbial communities (Kuznetsov et al., 1951;

1957; Sorokin, 1957; Egorova et al., 1952; Romanova, 1958a; 1959; Kriss and Chebotaryov, 1970). The authors of the first data on microorganisms in Lake Baikal were the prominent scientists S.I. Kuznetsov, Yu.I. Sorokin and A.E. Kriss. Their names are associated with outstanding achievements in hydrobiology, which have received worldwide recognition. S.I. Kuznetsov focused on the study of microorganisms in the cycle of matter and energy in lakes; he was the first to suggest using carbon and sulphur isotopes to study the intensity of microbiological processes. In 1953, S.L. Kuznetsov conducted experiments to determine values of photo- and chemosynthesis in the water column of Lake Baikal by adding labelled carbon to light and dark flasks. In the surface layer of the bottom sediment of Lake Baikal, he determined the total number of bacteria and found nitrogen-fixing, nitrifying and denitrifying bacteria (Kuznetsov et al., 1957). Sorokin was the follower of S.L. Kuznetsov, and he developed structural and functional direction in the studies of aquatic ecosystems. He initiated the radiocarbon method in hydrobiology, managed to cover a large number of problems concerning methodology and the ecology of aquatic microorganisms and made a significant contribution to the understanding of primary production and the role of bacterial chemosynthesis in the aquatic environment. At Baikal, Yu.I. Sorokin isolated and characterized sulphate-reducing bacteria from deep-water Baikal ooze (Sorokin, 1957). A.E. Kriss (1908-1984) was one of the founders of marine microbiology. He studied the role of microorganisms in corrosion of hydrotechnical structures, developed bacteriophage preparations, organised the first electron microscopy laboratory in the USSR in 1946 and discovered the presence of viruses in sea water (Kriss, 1947). A.E. Kriss identified the microzonal distribution of deep-water heterotrophic bacterioplankton in three basins of Lake Baikal (Kriss and Chebotaryov, 1970).

A.G. Rodina from the Zoological Institute of the Academy of Sciences of the USSR contributed significantly to studies of microorganisms in Lake Baikal. She described physiologically different groups of bacteria (ammonifying, nitrifying, denitrifying, nitrogen-fixing, sulphate-reducing, sulphur-oxidizing, aerobic, anaerobic and cellulose-decomposing) in the microbial community of the water column and the bottom sediments of Lake Baikal (Nechaeva and Salimovskaya-Rodina, 1935; Rodina, 1954). A.G. Rodina studied the number of microorganisms in bottom sediments and determined the productivity of bacteria in stony fouling in the littoral zone. In this regard, she can be considered as a pioneer in the studies of Baikal microbial communities in the benthos and periphyton (biofilms). Notably, A.G. Rodina was distinguished by a wide range of scientific activities. She also showed her worth in methodological issues. Thus, together with A.S. Troshin, she used, for the first time, labelled phosphorus to demonstrate the involvement of bacteria in the feeding of some freshwater animals. In addition, she used fluorescent dyes to enumerate the number of bacteria (Rodina, 1965).

At Baikal, A.P. Romanova used the well-known N.G. Kholodny method of fouling glasses. It showed the number and morphological diversity of microorganisms in the water column of the lake in different seasons of the year (Romanova, 1958b). A.P. Romanova devoted much attention to the bacteria of the nitrogen cycle in plankton and bottom sediments (Romanova, 1959; 1961; 1963). She performed a layer-by-layer study of bottom sediments, determined the total number bacteria and number bacteria involved in the nitrogen cycle and drew a conclusion about the very intensive processes of ammonification, nitrification and nitrogen fixation in sediments and poor denitrification, i.e. nitrate reduction.

After the reorganization of the Baikal Limnological Station into Limnological Institute Siberian Branch of the Academy of Sciences of the USSR in 1961, studies of microorganisms in Lake Baikal became planned, systematic and complex, and they were carried out mainly by the researchers from the newly created Laboratory of Applied Microbiology. M.A. Messineva was the first head of the Laboratory (1972-1980). In the 1950s, she came from Moscow and investigated the microorganisms in bottom sediments in Lake Baikal and their role in biogeochemical processes by measuring C_{org} and bacteria biomass (Messineva, 1957).

Researchers from the Laboratory continued to study the microorganisms in the water column and bottom sediments of Lake Baikal. The collective monograph "Microbiology heritage of the 20th century" by T.P. Vinogradova and others (2004) gives an overview of the most important results and publications of the researchers from the Laboratory of Aquatic Microbiology. A series of studies performed by T.A. Mladova on the quantitative and qualitative composition of bacteria in water and bottom sediments and their spatial distribution, seasonal dynamics and dependence on the content of organic matter in Lake Baikal were published in the 1970-90s (Vinogradova et al., 2004). A.I. Shtevneva determined seasonal dynamics of the abundance of the bottom microflora, the value of destruction of organic matter in bottom sediments and the activity of bacteria, which was higher than in the water column (Vinogradova et al., 2004). During this period, the significant works were the 1981-1985 studies of bacteria involved in the nitrogen cycle performed by V.A. Verkhozina, phosphorus – by V.V. Parfenova and aggregated bacterioplankton – by L.P. Spiglavov. N.A. Lapteva characterized in detail the oligocarbophilic bacteria in the water column of the lake (Vinogradova et al., 2004).

A special mention should be made of the results of a microbiological assessment of the water quality and bottom sediments in Lake Baikal carried out by the researchers from Limnological Institute, together with their colleagues from other institutes. In 1970-1975, G.A. Goman described for the first time, the microbial communities of contaminated bottom sediments in the area affected by wastewaters from the Baikal Pulp and Paper Plant (Vinogradova et al., 2004). Subsequently, studies were carried out in this area to estimate the

number of anaerobic saprophytes, sulphate reducers and methanogens, to determine the rates of protein and cellulose decomposition, as well as those of sulphate reduction and methane formation, and to predict the environmental deterioration that would ensue from further discharge of wastewater (Namsaraev et al., 1995a; 1995b; Zemskaya et al., 1997). In 1997-2002, V.V. Drucker and E.A. Panasyuk first determined the taxonomic composition and distribution of opportunistic bacteria in the littoral and pelagic zones of Lake Baikal and its tributaries (Drucker and Panasyuk, 2006).

Baikal studies of bacterioneuston, bacteria inhabiting the surface microlayer of water, became pioneering in the world of aquatic biology. Even now at this time, information about bacterial neuston in fresh waters is limited to a few studies. In the 1970-1980s, V.M. Nikitin determined the number and daily dynamics of bacteria from the surface microlayer, as well as the proportion of pigmented bacteria in neuston. He initiated studies on the destruction of oil products by hydrocarbon-oxidizing bacteria, which V.Ya. Andrukhova, V.I. Petrova, L.M. Mamontova, S.D. Taliev, and O.M. Molozhavaya continued in 1979-1986 (Vinogradova et al., 2004). In 1975-2000, I.A. Nechyosov studied the formation of microbiocenoses of Baikal bottom sediments, their enzymatic activity and the effect of tributaries on the ecosystem of the lake (Vinogradova et al., 2004).

Among the studies of bottom bacteria, the discovery of methanotrophic bacteria in mats should be noted (Kuznetsov et al., 1991). Further studies of bacteria in the carbon and sulphur cycles in Baikal bottom sediments, in particular, those of methanotrophic and sulphur-oxidizing bacteria, were headed by B.B. Namsaraev (Head of Laboratory of Microbiology from the Institute of General and Experimental Biology of the Siberian Branch of the RAS) in collaboration with T.I. Zemskaya (Namsaraev et al., 1994; Namsaraev and Zemskaya, 2000; Dagurova et al., 2004). During this period, the features of the distribution of microorganisms in the coastal and deep parts of Lake Baikal were determined under the conditions of a thermal bar formation (Drucker et al., 1997).

The current world stage of aquatic microbiology (from the 1980s to the present) is associated with technologies that have revolutionized microbiological research, e.g. flow cytometry and analysis of nucleic acids and proteins. Since that time, the state and advances of Russian microbiology have not been as significant as they were in previous years, largely due to the fact that new technologies and methods have been developed and implemented abroad.

Studies of microbial communities using methods of molecular genetic analysis began rather early at Lake Baikal in comparison with other waterbodies in Russia. These works set a new stage for investigations of microorganisms in the lake and became a real breakthrough in the understanding of the composition of bacteria in the water column, fouling and bottom sediments (Belikov et al., 1996; Belkova et al., 1996; Denisova et al., 1999). The period from 2000 to the present has been

a productive time for identifying the genetic diversity of the autotrophic picoplankton communities (Semenova, 2001; Tikhonova, 2006; Belykh et al., 1999; 2011), bacteria of the water column (Belkov, 2004), sponges (Kalyuzhnaya, 2012), the intestinal microflora and external integuments of fish (Sukhanova, 2012) and benthic cyanobacteria (Gladkikh, 2012). Genetic biodiversity and a complex characterization of microbial communities from bottom sediments, occurrence areas of gas hydrates, seepages of oil and hydrocarbon gas, bitumen structures and barrier inflow zones of the main Baikal tributaries were observed in a series of works headed by T.I. Zemskaya (Zemskaya et al., 2001; 2009; Zemskaya, 2007; Shubenkova, 2006; Chernitsyna, 2007; Lomakina, 2010; Likhoshway, 2011; Maksimenko, 2012) and O.N. Pavlova (Bukin et al., 2017).

Cultivation, microscopic, physiological, biochemical, analytical and genetic methods were used for studies of cultured and uncultured bacteria of the genera *Streptomyces* and *Micromonospora* (Terkina, 2004), *Pseudomonas* (Pavlova, 2004), *Caulobacter* (Kovadlo, 2006), *Bacillus* (Suslova, 2007) and *Enterococcus* (Kravchenko, 2009), as well as microorganisms oxidizing iron and manganese (Zakharova, 2007), the microbial community of biofilms (Malnik, 2010) and the intestinal microflora of molluscs (Shtykova, 2013), which were headed by V.V. Drucker and V.V. Parfenova. Most publications of the above-mentioned authors are on the website of Limnological Institute SB RAS. Most publications of the abovementioned authors are on the website of Limnological Institute SB RAS.

In 2009, V.V. Parfenova, Head of the Laboratory of Aquatic Microbiology, initiated the study of microbial communities in biofilms at the water-air and water-solid substrate interfaces, which are continuing successfully at present. These studies are aimed at determining the taxonomic composition, structural organisation, specifics of formation, and biotechnological potential of microbial communities from the surface microlayer of water, as well as biofilms formed on biogenic and abiogenic substrates. According to the world literature, J.W. Costerton and colleagues are considered to be pioneers in the study of biofilms. They showed that approximately 95%-99% of bacteria on the Earth exist as structured associations generally called 'biofilms' (Costerton et al., 1978; 1987). Biofilms are communities of bacterial cells attached to a substrate, surface, or to each other and surrounded by a polymer matrix they synthesize (Donlan and Costerton, 2002). Interest in the study of biofilms increased dramatically due to discoveries by medical microbiologists, who indicated the large-scale role of biofilms: 65% of chronic infections are caused by the formation of biofilms (Flemming et al., 2016). As mentioned above, before the official discovery of biofilms by J.W. Costerton et al. (1978; 1987) microbiologist N.G. Kholodny in the 1930s identified their existence in soil. At Baikal, A.P. Romanova described the formation of biofilms in the water column of the lake (Romanova, 1958). In oligotrophic habitats, the formation of biofilms by microorganisms on the surface of hydrobionts, organomineral

particles of detritus and minerals is of special importance. Each represents a unique microbial community with a very complicated intrinsic spatial structure, e.g. cyanobacterial mats, periphyton films, symbionts and associated microflora of the digestive tract and external integuments, bodies of hydrobionts and the surface microlayer of water. As a rule, biofilms develop in places of concentration or flow of organic matter, which determines the peculiarities of their composition, structure and metabolic characteristics. Microbial biofilms in the largest and deepest lake in the world, Lake Baikal, are of great value.

Researchers from the Laboratory of Aquatic Microbiology study biofilms in the littoral zone of Lake Baikal in several research areas, using an integrated methodological approach with methods of classical microbiology, microscopy, molecular biology, analytical chemistry etc. The study of genetic and taxonomic diversity of bacterial and viral communities of Baikal biofilms, their quantitative assessment and elucidating their role in the functioning of the Baikal ecosystem are one of the main research areas. In 2010, metagenomic analysis of communities was used for the first time to determine the diversity of microbiocenoses, when this research area had only begun to develop in Russia. The first results showed the high diversity and large metabolic potential of bacteria from biofilms of bottom substrates (Perfenova et al., 2008; 2013; Malnik, 2010; 2013; Gladkikh, 2012; Sorokovikova et al., 2013), sponges (Gladkikh et al., 2014; Jung et al., 2014; Seo et al., 2016) and the surface microlayer (Galachyants et al., 2016; Galach'yants et al., 2016; 2017). At the present time, an analysis of the genetic diversity of microbial communities of various habitats of Lake Baikal was performed using high-throughput sequencing (Fig. 1).

The surface microlayer covering all waterbodies occupies approximately 70% of the earth's surface and plays a great biospheric role. The neuston communities are of a special interest in terms of their function under conditions of rapidly changing weather factors and resistance to xenobiotics, including those of aerosol origin. The benthic microbiomes developing on the bottom substrates primarily attract attention with their richness of species composition, complex structure and high productivity, especially considering the last negative changes in the littoral zone of the lake. Like neuston microorganisms, the benthic microbial communities in freshwater are not well studied. In Baikal, the littoral zone occupies approximately 7% of the total area, and the microbial processes occurring there play an important role and have a significant impact on the ecosystem of the entire lake.

The Laboratory of Aquatic Microbiology was the first in Russia to study toxic cyanobacteria. Various types and variations of toxins, as well as toxin-encoding genes, were identified

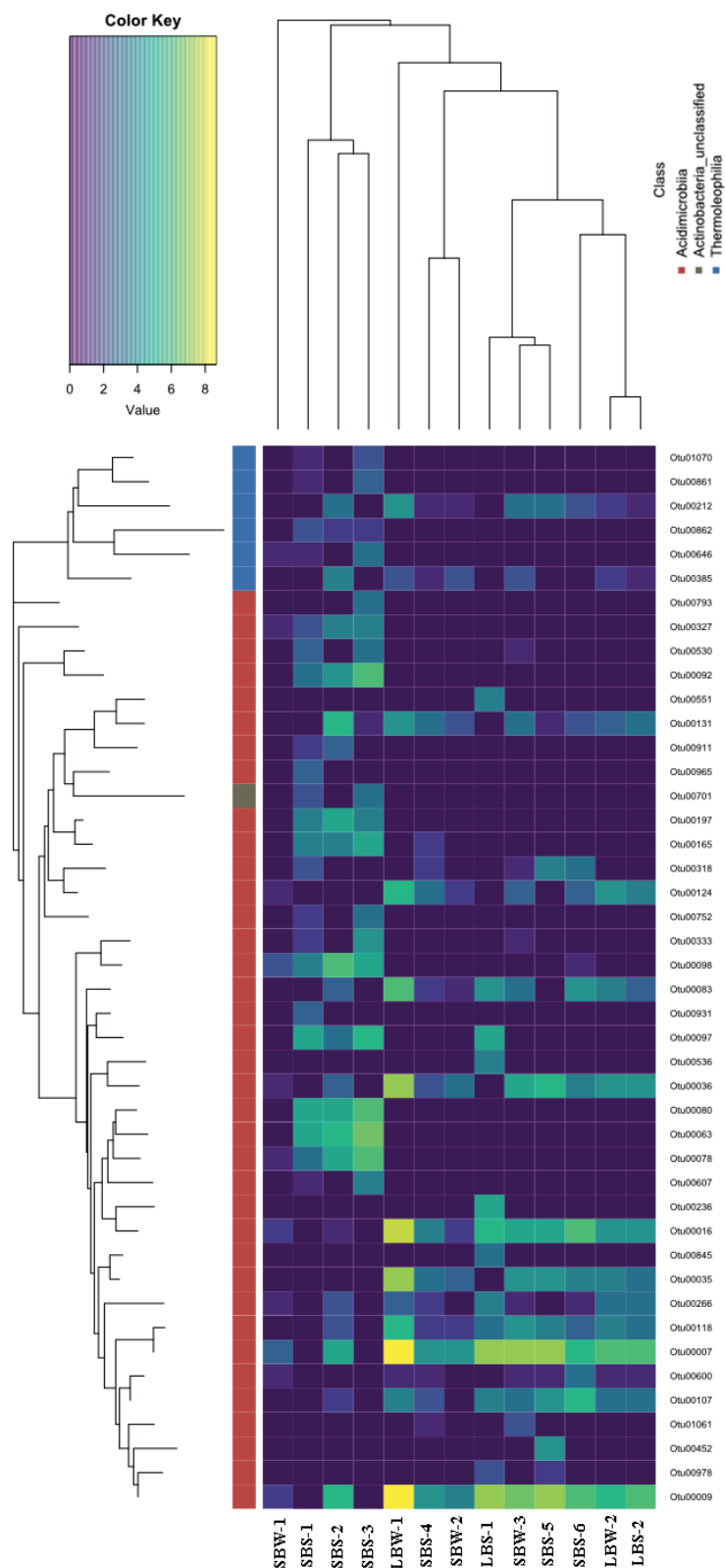


Fig. 1. Phylogenetic tree, heat map and UPGMA dendrogram of 16S rRNA gene fragment sequences of the Actinobacteria phylum in metagenomic communities of the water column, sponges and biofilms based on the high-throughput sequencing data.

(Tikhonova et al., 2006; Belykh et al., 2013; 2015a; 2015b; 2015c). The mass development of cyanobacteria in water ecosystems is one of the most pressing environmental problems in the world. The bloom of benthic cyanobacteria, including toxin-producing species, at Lake Baikal, which began in 2010, has now assumed the character of an ecological crisis and may have led to the mass death of Baikal sponges (Fig. 2) (Belykh et al., 2016; 2017; Timoshkin et al., 2016). The concentrations of microcystins and saxitoxins in the samples of plankton and benthos were detected, and the degree of threat to human and animal health was assessed. Cyanobacterial blooms are the most significant and characteristic consequence of the eutrophication of water ecosystems (Lampert and Sommer, 2007); they are increasingly registered all over the world. Global warming is considered to be one of the causes of blooms (Paerl and Otten, 2013). The causes of the intensive growth in the number of plankton and benthic cyanobacteria and their colonization of new substrates in Lake Baikal are unknown. Studying the influence of biotic and abiotic factors on the cultures of cyanobacteria that are developing intensively in the lake can be one of the ways to solve this problem. The species composition, abundance and physiological properties of bacteria involved in the nitrogen cycle (nitrogen-fixing, denitrifying and nitrifying bacteria) are studied using modern methods. According to the latest data, the nitrogen content in Lake Baikal tends to change (Obolkin et al., 2016). One of the themes of the Laboratory of Aquatic Microbiology is ongoing research on the phosphorus-mobilizing and phosphate dissolving bacteria. With the increasing phosphorus load on the lake, it is necessary to obtain answers to many questions concerning the involvement of bacteria in the phosphorus cycle. An important area of re-

search in the Laboratory of Aquatic Microbiology is the microbiological assessment of the water quality in the lake, considering the anthropogenic impact and based on the long-term data.

The genomes of Baikal heterotrophic bacteria have a high content of genes encoding synthesis of many biologically active substances, both those that are widely used in medicine and new metabolites that probably play an important role in the functioning of biofilms and are promising for biotechnological purposes (Sukhanova et al., 2017). The applied aspect of studying natural biofilms has arisen due to their powerful biotechnological potential, high adaptability and resistance to environmental factors, as well as their ability to colonize new biotopes. The objective of studying the biosynthetic abilities of freshwater microbial communities is extremely relevant. In this regard, poorly studied unique habitats, such as Lake Baikal, are particularly attractive. It is necessary to investigate the ability of microorganisms from Lake Baikal to degrade persistent organic compounds, determine the involvement in the biological self-purification of the waterbody by neutralization and oxidation of entering pollutants. At the same time, it is necessary to evaluate the negative effects of biofilm formation, which include economic damage to industry and harm to human and animal health.

The study of viral communities of Lake Baikal is of great interest. Many years of successful research of viral communities from the water column using electron microscopy resulted in the detection of wide morphological diversity and an abundance of plankton bacteriophages in different seasons of the year throughout the water column (Fig. 3) (Drucker and Dutova, 2006; 2009). In Baikal, the authors identified nine of ten DNA-containing bacteriophages known in

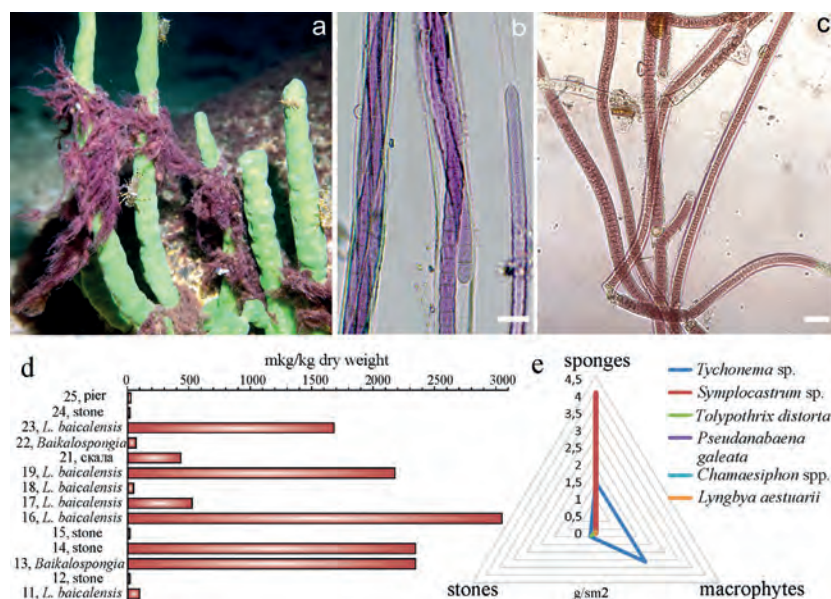


Fig. 2. The dominant species of cyanobacteria, the concentration of microcystins and the biomass of cyanobacteria species in the fouling of various substrates: sponges, submerged macrophytes and stones. a – a general view of the branched sponge *Lubomirskia baicalensis* with cyanobacterial fouling; b – *Symplocastrum* sp.; c – *Tolypothrix distorta*; d – concentration of microcystins based on enzyme immunoassay ($\mu\text{g/kg}$ dry weight) in cyanobacteria developing on sponges, stones, rocky outcrops, and pier; e – biomass of cyanobacteria (g/cm^2) in fouling of sponges, stones and macrophytes. a-c – light microscopy, scale bar 20 μm .

the international classification and discovered four endemic morphotypes, which had not been described by other researchers. The analysis of virome diversity was carried out by using signature genes sequencing (Fig. 4) (Butina et al., 2010; Potapov et al., 2018). The study of morphotypes of benthic and neuston bacteriophages, as well as the analysis of the biofilm viromes, has just begun. Investigations of surface microlayer and bottom biofilms viral communities are the first in the field of aquatic virology.

Lake Baikal, with its unique ecological features and rich biotopes, extreme diversity and high endemism of hydrobionts with their long evolutionary history (25 million years) can serve as a natural laboratory for studying the composition, structure, mechanisms of formation and functioning of bacterial and viral communities from biofilms, as well as a productive source of new and rare metabolites.

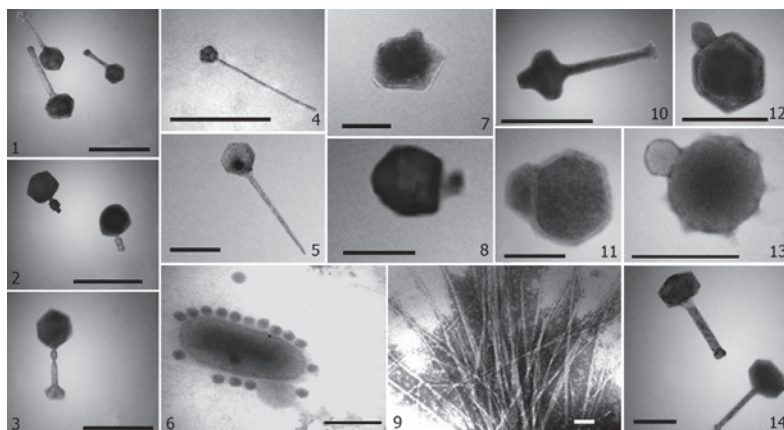


Fig. 3. The morphological and taxonomic diversity of bacteriophages in Lake Baikal. Bacteriophages of the families *Myoviridae* (1-3), *Siphoviridae* (4-5), *Podoviridae* (7-8), and *Inoviridae* (9), morphotypes of 'endemic' bacteriophages in the form of whirligig (10), shell ones (11, 12), head-shaped with spines (13), and hammer-shaped (14). 6 – bacterial cell surrounded by bacteriophages, transmission electron microscopy. 1-5, 7-14 – scanning electron microscopy. Scale bar: 1, 2 – 250 nm, 3, 10-12 – 200 nm, 4 – 500 nm, 5, 13, 14 – 100 nm, and 7, 8 – 50 nm.

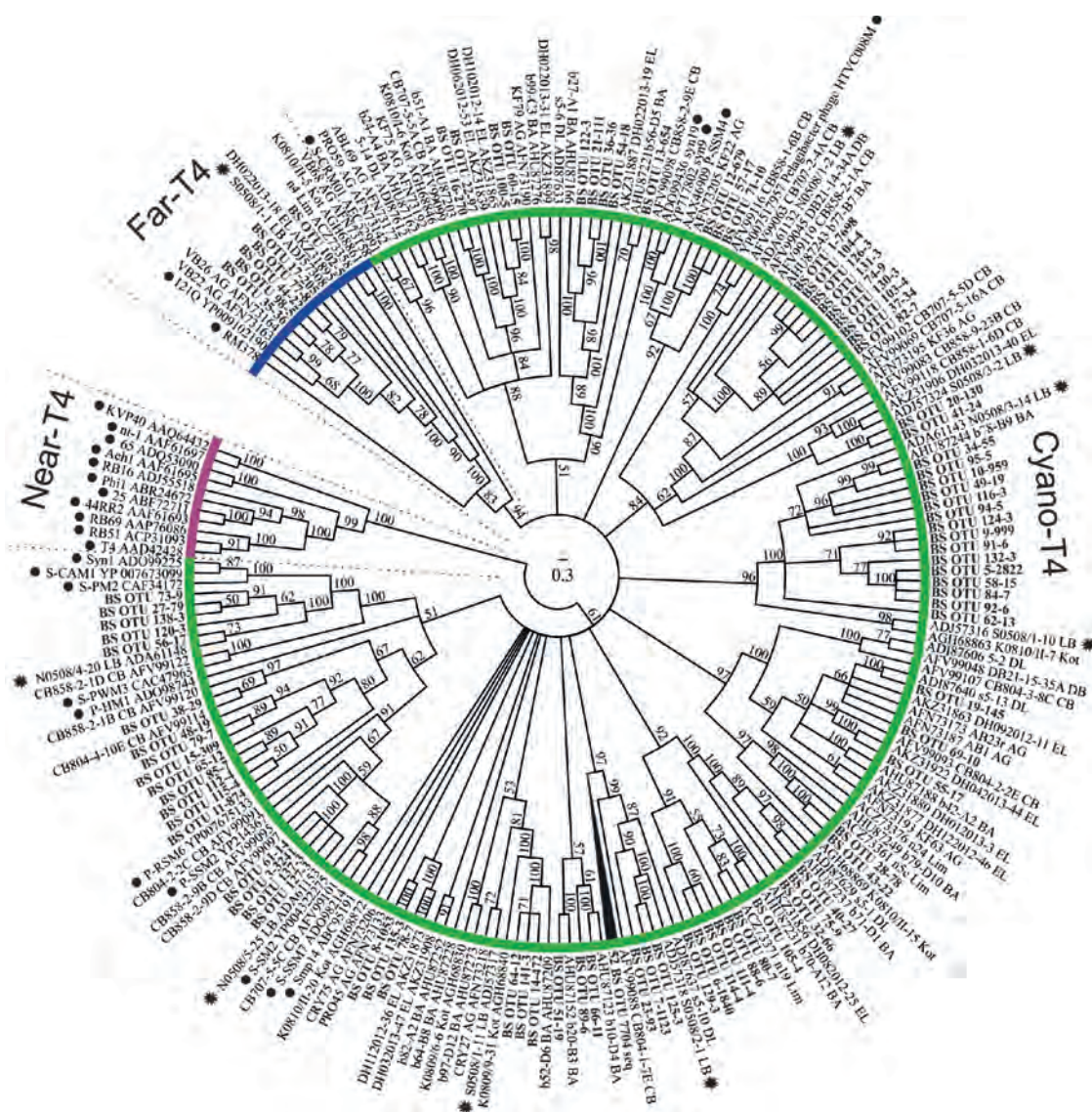


Fig. 4. Phylogenetic analysis of the fragments of the major gp23 capsid protein. Circles mark cultured bacteriophages. BS OTUs are highlighted in bold. Asterisk marks the Baikal sequences obtained by Sanger sequencing. Isolation sources: LB – Lake Baikal, EL – East Lake, CB – Chesapeake Bay, BA – lakes Bourget and Annecy, DL – Donghu Lake, DB – Delaware Bay, AG – Arctic glaciers, Lim – Lake Limnopol, Kot – Lake Kotokel.

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Promising ichthyologic studies in Lake Baikal: fundamental and applied aspects

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ABSTRACT. Promising areas of ichthyologic research in Lake Baikal have been examined. Particular attention is being paid to the technology of remote fish census, including hydroacoustic methods and the method of quantitative environmental DNA analysis. These technologies may provide information on the status of artificially reproducible populations in reservoirs for developing a proper fishing strategy. The areas of application of molecular genetic methods for solving applied problems in creating the biological bases of fisheries, studying diseases, associated microflora and parasitic fauna, as well as for identifying fish feed objects have been determined. Studies aimed at restoring and maintaining the populations of rare and endangered fish species using artificial reproduction methods, including breeding, hybridization, hormonal stimulation of spawning producers, cryopreservation of sexual products, individual tagging, etc., are discussed. Prospects of mobile remote underwater video monitoring systems, the EthoStudio software package and the holography method to simulate sensory reaction in studying mechanisms of adaptations of fish in natural environment and to develop criteria for assessing their stability in aquaculture conditions have been reviewed. Combining ethological studies and in-depth integrated morphological, molecular-genetic and physiological-biochemical screening of Baikal fish that would allow to better understand fundamental evolutionary processes responsible for the formation of behavioral adaptations, creation and maintenance of genetic diversity within and between populations in the natural environment, as well as developing recommendations on the introduction of state-of-the-art scientific monitoring approaches into aquaculture are very promising.

Keywords: ichthyological studies, hydroacoustic methods, environmental DNA, molecular genetic methods, common garden experiments, video monitoring systems, Lake Baikal

There are almost three hundred years of ichthyologic studies in Lake Baikal and in its catchment area. During this time, they moved away from brief faunal reviews and taxonomic descriptions towards integrated and interdisciplinary studies oriented to fish biodiversity, systematics, phylogeny, evolution, ecology, morphology and physiology. Results of these studies are published in many papers. They constitute a basis of the current knowledge about fish of Lake Baikal, and they will be still in demand in the future. The importance of the ichthyologic research in Lake Baikal is due to necessity to resolve several problems in the following areas: fish abundance dynamics, reproduction of fish stock, creation of a biological basis for fisheries and study of fish ailments. Hence, a very promising is to develop methods of a complex monitoring of fish in Lake Baikal.

Baikal omul *Coregonus migratorius* (Georgi, 1775) is the main commercial species of Lake Baikal, thus the census of its population size and biomass is a goal of the rational nature resource management. The urgency of

a more meticulous investigation of this species stock is driven by the current decrease of its abundance. Thereby the monitoring methods that do not need fish catch are in priority. Developing hydroacoustic methods, particularly to obtain equations of target strength dependence on omul size by reference to specific features of behavior of different morphoecological groups and age on a theoretical and practical basis would be promising. These methods were tested during a trawl-acoustic survey of Baikal omul stock assessment and distribution (Melnik et al., 2009).

The extension of the studies for the ice period will give new opportunities to improve the method in order to apply it as a monitoring technique of other pelagic fish species of Lake Baikal. A difficulty of evaluation of population size of pelagic sculpins (*Cottoidi*) by these methods is that the back echo strength from fish without a swim bladder is significantly lower than from those that have it. Besides, the sculpins are smaller than the omul, and they inhabit the maximum depths (up to 1600 m). Nonetheless, the hydroacoustic

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technique can be and has to be adapted to the issue of monitoring of all pelagic species as a whole.

The environmental DNA (eDNA) quantification analysis is a method of evaluation of biomass of hydriobionts which is rapidly growing nowadays in addition to the hydroacoustic one. For the first time the potential of the method to detect vertebrates (particularly fish) in water samples by means of eDNA was demonstrated in 2008 (Ficetola et al., 2008). This tool is particularly important in monitoring rare and endangered species of fish and water invertebrates. Moreover, this technique has a potential of improving the detection sensitivity and decreasing of cost in the field use (Goldberg et al., 2011; Jerde et al., 2011; Thomsen et al., 2012). Its advantage is also a possibility to quickly get a full information about the biodiversity of a waterbody that is very important due to the lack of time for a thorough fish sampling during the field work (Jerde et al., 2011; Dejean et al., 2012; Pilliod et al., 2013; Sigsgaard et al., 2015; Hänfling et al., 2016; Yamamoto et al., 2016). This method is now evolving in three directions: detection of endangered species, tracking of invasive species and optimization for the field and laboratory conditions (Mahon et al., 2013; Wilcox et al., 2013; Barnes et al., 2014). The testing of the eDNA analysis for evaluation and monitoring of Baikal omul stock showed that the peculiarities of its distribution revealed by hydroacoustic method allows the species to be taken as a model for developing of eDNA analysis methods. The both approaches are complementary and enables the correction in the case of a study of actively migrating species. The development of eDNA analysis methods may serve a basis for a study of genetic polymorphism of fish in assemblies of various kind and related to their space distribution (Kirilchik et al., 2018). The implantation of the improved technology of the distance fish inventories (together with spawning population and young fish monitoring data) will provide a precise information on the current state of artificially reproduced populations; the information that is necessary to elaborate a correct strategy of commercial fishing.

The priority area of our ichthyologic studies is a recovery and conservation of rare and endangered fish species by means of artificial reproduction and development of innovative methods of cold-water breeding of Salmonidae and Acipenseridae in Siberian region (Sukhanova et al., 2011; Smirnov et al., 2015). Research in this direction suggests: creating test sites for short and long-term experiments on artificial reproduction and aquaculture on the basis of existing fisheries and aquarium complexes (upgrading the experimental base); carrying out fish-breeding and reclamation works on the ways of spawning migrations, restoring natural spawning sites; improving the methods of brood fish sampling for breeding purposes; creating of replenishable replacement and spawning schools of Baikal fish species that are endangered or decrease in number; developing technology of natural spawning in the channels of the cage culture fisheries. Development of aquaculture needs selection, diversification and increase of volume of high quality fish seed, e.g. by hybridization of whitefishes and introduction of the most robust and

promising hybrids (Sukhanova et al., 2017).

Selection, hybridization and creation of a replenishable cryobank need development, introduction and improvement of methods of hormonal stimulation of spawning fish, cold storage of reproductive products, individual fish tagging (Sukhanova et al., 2015; Sukhanova et al., 2017). Very promising can be the interspecies germ cell transplantation (GCN) adapted for whitefishes both for the study of reticulate evolution, conservation of species and populations of whitefishes, and for creation of new promising fish cultures. The innovative molecular GCT biotechnology allows a more effective use of females because the species of small size that quickly grow up and are easy to keep are selected as recipients (Majhi et al., 2014), and therefore contributes to optimization of many stage in artificial fish breeding and to preservation of biodiversity.

The rapid progress of fisheries worldwide confirms the importance of aquaculture for meeting the alimentary needs. The effective fish farms use innovative approaches based on progressive biotechnologies, new industrial techniques, dynamic marketing and competitive advantage. The studies of pathogenic bacteria in fish microflora and factors contributing to prevention of fish infectious diseases are necessary for science-based control of quality and safety of primary and final products (Abramova, 2004; Buller, 2004; Kirichenko et al., 2004). Change of trophic status of artificial ponds during the fish breeding causes blooms of saprophytic microorganisms, which lead to growth of incidences and excessive mortality among the fish. Therefore, the determination of key indicator species of regional pathogens by state-of-the-art methods of detection is an urgent and promising scientific objective. (Sukhanova et al., 2010; Dzyuba et al., 2011a; 2011b; 2012; 2013; 2014a-b; Belkova et al., 2014). Another rapidly developing area is the study of the associated microflora – an informative method for analyzing both the ecology of endemic Baikal species (Dzyuba et al., 2014a; 2014b; 2016; Belkova et al., 2015), and the objects of aquaculture (Belkova et al., 2017).

Molecular genetic methods are a reliable and convenient tool for solving many applied problems. In addition to the studies of the associated microflora, they are successfully used in the investigations of fish parasites (Denikina et al., 2016), and in the identification of fish prey (Kuznedelov and Dzyuba, 1999; Carreon-Martinez et al., 2011; Harms-Tuohy et al., 2016). This tool is very useful in analysis of highly digested gastric content, fish eggs, invertebrate cocoons etc. The advantage of the molecular genetic methods is the analysis of short DNA fragments present in the contents of the gastrointestinal tract of fish, even in the absence of material suitable for identification by morphological features. Molecular genetic methods are promising, and sometimes the only tool when conducting legal expertise, for example, of fish eggs or products during investigations of crimes against wildlife during the illegal fishing of valuable commercial fish.

Equally promising is the development of mobile remote systems for underwater video monitoring (Khanaev et al., 2000), prototype of instrument based

on the EthoStudio software and holographic approach imitating tactile reaction on Baikal fish inhabiting different environments (Sapozhnikova et al., 2016; 2017a; 2017b). Possible consequences of anthropogenic impact include behavioral changes, worsening of sensory sensitivity, increased stress and lower growth rates of individuals (Sapozhnikova et al., 2017b). In turn, the adaptation of fish to different conditions (noise level, pressure, ground heterogeneity, changes of physical parameters with depth) causes a large variety of adaptations of the sense organs. The conditions of keeping rare and endangered fish species during artificial reproduction determine their further survival and affect the efficiency of the replenishment of commercial stock. The study of the mechanisms of adaptation of fish in the natural environment will allow the development of criteria for assessing their stability in aquaculture with constant monitoring of the environment (common garden experiments) (Sapozhnikova et al., 2017b; Sukhanova et al., 2017). The combination of ethological studies and morphological screening of fish sensory system during artificial cultivation helps to identify the most stress-resistant individuals and makes it possible to develop a system of ecological and morphological certification of fish, to select forms that are promising for high-tech industrial aquaculture. Thus, at present, studies of physiological processes affecting the behavior of fish become relevant. The combination of ethological studies and in-depth comprehensive morphological, molecular-genetic and physiological-biochemical screening of Baikal fish will help to solve the problems of biodiversity conservation under the constantly increasing anthropogenic load. Theoretically, such integrated work will allow a better understanding of the fundamental evolutionary processes responsible for the formation of behavioral adaptations, the creation and maintenance of genetic diversity within and between populations in the natural environment, as well as the development of recommendations for the introduction of state-of-the-art scientific monitoring into aquaculture.

The implementation of these research areas will bring the work on the study and preservation of fish biological diversity, as well as monitoring and management of the Baikal fish stock to a new orbit. In addition, the information obtained will provide a scientific and technological basis for the development of aquaculture in the Baikal region at the modern international level. However, all these are just a few initiatives that preserve the continuity of ichthyologic studies in Lake Baikal and testifying their significance in the new millennium.

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Mechanisms of fast transformations of Baikal biota: multidisciplinary approach

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ABSTRACT. We discuss the current state of investigations concerning the impact of dramatic ecosystem rearrangements in Lake Baikal on the evolution of the lake's biota, as well as the approaches that should be used to achieve further progress in this area.

Keywords: evolution, modelling, climate change, Lake Baikal

It is difficult to find more compatible entity than Lake Baikal with long-term stability of environment (Grachev et al., 1998; Kuzmin et al., 2001; MacKay, 2007). High variability of the crucial natural conditions defining physical ecological niches and continuous environmental challenges are the major factors of survival of all components of this giant and ancient eco-system (Sherbakov, 1999; Dynesius and Jansson, 2000 etc.). Therefore, fast and dramatic transformations of the physical conditions should influence significantly the evolutionary processes of the biotic component of the lake ecosystem. Moreover, the environmental oscillations of such scale are important per se and require specific adaptations of organisms (Margalef, 1978). Fig.1 shows possible evolutionary consequences of environmental oscillations indicating that the different rates and spans of morphological evolution may cause different outcomes ranging from speciation to the development of a generalist species.

Unfortunately, the role of fast environmental changes as the force forming the evolution of Baikal biota has been underestimated and understudied until now in spite of the availability of excellent paleontological record providing firm evidences of its importance (Grachev et al., 1998; Kuzmin et al., 2001; Karabanov et al., 2004). On the other hand, the problems concerning long and short term consequences of dramatic environmental processes in Lake Baikal become increasingly important scientifically and attract attention of general public due to the last events, including blooming of filamentous green algae (Romanova et al., 2013; Kravtsova et al., 2014) and spreading sponge disease (Khanaev et al., 2018; Kulakova et al., 2018).

Any study aimed at the elucidation of evolution-

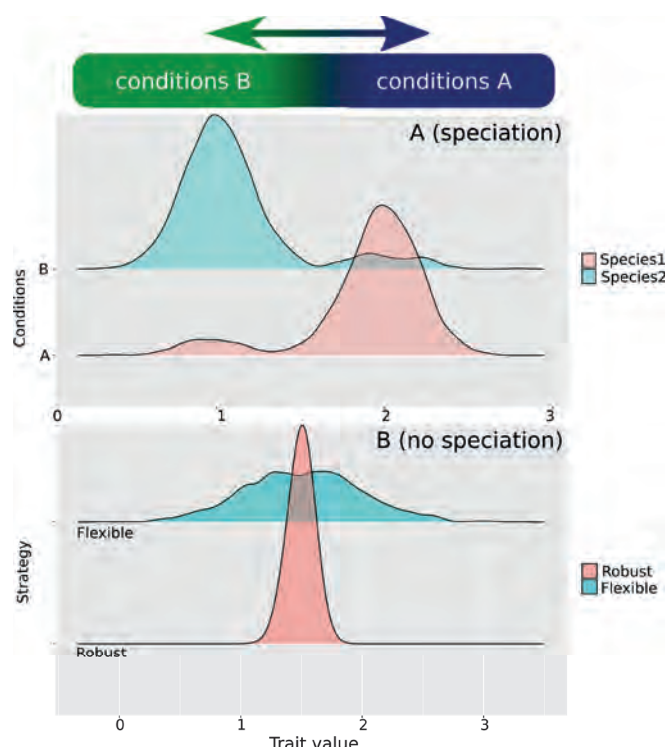


Fig. 1. Different evolutionary consequences of oscillating environment. Horizontal bar at the top shows to sets of conditions between which the oscillation of environment occurs. A: the case of speciation of a single common ancestor (not shown) splits into two sister specialist species adapted to the opposite conditions but able to survive unfavourable periods; B: the two cases when the oscillations do not cause speciation due to the contrasting adaptation strategies. The flexible (upper curve) strategy causes evolution towards single species of extreme morphological diversity similar to the case described by Johnson and Stankowski (2018), the second strategy is a selection of robust specialist able to survive the extreme conditions.

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ary mechanisms, which make complex assemblages of organisms sustaining the challenges offered by global and/or local processes, must be multidisciplinary. Now, we are going to adopt different points of view at the most crucial problems concerning the sustainability mechanisms of the Baikal biodiversity with respect to the stream of environmental challenges it used to face so successfully until now.

Classic ecological and hydro-biological approaches are indispensable, both in justification of new studies and in the previous knowledge about the ecosystem. Taking into account almost perfect isolation of Baikal species from the neighbouring biotopes, one may speculate that the rate of species description from the lake should decrease from now on, and the taxonomy of major groups should reach saturation. This means that the past century period of extensive study of the lake (Timoshkin, 1997) is about to be finished, and the main focus will shift towards the organization of organisms at higher levels, and future findings should be expected in the field of organismal interactions in such complex communities typical for Lake Baikal.

Indeed, to date 34 distinctive species communities of macroinvertebrates have been described in the Southern Basin of the lake. These communities are dominated by amphipods, oligochaets, trichopterans, chironomids, mollusks and representatives of other groups. Species diversity of all communities is high: their Shannon index varies from 2.9 to 4. Number of species forming invertebrate communities range from 36 to 144 species, and from 5 to 17 of them may dominate by biomass that is evidence of a complex structural organization of the communities (Kravtsova et al., 2004). This complexity suggests high stability. However, it is still not clear, if they remain stable during evolution. One may expect that if inter-specific interactions are taken into account, the picture will become even more complicated. No wonder that some peculiarities in general setup communities in Lake Baikal had been already described, but they had to be explained. Moreover, coevolution between most species in communities has not proved yet.

Analysis of the coordination between evolutionary processes in species belonging to the same community means that the complexity of data analysis increases dramatically in comparison with “mere” description of biological phenomena. Therefore, more sophisticated methods of data analysis and statistical assessments are required. Theoretical treatment of evolutionary problems becomes increasingly important. The most common theoretical approach includes some kind of modelling of processes studied in a way that the result of modelling repeats the experimental data used. This approach was developed as early as in 2004 (Semovski et al., 2004a; Bukin et al., 2007; Fazalova et al., 2010) and used successfully to elucidate several cases of complex micro-evolutionary events that generated peculiar molecular phylogenies. Fig. 2 show one of these examples. Individual-based model was used to show that the F_{st} metrics designed to describe pair-wise differentiation between populations; it is still useful in case of many populations. Application of this approach

is justified for the description of population subdivision of *Gmelinoides fasciatus* (Gomanenko et al., 2005; Bukin et al., 2018). The same approach was effective in case of demographic changes of population. It allowed estimating the number of sequences for elucidation of a real-world problem (Semovski et al., 2004a; 2004b). In all simulations, the environmental changes were modelled as changes of “environmental capacity”, which was defined as the maximum number of individuals sustained at biotope under given conditions. Fig. 2 shows the individual-based modelling of genetic differentiation between four linear populations of organisms with different mobility (average distance of migration from the birthplace). In further studies, the modelling approach will be applied to newer kinds of data, such as multiple SNPs and microsatellite loci, which will be added to the individual characteristics of objects.

In general, new possibilities in studies of biodiversity appeared, when high throughput DNA sequencing techniques became feasible for environmental and/or evolutionary studies of invertebrates, protists and algae (Sauvadet et al., 2010; De Vargas et al., 2015; Leray and Knowlton, 2015). Additionally, the diversity of protists in nature was underestimated significantly, and metagenomics approach was absolutely necessary for describing biodiversity (Leray and Knowlton, 2016). Species composition of protists was shown to be very specific. The study of eukaryotic metagenomes is es-

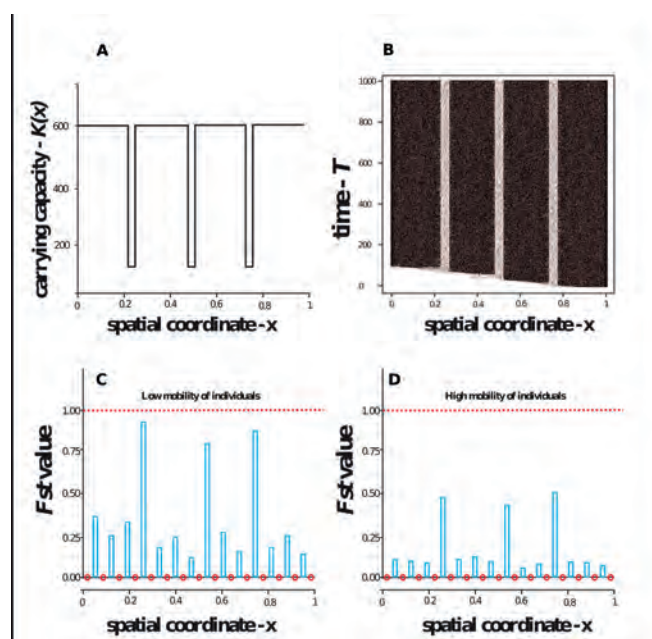


Fig. 2. The semi-transparent barriers between the populations arranged as shown on the panel A. Panel B shows the initial expansion of the individuals (each dot corresponds to an individual) filling the whole range and forming the distribution stable in time. Semi-transparent barriers between the populations are much less densely populated. Red circles on panels C and D designate sampling points along the spatial coordinate (x). F_{st} values were calculated by splitting the simulated data into two sets left and right from every stretch linking neighbouring “sampling points”. Panel C shows F_{st} peaking on the barriers in case of low organism mobility, D - the same in case of high organism mobility. The simulation illustrates that F_{st} may be useful for finding barriers between more than two populations.

pecially important, since recently occurring fast local transformations in Lake Baikal changed environment of near-shore zone of the lake most likely due to antropogenic impact, although natural reasons cannot be fully eliminated (Kravtsova et al., 2014). One of the most obvious features of the changes is bloom of filamentous green algae in many cases dominated by species of *Spirogyra* (Romanova et al., 2013). Representatives of this genus were described as very rare species in Lake Baikal in the middle of the 20th century (Izhboldina, 2007). It is important to understand, whether the current blooming is caused by the local opportunistic species responding to the favourable conditions, or by the exotic species that invaded Lake Baikal. Unfortunately, it is difficult to rely on morphologically-based species diagnoses due to lack of morphological traits in this group (Wongsawad and Peerapornpisal, 2015).

Our preliminary metagenomic data suggest that there are, in fact, several sequences of *Spirogyra* possibly of the species rank, and at least some of them are very close to non-Baikal species (in press).

Notably, the application of modern methods of high throughput sequencing in studies of Baikal biota have begun relatively recently (Ravin et al., 2010). Studies in this direction have revealed many unexpected findings, some of which are difficult to explain (Romanova et al., 2016; Kulakova et al., 2018). For instance, an extremely high level of gene order rearrangements in mitochondrial genome (as exemplified in Fig. 3) in comparison with the non-Baikal sister taxa (Cormier et

al., 2018). The most rearranged mitochondrial genome was detected in shallow water amphipods *Gmelinoides fasciatus*. This finding appears to be especially mysterious in combination with the data on extreme longevity and evolutionary stability of amphipods species (Bukin et al., 2018). The habitat of this amphipod species was affected in the course of global ecosystem changes through the whole history of a large lake within the confines of current Baikal. In spite of dramatic ecosystem transformations, this species has survived and kept its identity.

Acknowledgments

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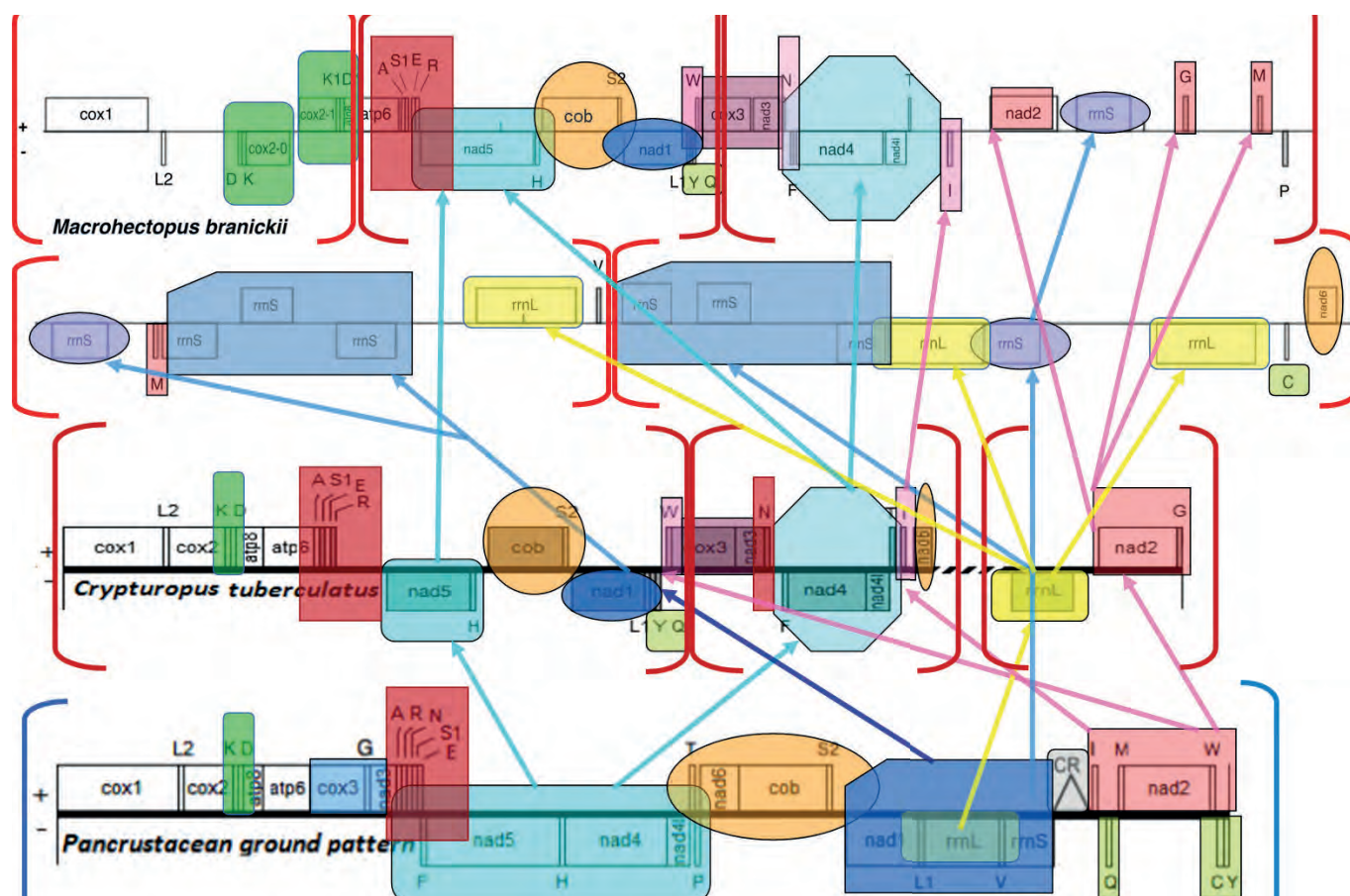


Fig. 3. Gene order rearrangements in Baikal amphipods *Crypturops tuberculatus* and *Gmelinoides fasciatus* compared to the Pancrustacean ground pattern shared among most of Crustacea.

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Mass disease and mortality of Baikal sponges

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ABSTRACT. In recent years, significant changes in the ecological system of the coastal (littoral) zone, including mass death of the endemic representatives of the freshwater sponges of the Lubomirskiidae family, have been an urgent problem of Lake Baikal. Similar problems are known all over the world. Thus, mass disease and death of corals and sponges are indicated in the Mediterranean, Adriatic, Caribbean and other seas, which raises serious concerns about the future of these biocenoses (Olson et al., 2006; Webster, 2007; Wulff et al., 2007; Stabili et al., 2012). In Baikal, diseased sponges were first found in 2011. The area of sponge disease is constantly expanding, and, to date, dying specimens have been found throughout the lake. The mass death of sponges occur in presence of the large-scale violation of the spatial distribution and the structure of phytocenoses in the littoral zone, but the causes of these phenomena are unknown.

The relevance of the problem arises from the fact that changes in the littoral zone of Lake Baikal can significantly affect the productivity and composition of planktonic organisms and zoobenthos, which are the food base for fish, as well as the quality of drinking water. The deterioration of the ecological state also affects the attractiveness of the lake for tourism. At the international level, serious intellectual and financial resources were mobilized to solve similar problems. Despite the obvious relevance, in Russia such studies are carried out irregularly by small groups of researchers.

Keywords: Baikal, sponges, mass death, metagenome, dysbiosis

1. Introduction

Lake Baikal is sometimes called The Galapagos Islands of Russia because of its exceptional biodiversity and importance for the evolutionary science. The age, isolation and deep oxygen-saturated waters of Lake Baikal formed one of the world's richest freshwater ecosystems. Lake Baikal located in the centre of Eurasia has many features inherent to the ocean: abyssal depths and a huge mass of water, internal waves and seiches, strong storms and high waves, upwelling, expansion of the basin due to the separation of the coast similar to the separation of the continents of Africa and South America, large magnitudes of magnetic anomalies, etc. (Kondratyev, 1992). Lake Baikal is the only deep-water lake, where the water saturated with oxygen stretches to the very bottom, like in the ocean.

The ecosystem of Lake Baikal is also close to oceanic ecosystems by structure and other characteristics, e.g. the presence of a pelagic community of the organisms and zones with high productivity of macroplankton in the area of the slope similar to the highly pro-

ductive zones of upwelling in the ocean. The benthic community is characterized by extraordinary species diversity and high production, and it mainly consists of oligochaetes, molluscs, amphipods, chironomids and sponges. The biomass of sponges exceeds tenfold the biomass of all littoral macroorganisms (Kozhov, 1972; Masuda, 2009).

The biological diversity of plants and animals inhabiting Baikal is greater than in other lakes of the world. For example, the number of known species and subspecies is more than twice higher than in Lake Tanganyika, which is comparable to Baikal in dimensions and origin. At present, there are approximately 2500 species of animals and more than 1000 species of plants in Lake Baikal. The Baikal fauna is formed autochthonous, i.e. the diversity of endemics has developed in Lake Baikal itself. Baikal is one of the most transparent lakes in the world. The amazing transparency (approximately 40 meters) has resulted from the low content of mineral salts in the lake and a great filtering ability of sponge communities and other filtration organisms.

Recently, Lake Baikal is facing increasing threats to environment. Global climate change can threaten its

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ecosystem; thus, the temperature of the surface water layers and the ice cover have already changed. Other threats are the industrialization of Russia and Mongolia, as well as the explosive boom of tourism. The huge increase in tourists on the shores of the lake does not lead to a real understanding by the authorities of how the waste products of tourists affect Baikal, and the locals do not have the adequate waste management systems for the tourist business. However, the largest threats are the changes in the biological community of the coastal zone.

Thus, the abundant growth of the filamentous algae *Spirogyra* was first recorded in 2010 in Bolshiye Koty Bay, which subsequently spread to other areas (Timoshkin et al., 2014). Mass reproduction of *Spirogyra* is found in shallow water, in areas with an increased content of biogenic elements. Filaments of dying off algae are washed out in shallow water, where they rot releasing the toxic substances uncharacteristic for the lake that affect the water quality.

Almost simultaneously with the onset of the abundant growth of the filamentous algae in the littoral zone of the lake, diseased sponges of anomalous pink colour were found. The disease of freshwater sponges in Lake Baikal first appeared in 2011 (Bormotov, 2011) and was accompanied by the death of the symbiont green microalgae changing the green colour of the sponges to pink. Sponges with anomalous colouration were found only in Central Baikal at depths of 25-55 m. In subsequent years, the external signs of the disease changed, and now sponges are found throughout the lake with different symptoms of body lesions, such as discolouration, tissue necrosis, the formation of brown plaque and dirty purple bacterial covers of separate branches. The number of sponges *Lubomirskia baicalensis*, which are the most sensitive to the disease, reduced by a third. In addition, 10-20% of annually registered sick sponges died during winter (Timoshkin et al., 2016; Khanaev et al., 2018).

2. Analysis of diseases of marine sponges

Healthy sponges are in dynamic equilibrium between the macroorganism, symbiotic microorganisms and microorganisms from the environment, the quantitative and species composition of which can vary greatly during drastic changes. The impact of stress on sponges can cause dysbiosis, i.e. a violation of the symbiotic community. In sponges, it is often characterized by increased alpha diversity (Luter et al., 2012) or the appearance of opportunistic pathogens (Simister et al., 2012). The stress can threaten the physiology and immunity of the host (Ghanbari et al., 2015; Pinzón et al., 2015; Liu et al., 2016), which further leads to loss of control of the microbiome and death of sponges. Thus, dysbiosis is probably not the cause of the disease, but may be a response of sponges to stress.

Recently, an unprecedented increase in the number of diseases of benthic organisms, including sponges, corals and algae, has been observed worldwide (Webster, 2007; Bourne et al., 2009; Burge et al., 2014), some of which led to mass mortality (Garrahou et al.,

2009). For example, in the Mediterranean in 2008 and 2009, 80-95% of *Ircinia fasciculata* and *Sarcotragus spinosulum* samples died. The sponge disease *Ianthella basta* and *Rhopaloeides odorabile* was widely spread on the Great Barrier Reef (Luter et al., 2010a; 2010b). From the latter sponge, the pathogenic strain NW4327 *Pseudalteromonas agarivorans* was isolated (Webster et al., 2002; Choudhury et al., 2015). However, in many other studies, only an imbalance of microorganisms was found causing a greater variety of microorganisms in diseased sponges in comparison with healthy individuals. The disease was accompanied by the development of opportunistic, often polymicrobial infections (Lesser et al., 2007; Coma et al., 2009; Bourne et al., 2016). In most cases, researchers only state dysbiosis, i.e. a shift in the microbial community of diseased sponges, without isolating a pathogenic agent (Webster et al., 2008; Gao et al., 2015; Blanquer et al., 2016; Luter et al., 2017; Deignan et al., 2018).

At present, symbiotic communities consisting of a host macroorganism (animals, plant and algae) and its associated microflora have been proposed to be called holobionts, a complex ecosystem, which symbiotic partners are closely interrelated by functions that cannot be performed by individual organisms (Rohwer et al., 2002; Bosch and McFall-Ngai, 2011; McFall-Ngai et al., 2013; Bordenstein and Theis, 2015). Only a single harmonious system of host, together with symbiotic microorganisms, ensures interaction with the environment and affects the health and functioning of the entire ecosystem. Sponges are one of the most diverse and complex aquatic habitat holobionts.

Sponges are simple, but successful organisms, which evolutionary age exceeds 600 million years, and they are common everywhere (Van Soest et al., 2012; Maldonado et al., 2015). Sponges are sessile organisms that filter large amounts of water for nutrition (Gili and Coma, 1998; de Goeij et al., 2013; Kahn et al., 2015; Maldonado et al., 2015); therefore, they are strongly affected by waterborne viruses, bacteria, archaea and eukaryotic microorganisms (Thomas et al., 2016; Moitinho-Silva et al., 2017). These microorganisms are the main source of food for sponges, but, at the same time, there are various symbiotic microbial partners in the body of sponges that have avoided the effects of the immune system. Microbial communities contribute to the nutrition, protection, immunity and development of the host, collectively affecting its functioning and health (Koropatnick et al., 2004; Eberl, 2010; Nicholson et al., 2012; Flórez et al., 2015). Symbionts of sponges are species-specific and are divided into two clusters: the core microbiome consisting of microorganisms found in most species of sponges, and the variable microbiome consisting of 'focused specialists', which differ in their relative numbers and are rarely found in other species (Erwin et al., 2012; Hester et al., 2015; Thomas et al., 2016). Unfortunately, the formation patterns of these two groups remain unknown (Moitinho-Silva et al., 2017). A sponge holobiont is a dynamic ecosystem that reacts to changes in the environment, particularly, anthropogenic stressors that threaten the holobiont stability and lead to dysbiosis, illness and death of sponges.

So far, the causes of death among sponges and other sessile filtering organisms recorded worldwide are not precisely known, and methods of combating this phenomenon, as well as ways to prevent it have not been found. Recently, methods of a next-generation sequencing and analysis of interactions in the host-microorganism system have been widely used to study the diversity of eukaryotic microbial symbionts and ways of structuring microbial communities (Costello et al., 2012). In the study of diseases and death of sponges and corals, metagenome sequencing is most often used to study changes in the composition and activity of bacterial communities. The key unresolved problem is whether the functional features of symbiotic microorganisms can be predicted based on their taxonomic position. Understanding of these ecological problems will provide answers to long-standing evolutionary questions, particularly, whether microorganisms can influence speciation models and evolutionary diversification of their hosts (Brucker and Bordenstein, 2013).

To identify the causes of infection, 16S RNA sequencing of sponge symbionts (Webster et al., 2002; Cervino et al., 2006; Angermeier et al., 2011; Stabili et al., 2012; Choudhury et al., 2015; Gao et al., 2015; Sweet et al., 2015; Blanquer et al., 2016), amplicon sequencing of other genome sites of bacteria and eukaryotes (Choudhury et al., 2015; Sweet et al., 2015), whole genome sequencing of a bacterial pathogen (Choudhury et al., 2014), and culturing of pathogenic bacteria (Stabili et al., 2012; Choudhury et al., 2015) were used in these studies. The significant changes in the composition of the microbiome were observed in all cases of sponge disease described. Only in some studies these experiments allowed identifying the pathogen or group of pathogens that caused sponge infections (Webster et al., 2002; Cervino et al., 2006; Stabili et al., 2012; Sweet et al., 2015), as well as biochemical mechanisms through which infection developed (Webster et al., 2002; Choudhury et al., 2015). However, in case of Baikal sponges disease, as in some other cases of sponge disease (Angermeier et al., 2011; Blanquer et al., 2016), such analysis was not effective enough to identify the cause of the disease.

3. Studies of the disease of the Baikal sponges

3.1. Field studies

In May-June 2015, 11 transects were laid throughout the water area of Lake Baikal: transects Nos. 1-3 – near Varnachka, transect No. 4 – Ulanovo settlement, transect No. 5 – near Olkhonskiye Voro-ta Strait, transect No. 6 – Cape Ukhan, transect No. 7 – Cape Elokhin, transect No. 8 – Cape Turali, and transects Nos. 9-11 – in the Listvennichny Bay. During annual expeditions around Lake Baikal, at these transects quantitative and qualitative collection of sponge samples was carried out. We have developed a new method, which allows us to take not the whole sponge, but only its small part, after which the sponge remains

viable. In 2015, sponges were mapped using photo and video documentation, and approximately 1800 sponge samples were collected. The data obtained in 2015 were processed and published in the Journal of Great Lakes Research (Khanaev et al., 2018). Subsequently, similar expeditions with the collection of the sponge samples were conducted in 2016-2018. The diseased sponges were found throughout Baikal, but the degree and intensity of the disease varied depending on the lake areas and the depths. Percentage of the projective cover of the bottom by diseased and healthy sponges varied in the basins. Thus, the ratio of diseased sponges to healthy ones (1 m²) in Southern Baikal Basin varied from 22.3 to 51.4%, in Central – 8.4-11%, in Northern – 8.5-11%. The data we obtained are basic for studying the dynamics of the state indicators of spongi fauna in the future.

3.2. Analysis of Baikal sponge metagenomes

The endemic freshwater Baikal sponges of the Lubomirskiidae family dominate the littoral zone of the lake, and their biomass is more than 700 g per m² (Kozhov, 1972; Pile et al., 1997; Semiturkina et al., 2009). Such sponge biomass is unusually high for the freshwater body (Bailey et al., 1995), but comparable to coastal Antarctic benthic communities (Dayton et al., 1974) and some reefs (Wilkinson, 1987). *L. baicalensis* has a rich green color due to the presence of a large amount of symbiotic green algae, probably *Choricystis* sp. (Trebouxiphyceae).

Fresh samples of sponges *L. baicalensis* were collected by scuba diving during field trips conducted in 2010, 2011 and 2015 from the Southern, Central and Northern Baikal Basins. The samples were frozen at -20 °C immediately after lifting and transported to the laboratory in refrigerator for subsequent DNA isolation and sequencing analysis.

Total DNA was isolated using TRIzol LS reagent (Invitrogen, Ambion, USA) according to the manufacturer's protocols. The universal bacterial primers 518F and 1064R (Huber et al., 2007) were used to amplify the V4-V6 hypervariable region of the bacterial 16S rRNA gene using the 454 GS Junior sequencing System and with GS FLX Titanium series reagents in Irkutsk Anti plague Research Institute of Siberia and the Far East. The raw sequencing reads are available under BioProject ID: PRJNA369024.

In the aggregate processing of data files obtained using two different sequencing technologies, an open-reference OTU picking implemented in the QIIME package (Caporaso et al., 2010) was used. Within the QIIME platform, the sortmerna_sumaclus option (Kopylova et al., 2016) was used as a method, and the subset of SILVA database gg_13_5 compatible with the Picrust package (Mukherjee et al., 2017) was used as a reference.

Fig.1 shows the distribution histogram of families of microorganisms in the sponge samples.

The analysis of the histogram indicated that the content of green alga (Trebouxiphyceae) in sponge samples varies greatly from 90 to 0%. At the same time,

there was no clear correlation between the content of algae and the health of sponges. In sponges with external signs of the disease (see samples marked with purple blocks), the content of green algae could be comparable to a healthy sponge, as in 2010, decrease or disappear. Another family of crustal microbiome, the content of which varies significantly in different sponge samples, is *Chitinophagaceae*. The maximum number of these bacteria was observed in sponges without external signs of the disease, but with a reduced number of green algae. Sponges with an increased content of green algae relative to a healthy sponge in 2010, as well as

sponges with a very low content of *Trebouxiophyceae*, had a low content of *Chitinophagaceae*. The remaining 5 families of the core microbiome did not change significantly, and the number fluctuations did not clearly depend on the health of the sponges. The bacteria *LD19* (*Verrucomicrobiaceae*) were an exception, the high content of which was found in a diseased pink sponge collected in 2011. Much smaller amounts of *LD19* were found in samples of healthy sponges collected in 2010 and 2011. Hence, a change in the composition of the microbiome may not lead to the appearance of external signs of the disease.

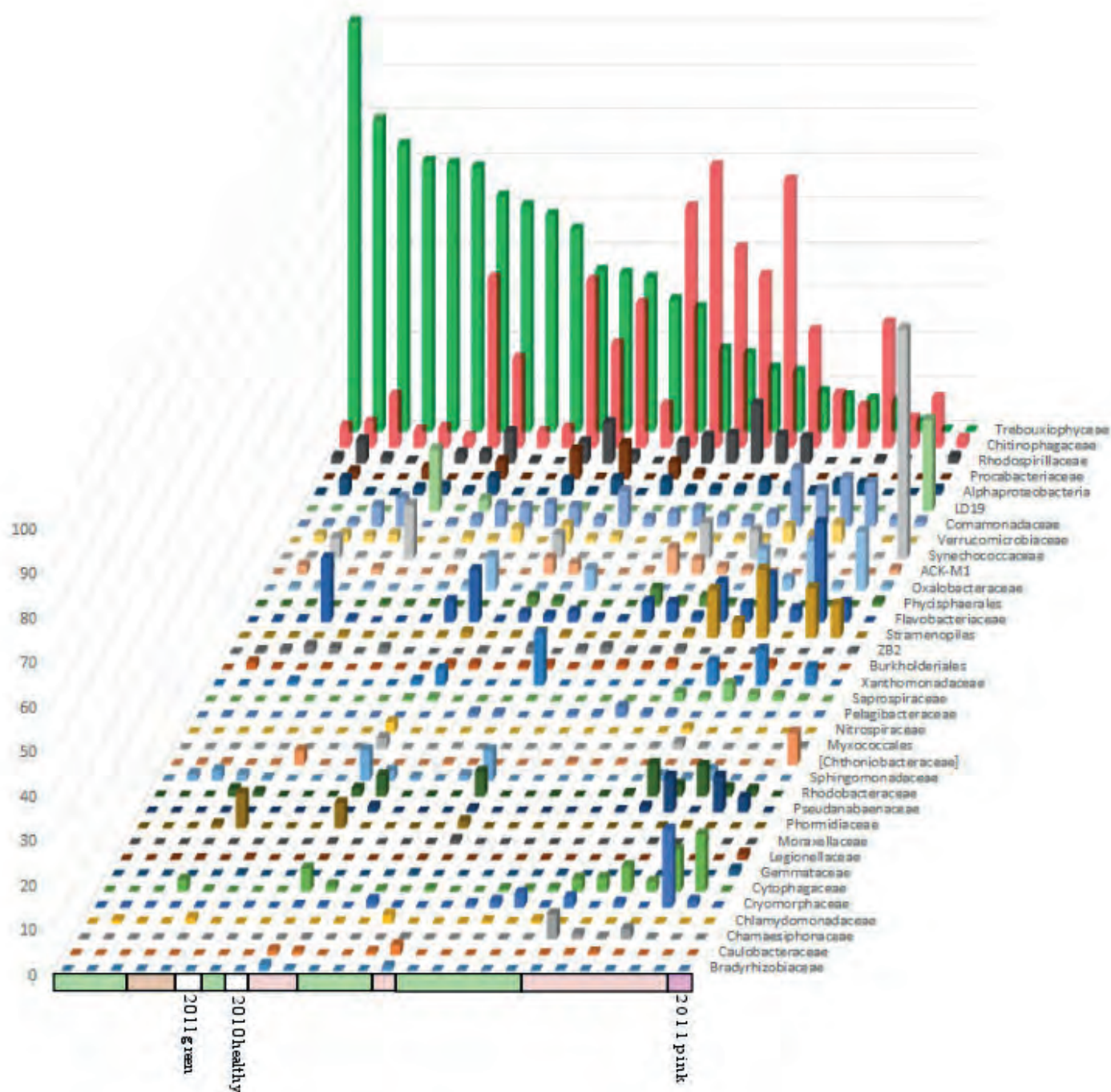


Fig. 1. The changes of the Baikal sponge microbiomes. The composition of microbiomes of Baikal sponges collected in 2015, ranked by reducing the amount of symbiotic green algae. Sponges with no visible signs of the disease are marked with a green rectangle; sponges with signs of the disease are purple. As a comparison, the compositions of microbiomes of a healthy sponge 2010 (2010 healthy), a diseased pink sponge 2011 (2011 pink) and a sponge without external signs of the disease 2011 (2011 healthy) are given. The analysis included families of bacteria whose content exceeds 0.5%.

4. Conclusion

Therefore, the composition of the microbiome in Baikal sponges differs from the microbiome of sea sponges, but the recorded changes in the microbiomes leading to dysbiosis are apparently not the cause of the disease. The most likely causes are stressful effects, such as rising temperatures, increasing concentrations of nutrients, and other unknown factors.

At the same time, ecological stress accompanied by changes in the symbiotic microbial community can lead not to death, but to the adaptation of the holobiont to new conditions (Webster and Reusch, 2017). Thus, symbiotic microorganisms of corals influence their ability to acclimate to the changing environmental conditions (Reshef et al., 2006; Ziegler et al., 2017). Changes in the genomes of microorganisms are possible, but they rarely occur, and speciation proceeds slowly. However, the composition of the sponge symbionts can rapidly change by capturing new microorganisms from the environment. Additionally, genetic changes in the associated microbiome are also possible, for example, by accelerating horizontal gene transfer (Thomas et al., 2010; Fan et al., 2012; Gao et al., 2014). Horizontal gene transfer can quickly result in acquisition of new functions without changes in taxonomic composition (Rosenberg et al., 2007; Putnam et al., 2017).

In recent years, some scientists engaged in microbiology of sponges have joined the project (The Sponge Microbiome project) in order to develop the standardized protocols and unified databases of the microbial diversity of symbionts in marine sponges (Thomas et al., 2016; Moitinho-Silva et al., 2017). Joining to this project may be reasonable to find causes of the disease of Baikal sponges. Comparison of diseases in marine and freshwater sponges is not only of practical, but also of theoretical interest to clarify the role of the host as the basis of the ecosystem and to determine mechanisms underlying the interaction between sponge and microorganisms. Therefore, a comparison of marine and freshwater holobionts can facilitate understanding of the relationship between the stress, dysbiosis and the disease or adaptation of sponges in order to develop a strategy for managing a situation.

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POPs monitoring system in Lake Baikal – impact of time or the first need?

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ABSTRACT. Persistent organic pollutants (POPs) were first found in the fat of the Baikal seal “*Phoca sibirica*, Gm” in 1986. In subsequent years, studies of POPs at Baikal did not lead to the creation of a monitoring system, despite its high demand for Lake Baikal as a source of drinking water of the world value. We have proposed a solution to the scientific methodological problem of POPs control in Lake Baikal by selecting priority POPs, the optimal sampling and developing methods for determining POPs in a range of concentrations corresponding to their content in Baikal waters and meeting the requirements of serial analysis. Three classes of pollutants were selected as priority POPs, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and o-phthalic acid diesters (phthalates). The water-sampling scheme in the pelagic zone of the lake included five directions from the west to the east coast in three lake basins, the southern and northern extremities of the lake, the delta of the Selenga River, Maloye More strait, and Chivyrkuy and Barguzin bays. The determination of priority POPs was based on a comprehensive analysis of one sample, the volume of which did not exceed 1.0 L, and the GC-MS/MS method in the analytic ending. Depending on the POPs concentration, the accuracy of their determination was estimated at a range of $\pm \delta$ from 10 to 35%. The testing of the methodology has indicated that the POP content in the Baikal waters at the modern stage is characterized by phthalate concentrations ranging from 0.03 to 3.7 $\mu\text{g/L}$, total PAH concentrations from 7.0 to 36 ng/L , and PCB congeners from 1.4 to 7.2 ng/L .

Keywords: Lake Baikal, POPs monitoring system, PCBs, PAHs, phthalates

1. Introduction

The first data on the presence of persistent organic pollutants (POPs) in Baikal were presented in Ts.I. Bobovnikova et al. (1986). The authors reported the discovery of polychlorinated biphenyls (PCBs) and organochlorine pesticides in the fat of the Baikal seal “*Phoca sibirica*, Gm”. A wider range of POPs detected and investigated in Baikal was shown in a series of works carried out within the framework of the Baikal International Center for Environmental Research (Kucklik et al., 1994; 1996; Iwata et al., 1995; Nakata et al., 1995) and summarized later by M.A. Grachev (2002). At this stage researcher were mainly interested in organochlorine pollutants, since the compounds from this group are stable in environmental objects and toxic for wildlife and humans. At that time, the POP content in the lake was assessed as corresponding to the global reference level.

The monitoring studies conducted in 1992-1993 indicated that organochlorine pollutants, depending on the levels of their accumulation in the fat of the seal

and in the muscles of the Baikal omul, can be represented as follows:

PCB ~ DDT > Chlordane > HCCH > HCB > > > PCDD, PCDF (Kucklik et al., 1994; Nakata et al., 1995), where: DDT is the sum of dichlorodiphenyltrichloroethane and its metabolites, HCH – hexachlorocyclohexane, HCB – hexachlorobenzene, PCDD – polychlorinated dibenzodioxins and PCDF polychlorinated dibenzofurans.

In Baikal water, the total concentrations of PCB congeners (upper water layer) were determined in the concentration range from 0.02 to 0.59 ng/L (Iwata et al., 1995) and from 0.13 to 1.9 ng/L (Kucklik et al., 1996) with the trend of their increase from the northern to the southern basin of the lake. Based on the similarity of PCB congener profiles found in the water and in the technical product “Sovol”, which was produced at that time in Russia, it was concluded that PCBs enter the lake from local sources.

In subsequent years, POPs studies at Lake Baikal was focused on the catchment basin of lake, i.e. water and bottom sediments of the tributaries, soils on the coast; identification of sources; assessment of regional

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transport of POPs from the industrial zone of the Baikal region to the water area of the lake (Gorshkov and Marinaite, 2000; 2002; Marinaite and Gorshkov, 2002; Batoev et al., 2003; Tsydenova et al., 2003; 2004; Gorshkov et al., 2008; Marinaite et al., 2013; Shirapova et al., 2013a; 2013b).

2. Results and Discussion

In 2013-2014 within the framework of the Federal Target Program “Protection of Lake Baikal and the socio-economic development of the Baikal natural territory for 2012–2020”, the specialists from the Scientific and Production Association “Taifun” and LIN SB RAS carried out monitoring of organochlorine pollutants. The study objects were the surface layer of the atmosphere, the upper water layer, bottom sediments and lake biota, and the coastal soil. According to the monitoring results, the content of PCDD and PCDF in three lake basins did not exceed 0.01 pg/L; PCBs were assigned to the main organochlorine POPs in Baikal water. The monitoring also revealed the following changes in the “fate” of PCBs in Baikal over the past 20 years (Gorshkov et al., 2017a; Samsonov et al., 2017):

- the concentration of PCBs in water was 4.5 times higher relative to the maximum levels and 20 times higher relative to the minimum values recorded in 1991-1992;
- water from the middle basin of the lake differed in the minimum PCB content (Fig. 1).

The increasing concentration of PCBs in Baikal water, arose the following questions: a) an increase in the level of PCB content in Baikal waters is the result of their slow accumulation over the entire period or a salvo injection during a short period of time, b) the current level of PCB content in Lake Baikal will continue in the future because of the sustainability of pollutants of this class in aquatic ecosystems, or its gradual decrease is possible. Due to the lack of a monitoring system for POPs in the lake waters and systematic monitoring data, there are no answers to these questions (Fig. 2).

Identification of PCB sources, which determined the growth of PCB content in the lake waters, has not been completed as well. According to (Samsonov et al., 2017), the global atmospheric transport is the dominant channel for the entry of PCBs into Baikal waters, since “there are no significant variations in the qualitative composition of congeners identified in the composition of water samples” collected in the pelagic zone of the lake. At the same time, an increased content of congeners with a high degree of chlorination (11–19% of the total amount of PCB) in water samples from Southern Baikal indicating the persistence of local PCB sources was observed in (Gorshkov et al., 2017a).

The group headed by E.N. Tarasova contributed significantly to the study of superecotoxicants - PCDD and PCDF, in the Baikal ecosystem (Mamontova et al., 1997; 2001; 2013; Tarasova et al., 1997; Mamontov et al., 2000; 2013; Kuzmin, 2005). In particular, should note an estimate of the proportion of coplanar PCBs in

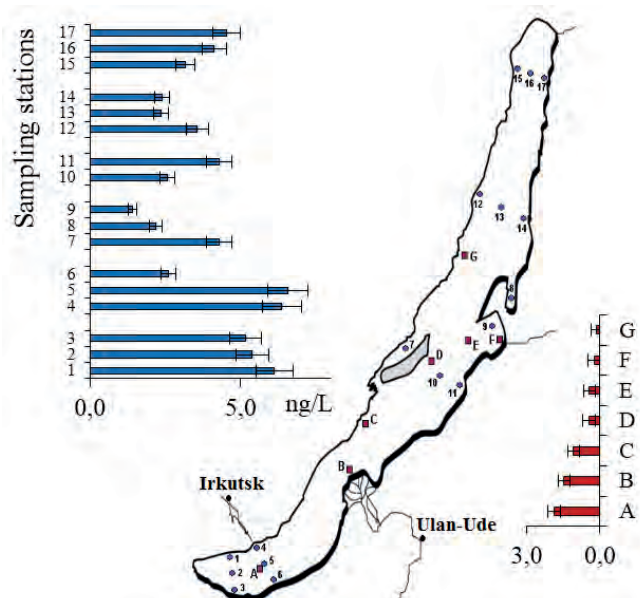


Fig. 1. Map of water sampling and total concentrations of PCB congeners (ng/L) detected during the expeditions conducted in 1993 – ■ (Kucklick et al, 1996) and 2015 – ■ (Gorshkov et al., 2017a).

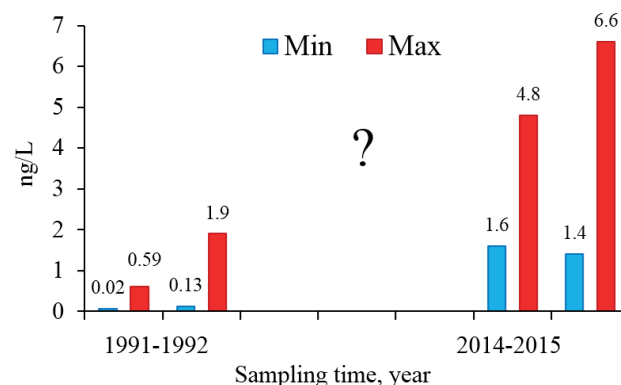


Fig. 2. Levels of total PCB concentrations (ng/L) detected during independent expeditions in 1992-1993 (Iwata et al., 1995; Kucklick et al, 1996) and 2014-2015 (Gorshkov et al., 2017a; Samsonov et al., 2017).

the total equivalent toxicity of PCDD, PCDF and PCB (TEQ1998), which exceeds 70% in commercial fish (Mamontov et al., 2000) and 50% among the people examined in the Baikal region (Shelepchikov et al., 2012).

According to a study conducted by LIN SB RAS, polycyclic aromatic hydrocarbons (PAHs) can be also included in the Baikal waters purity control system. The presence of PAHs in the lake waters is associated with both anthropogenic sources: organized emissions of boiler houses burning coal in cities and settlements on the coast; emissions from the Baikal fleet, as well as from natural sources. The latter include forest fires at the Baikal natural territory and natural oil shows.

In the upper water layer of the pelagic zone of the lake, PAHs showed rather narrow range of total concentrations, from 7.0 to 36 ng/L (results of PAH monitoring in the waters of Baikal in the spring of 2015 and 2016). The composition of PAHs was dominated by naphthalenes; their total quantity reached 15 ng/L. In the fraction of detected PAHs, this value was 50-

70%. During the monitoring of Baikal waters in 2016 (autumn), the samples from the upper water layer of the southern and middle basins were characterized by a high content and extreme quantity of PAHs in individual samples, exceeding the average level for lake waters, by 5–15 times (Fig. 3). The composition of PAHs in these water samples also differed essentially: phenanthrene, pyrene, fluoranthene dominated in the PAHs fraction (up to 90% of the detected Σ PAHs). Considering that during the water sampling the Siberian taiga was burning on the coast of the lake, and pyrene and fluoranthene are indicators of wood burning products, an increase in the PAH content in the upper water layer may be a result of their entry from the atmosphere polluted by forest smoke.

During 2006–2016, from 10 to 32 cases of fires occurred annually; the forest area covered by fires estimated from 116 to 11,000 hectares per 1 million hectares of territory. For the period from 2014 to 2016, the total area of forest fires covered 26,720 hectares of territory representing 80% of the total area of all fires for the past 10 years (State Report, 2017). In the absence of a system of continuous control, it is difficult to assess the contribution of PAHs in Baikal waters from forest fires, considering the spatial and temporal variability of this natural source.

Another natural source of PAHs in the Baikal ecosystem is natural oil seepages characterized by oil entering the lake water in a volume of 2 to 4 tons/year (Khlystov et al., 2007; Kontorovich et al., 2007). In Central Baikal (Cape Gorevoy Utes), the pollution of surface water with oil hydrocarbons was recorded for an area of no more than 1 km². The study of this Baikal phenomenon (Gorshkov et al., 2010) indicated that oil is fractionated at the bottom-water interface, and the segregation of heavy oil fractions takes place forming asphalt towers. A high-molecular PAHs, including such maximally toxic ones as benzo[a]pyrene, and dibenzo[a, h]anthracene, are accumulated in the asphalt towers, thus preserving the water purity of Lake Baikal. A light fraction of oil enriched with *n*-alkanes migrates to the water column and to the water surface

of the lake, where *n*-alkanes are readily biodegraded by the Baikal microbial community (Pavlova et al., 2012). Data on laboratory experiments showed PAHs accumulation by phytoplankton from the light oil fraction (Shishlyannikov et al., 2017). Obviously, these processes limit of the area of pollution of Baikal waters with petroleum hydrocarbons, the purity of water, the composition and quantity of PAHs outside the boundaries of oil seepages.

The study of the diesters of *o*-phthalic acid (phthalates) in the aquatic ecosystem of Lake Baikal allowed their including in the POPs control system as priority ones. Phthalates are the most important products of the chemical industry. The volume of their production in the world reaches 5-6 million tons per year, up to 80% of synthesized phthalates are used as plasticizers for plastics. Plasticizers do not form chemical bonds with the polymer and are capable of passing into the environment through free migration during the operation and recycling of polymer products. Moreover, the entry of phthalates into water bodies is possible not only from anthropogenic sources, but also as a result of the activity of bacteria and algae in aquatic ecosystems (Chen et al., 2004; Namikoshi et al., 2006). The first monitoring data of phthalates in Baikal showed a high heterogeneity of their distribution in the three depressions of the lake, sharp seasonal variability of concentration levels, which suggested various sources of this group of pollutants, anthropogenic and biogenic (Fig. 4) (Gorshkov et al., 2017b).

Notably, the POPs studies at Lake Baikal conducted since their discovery in the lake ecosystem but have not ended with the creation of a monitoring system, despite its high demand for Lake Baikal as a source of world-class drinking water containing up to 23,000 km³ of water or ~ 20% of surface fresh water of the world. A number of organizational issues in this direction were resolved by Irkutsk Hydrometeorology and Environmental Monitoring Department, but the scientific methodological problem of POPs control at Lake Baikal remained, which consists of the following:

- certified methods, state and international standards do not meet the requirements of POPs monitoring in Baikal water, since the lower limits of detectable concentrations of pollutants in these regulatory documents are higher than their content in samples of Baikal water (RD 52.44.590, 2016; GOST R 54503-2011; ISO 28540:2011; MP UVK 1.89-2014);
- an increase in the volume of samples taken to determine POPs at the trace and ultra-trace concentrations is practically unrealizable within the framework of monitoring considering the volume of the water body of Baikal and the required number of samples taken to obtain reliable information about the level of the lake water pollution.

The stated problem of POPs monitoring can be eliminated by solving the following tasks: a) selecting priority POPs for monitoring in Baikal waters; b) search for the optimal sampling option; c) development

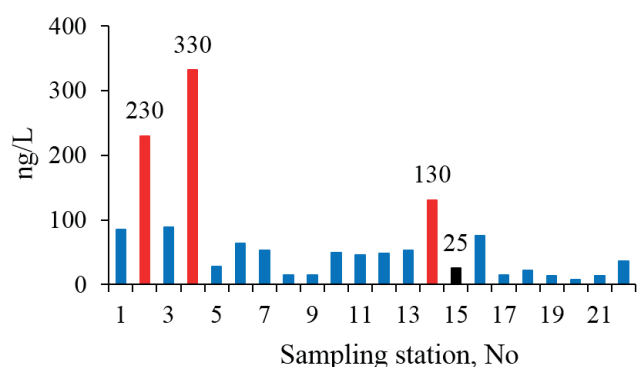


Fig. 3. Total PAH concentrations (ng/L) in the upper water layer of the Baikal pelagic zone (5 m). Water sampling in 2016 (September-October). ■ - samples with an extreme content of PAHs; ■ - PAHs content in the upper water layer (5 m) in the natural oil seepages of Central Baikal (Cape Gorevoy Utes).

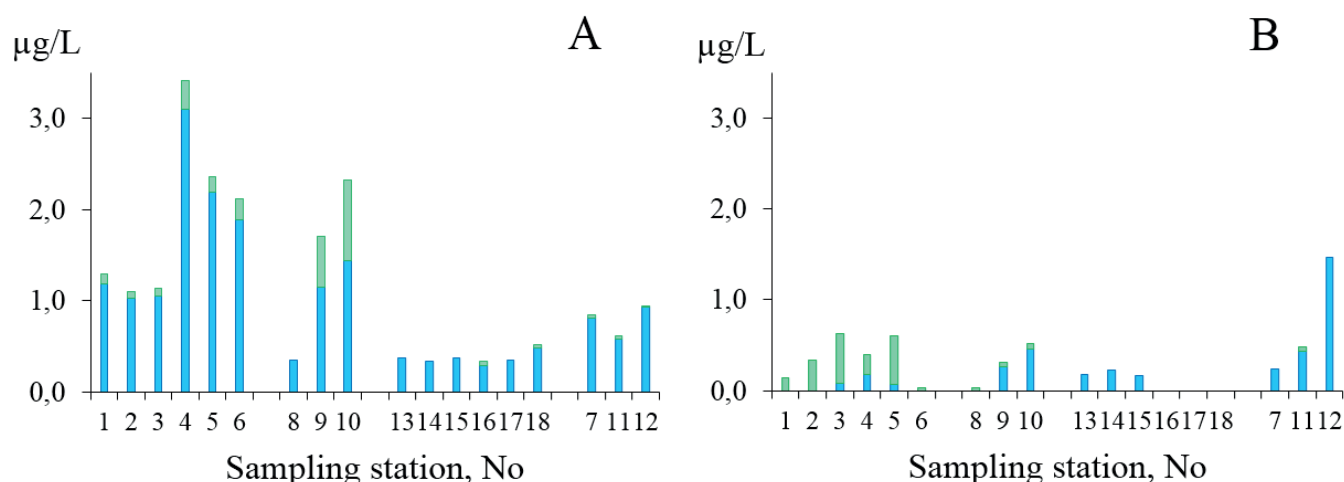


Fig. 4. The total concentration of phthalates ($\mu\text{g/L}$) in the Baikal pelagic zone, horizon 5 m. Monitoring: 2016, spring (A) and autumn (B). Phthalates: DBP - ■ and DEHP - ■

of methods for the determination of POPs in a range of concentrations corresponding to the content of POPs in the waters of Baikal and meeting the requirements of serial analysis.

The priority pollutants in the POPs monitoring system in Baikal waters should include three classes of compounds, PCBs, PAHs and phthalates, classified by the Stockholm Convention as POPs. PCBs were distinguished as the dominant organochlorine pollutants, and PAHs by the presence of natural sources. Phthalates in the water of Baikal were characterized by a level of two to three orders of magnitude higher than the concentrations of PAH and PCB, as well as sharp seasonal and spatial variability. Under these conditions, the determination of trends and dependences of the presence of POPs in the lake ecosystem is possible during their systematic monitoring.

We proposed the water sampling (Fig. 1) according to a scheme that includes 5 sections in three basins of the lake, three station at each section, 3 km from the western shore, 3 km from the eastern shore and 1 point in the center of the section, as well as sampling station in northern and southern extremity of the lake, in the area of the Selenga delta, Maloye More strait and in Chivyrkuy and Barguzin bays. In the three basins of the lake, we selected station for sampling from deep horizons (central points of the sections) from 4 to 6 samples depending on the depth of the lake at the sampling site.

To determine the priority POPs in Baikal waters, we have developed methods based on the extraction of pollutants in hexane and analysis of extracts using the GC-MS/MS method in the analytical ending. We suggest the determination of PCB, PAH and phthalates as part of a comprehensive analysis of a single sample, the volume of which does not exceed 1.0 L. The accuracy of measurements was estimated by the range of ± 8 from 10 to 35% depending on the analytes concentration (Gorshkov et al., 2017a; Izosimova, 2018; Kustova and Gorshkov, 2018). The GC-MS/MS method is highly sensitive and reliable for determination POPs, as well as included in the number of arbitration methods selected for the analysis of environmental objects.

This methodological approach was tested during the 2015-2016 expeditions around Baikal. The results indicate a modern level of priority POPs content in Baikal waters:

- two phthalates were found in the water of Baikal, di-*n*-butyl phthalate and di-(2-ethylhexyl)phthalate, the content of which corresponds to the concentration range from 0.03 to 3.7 $\mu\text{g/L}$;
- the content of ΣPAHs in the upper water layer of the Baikal pelagic zone ranged from 7.0 to 36 ng/L. The composition of PAHs was characterized by the dominance of naphthalenes, and their number reached 50-70% in the fraction of PAHs. There was no benzo[a]pyrene at the level of 0.1 ng/L, which was 50 times lower than maximum permissible concentration determined for this carcinogen in drinking water;
- in the waters of the lake, from 24 to 34 PCB congeners were identified (ΣPCB 1.4–7.2 ng/L). For continuous monitoring, 7 indicator congeners were introduced into practice.

3. Conclusion

It should be stated that the POPs monitoring system for the waters of Lake Baikal is not impact of time, but a first need. Choosing PCBs, PAHs and phthalates as priority pollutants, optimal scheme for water sampling, and new analytical solutions developed for determining POPs in the lake water can serve as a scientific basis for the POPs monitoring system in Lake Baikal. The data on the POPs content in Baikal at the present stage obtained from testing the proposed control system can be used as a starting point when considering trends in the “fate” of POPs in the future.

Acknowledgments

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Aerosol in the atmosphere of the Baikal region: history and contemporary researches



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ABSTRACT. The results of long-term studies (1995-2017) of the physical properties and chemical composition of atmospheric aerosol in the Baikal region are considered. These studies are important for understanding the role of the atmosphere in the formation of the chemical composition and quality of the waters of Baikal and its inflows. Over the past two decades, Limnological Institute SB RAS (Irkutsk, Russia) have conducted various joint studies of the characteristics of atmospheric aerosol with Russian and foreign groups. The chemical composition, biological component, size distribution, spatial distribution and aerosol sources over the lake were investigated. The interannual variability of the main chemical compounds in the aerosol was estimated. So, over the period studied, there was a gradual decrease observed in the concentrations of the main ions in the aerosol composition: in Irkutsk by 2.1 times, Listvyanka - by 4.0 times, at the background site of Mondy - by 3.5 times. In addition to quantitative changes, there is a change in the ratio of the main ions in the aerosol at Listvyanka site (south-west coast of Lake Baikal) and at the background site of Mondy. Upon condition of no external influences the content of the main ions in the aerosol on the surface layer of Lake Baikal is significantly lower ($0.33\text{--}5.2\ \mu\text{g}\cdot\text{m}^{-3}$) than during of extreme conditions ($2.2\text{--}5.2\ \mu\text{g}\cdot\text{m}^{-3}$). Forest fires near Baikal cause an increase of the concentrations of Na^+ , K^+ , NH_4^+ , Cl^- , and NO_3^- ions in Baikal aerosols. Components of soil-erosion origin (Al, Fe, Zn, Cr, Ba) dominate in the elemental composition of the aerosol. Their concentration increases during forest fires up to 1.4 - 6 times at average. The flows of sulfur and nitrogen, the main acidifying components, differ in different years of observations both at the monitoring sites and over the surface of Lake Baikal.

Keywords: Atmospheric aerosol, Baikal region, Chemical composition, Fluxes, Sources

1. A brief history of the study of atmospheric aerosol in the Baikal region

Studies of the air basin of the Baikal region in the 1970s-80s were mainly focused on studying of the chemical composition of atmosphere precipitations and its role in the chemical balance of Baikal waters (Khodzher, 1983; Vetrov et al., 1985; Obolkin et al., 1990; Anokhin et al., 1991). It was shown that the long-term ingress of acid components with atmosphere precipitations and their high accumulation in the snow cover and soils of the inflows' basins of the Southern Baikal caused to a shift of the main ions balance in the waters of the Southern Baikal inflows towards an increase of content of sulfates and a decrease of content of hydrocarbonates (Sorokovikova et al., 2004; Khodzher, 2005; Obolkin et al., 2016). Studies have shown the significant role of the atmosphere in the ingress of substances on the underlying surface. Studies of atmo-

spheric aerosol, one of the most dynamic components of the atmosphere, began a while later, after 1993. The areas to be studied were selected with due regard to varying degrees of anthropogenic influence (Khodzher et al., 1997a). In the 1990s – 2000s, with the participation of well-known Russian and foreign aerosol research groups, within the framework of RFBR, INTAS, ISTC projects, the «Siberian Aerosols» complex project and others, numerous field works were carried out to investigate various characteristics of atmospheric aerosol over Siberia and over Lake Baikal. The scientists of Limnological Institute (Irkutsk, Russia), of Voevodsky Institute of Chemical Kinetics and Combustion SB RAS (Novosibirsk, Russia), of Karpov Institute of Physical Chemistry (Moscow, Russia), University of Antwerp (Belgium) and Johannes Gutenberg University Mainz (Germany) they all studied in detail the composition of aerosol of various size fractions as of the size of several angstroms to the size of tens of microns (Koutsenogii et. al., 1993; Van Malderen et al., 1994; 1996). The

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biological component of the Baikal aerosol was investigated, its content can reach 60-80% of the total aerosol mass. In the seasonal large particles dynamics an intense spring-summer maximum is traced due to birch pollen (in April), coniferous trees (in June and July). In the composition of the biological component also various bacteria, viruses, etc. were revealed (Mattias-Maser et al., 2000).

In 1998, three sites for the continuous monitoring of the atmosphere were established, which since 2001 were included into EANET's international network sites of acid deposition monitoring in Southeast Asia (Acid Deposition Monitoring Network in East Asia) (EANET, 2000). Precipitation and weekly aerosol samples are taken at the stations all year round. In addition to these observations of atmospheric impurities there are summertime regular researches conducted above the water area of the lake using the research vessels owned by Limnological Institute SB RAS. Spatial characteristics of chemical and physical properties of the atmospheric aerosol above the Baikal waters are analyzed. This has made it possible to estimate the mass fraction of the aerosol attributable to the submicron part ($d < 1 \mu\text{m}$) and the coarsely dispersed fraction ($d > 1 \mu\text{m}$). Baikal aerosol is mainly represented by small particles. Their origin greatly depends on the seasons of the year, although soil and the crust generally remain the dominant sources. High concentrations of Al, Ca, Na, Mg, Cl indicate the predominance of natural sources of aerosol over the lake. In the cold period of the year, due to the increase of burned combustible amount and to the degradation of the property of the atmosphere to disperse, the anthropogenic contribution to such elements as As, Sb, Co increases (Obolkin et al., 1994; Van Malderen et al., 1994; Belan et al., 1996; Khodzher et al., 1997b). The main components of the soluble fraction of aerosols (SO_4^{2-} , NO_3^- , NH_4^+ , Ca^{2+}) were identified (Khodzher et al., 1994; Golobokova et al., 2002; 2004). Using of statistical methods and the analysis of synoptic processes in the atmosphere has made it possible to separate the contribution of natural and anthropogenic sources in the composition of aerosols. Concentrations of alkali and alkaline-earth metal ions in aerosols increase when air flows from continental regions of Mongolia, sulphate and nitrate ions concentration increases when air masses are transported from industrial areas of Siberia (Golobokova et al., 2005; 2006). The researches have an important practical application since the role of the atmosphere in the formation of the chemical composition and quality of the waters of Lake Baikal and its inflows has increased in recent years.

The contribution of regional industrial centers to the atmospheric pollution of Lake Baikal was studied within a framework of the Integration Project of the SB RAS "Assessing of the anthropogenic sources impact on the Baikal region atmosphere quality based on the experimental observations by means of laser sounding and mathematical modeling methods" with the participation of the Institute of Physical Material Science of SB RAS (Ulan-Ude, Russia), V.E. Zuev Institute of Atmospheric Optics of SB RAS (Tomsk, Russia), Institute of Computational Mathematics and Mathematical

Geophysics of SB RAS (Novosibirsk, Russia). The distribution of the aerosol was investigated not only in the surface layer, but also in the upper atmosphere up to 2-3 km from the earth's surface (Balin et al., 2016; Obolkin et al., 2017).

This article summarizes the results of long-term observations of only one of the characteristics of atmospheric aerosol - the chemical composition of the soluble fraction of the Baikal aerosol. It is exactly that fraction of the aerosol in condition of dry precipitation and atmospheric precipitations which participates in the formation of the composition of the main water ions of the lake.

2. Materials and methods

Atmospheric aerosol was sampled in the near-water layer of the atmosphere above the Lake Baikal and at three sites upon the EANET international program: Irkutsk (52.3 N; 104.4 E) is a city site which characterizes an urbanized territory; Listvyanka site (51.9 N; 104.7 E) is a site on the southwest coast of Lake Baikal which reflects the rural area conditions; Mondy (51.6 N; 101.0 E) is a background site remote from industrial centers of more than 300 km, at an altitude of 2005 m above sea level in the mountainous area between the ridges of the Eastern Sayans and Khamar-Daban. In the near-water layer of the atmosphere of Lake Baikal the aerosol was sampled according to the expedition trip routes of the research vessels owned by the Limnological Institute of SB RAS - «G.Yu. Vereschagin» and «Academic V.A. Koptug». During the period of 1993–1999 at the monitoring sites the atmospheric aerosol was sampled on filters "Whatman 41". Since the sites have been included into the EANET monitoring network, aerosol sampling is being performed by a universal method applied by the EANET and EMEP international networks (European Monitoring and Evaluation Programme). According to the EANET (2003), the samples were being taken on filters of 4 types. The filters were arranged in a successive order on a block which was tightly connected to a pump and a gas meter. The aerosol substance was collected on an external (first) teflon (PTFE) filter with a pore size about $0.8 \mu\text{m}$ in diameter. Different gaseous impurities were sorbed on filters 2-4. In the aqueous extracts of the first filter, the determination of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , NH_4^+ , NO_3^- , Cl^- , SO_4^{2-} ions was carried out. In the aqueous extract of Filter 2, the ions SO_4^{2-} , NO_3^- , Cl^- , NH_4^+ were determined. Filter 3 was extracted with 0.05% hydrogen peroxide solution and the ions SO_4^{2-} , Cl^- were determined. In the aqueous extract of Filter 4, NH_4^+ ions were determined. The corresponding gaseous impurities of SO_2 , HNO_3 , and NH_3 were calculated and summarized from the concentrations of ions in the filtrates of filters No.2-4. To ensure the comparability with data from other regions of the world, ion analysis was carried out by modern analytical methods recommended by monitoring networks such as: atomic absorption, high-level liquid- and ion chromatography. The introduction of ions determining method by ion chromatography method made it possible to obtain the measurement results with a con-

fidence level of $P=0.95$ accurately to within 4%. To compare the data, in 2000 the aerosol was collected on filters of 2 types - "Whatman 41" and PTFE. Relative determination error did not exceed 20%. In an aqueous extract of an aerosol sample the composition of microelements was analyzed with the use of an inductively coupled plasma mass spectrometer produced by Agilent 7500 ce (USA). The quality of the analyses has been approved by inter-laboratory comparative experiments performed within the framework of international programs Global Atmosphere Watch (GAW, 2004) under the aegis of the World Meteorological Organization (WMO) and Acid Deposition Monitoring Network in East Asia (EANET). The results of these analyses were included in the reports of GAW (QA/SAC-Americas) and EANET (EANET, 1998-2017).

3. The main result of studying of soluble fraction of atmospheric aerosol in the Baikal region

3.1. The Baikal region monitoring sites

The main contribution to air pollution in the Baikal region is made by stationary sources, including enterprises of the fuel and energy complex, chemical, metallurgical, pulp and paper production, woodworking and construction materials production (State Reports, 1999; 2008-2017). As the Figure 1 illustrates, in the period of years 1991–1999 total emission amount produced by stationary sources into the atmospheric air in the Irkutsk region almost halved. The least amount of impurities entered the atmosphere in 2000-2011. In 2012-2017 there was observed an increase of emissions by an average of more than 20% in comparing with the period of 2000-2011. A significant contribution to air pollution has been made by motor vehicle emissions, the amount of which increases annually by more than 10 thousand units (BSM, 2016). An additional contribution to air pollution comes from forest and peat fires which is difficult to quantify.

The most detailed studies of the chemical composition of the soluble fraction of the aerosol at the mon-

itoring sites began to be carried out since 1995. Figure 1 shows the average annual concentrations of the total content of ions in the composition of the soluble aerosol fraction at the atmospheric monitoring stations over the 16-year observation period. Long-term annual average of ion concentrations varied within the wide range and amounted to $4.0\text{--}11.1\ \mu\text{g}\cdot\text{m}^{-3}$ for Irkutsk, Listvyanka - $1.2\text{--}9.6\ \mu\text{g}\cdot\text{m}^{-3}$, and Mondy sites - $2.2\text{--}2.3\ \mu\text{g}\cdot\text{m}^{-3}$. Changes in ion concentration of the aerosol are in fair agreement with the dynamics of atmospheric emissions from stationary sources in Irkutsk region, especially at sites Irkutsk and Listvyanka. There is a decrease in the concentration of ions of the aerosol observed at all stations from 1998 till 2006-2017. The average ion content in the aerosol of Irkutsk decreased by 2.1 times, Listvyanka - 4.0 times, at Mondy site - 3.5 times.

In the seasonal aspect the highest ion concentrations in the aerosol at Irkutsk and Listvyanka stations were observed during the cold season. At Mondy site the annual course of ion concentrations is less pronounced. At this site a distribution with its maximum during the warm season was predominant. That indicates a predominant soil-erosion source of impurities in the atmosphere of this region. In some years (2004, 2007, 2009, 2014, 2017) some elevated concentrations were observed either in December or in January. In 2016 the annual course of the sum of ions at Mondy site was similar to those at Irkutsk and Listvyanka sites with the maximum during the entire cold period. This may indicate an increase of the influence of global transportation of pollutants in the atmosphere of the background area due to increased production volumes (Overview, 2017).

The dominant in the composition of the aerosol of the Baikal region were ions of continental origin both natural and anthropogenic character, NH_4^+ , Ca^{2+} , NO_3^- , SO_4^{2-} . Earlier the authors noted that in 2005-2015 compared to 1995-1999 a qualitative change occurred in the composition of the predominant ions at Listvyanka and Mondy sites against the changes in the quantitative characteristics of the aerosol. The role of alkaline-earth components decreased in Listvyanka aerosol, the main ions became NH_4^+ , NO_3^- and SO_4^{2-} . At Mondy site the influence of the alkaline components K^+ and Na^+ , as well as the ions Cl^- , SO_4^{2-} , NO_3^- , oppositely increased (Golobokova et al., 2018).

The most relevant is the state of atmospheric aerosol in the present period. The dynamics of ions in the aerosol at the monitoring sites in 2014-2017 was being considered. Similar seasonal dynamics of ion concentrations in the aerosol composition is observed at Irkutsk and Listvyanka sites with a minimum of concentrations in the warm period of the year and an increase of the concentrations in the cold one. Despite the decrease of ion concentrations in the warm period of the year, the concentrations increase at Listvyanka site in July. At Mondy site there are two maximum of concentrations of ions - in March and August with a higher content in March. It is characteristic that the increase in ion concentrations in Listvyanka and Mondy in the summer period is observed during the season of increased precipitation at the sites. The difference in con-

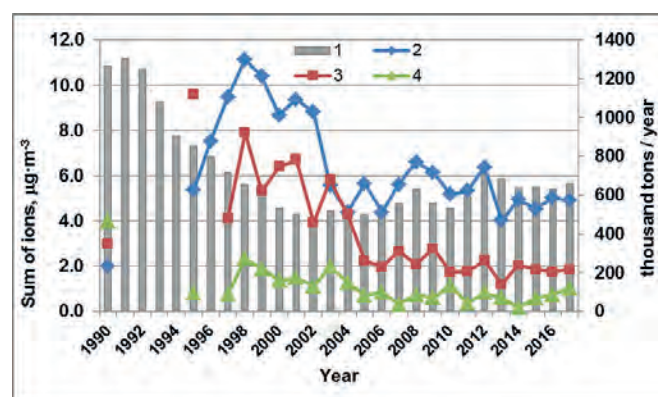


Fig. 1. Emissions of pollutants into the atmosphere (1) by stationary sources (total for the region, thousand tons/year) and the dynamics of interannual total concentrations of ions in the atmospheric aerosol at monitoring sites: Irkutsk (2), Listvyanka (3), Mondy (4), $\mu\text{g}\cdot\text{m}^{-3}$

centrations in the aerosol between the sites of Irkutsk and Mondy averages 7.6 times, between Listvyanka and Irkutsk – 2.6. The total content of ions in 2014-2017 at the site of Irkutsk varied within $4.5\text{--}5.0\ \mu\text{g}\cdot\text{m}^{-3}$, at Listvyanka site – $1.3\text{--}2.0\ \mu\text{g}\cdot\text{m}^{-3}$, and at Mondy site there was noted an increase in the total concentration of ions by 2017 from $0.17\ \mu\text{g}\cdot\text{m}^{-3}$ to $1.04\ \mu\text{g}\cdot\text{m}^{-3}$.

As the Figure 2 shows, in 2014-2015 at Mondy site sulfates and chlorides prevailed among anions, ammonium and calcium prevailed among cations, in the summer period the role of sodium ions increased. In 2016 the proportion of sulfates increased and the role of chlorides decreased, the proportion of ammonium increased noticeably, in 2017 there were no noticeable changes among anions and the proportion of sodium among cations increased especially in spring. At the site of Irkutsk there were no significant changes during 2014-2016. In 2017 there is a decrease in the proportion of ammonium was noticed and an increase of sodium and potassium was observed. Similar changes in 2017 occurred at Listvyanka site.

Such multidirectional trends in the composition of the aerosol that emerge at the monitoring sites in 2014-2017 can be explained by the different synoptic conditions of each particular year, although in general the overall picture remains: the distribution of the ionic composition with the dominance of sulfates, ammonium and calcium.

3.2. Aerosol of the near-water layer of the atmosphere of Lake Baikal water area

The aerosol over Lake Baikal water area was collected in the spring (May / June) and summer (July / August) periods of 2010–2017. Early studies (2005–2008) showed that in the central part of Central and Northern Baikal the submicron aerosol fraction dominates with a total ion concentration of $0.1\text{--}0.7\ \mu\text{g}\cdot\text{m}^{-3}$ and NH_4^+ and SO_4^{2-} ions predominant in its composition (Golobokova et al., 2011). These parameters allow to refer the Baikal aerosol to the background one. The change of its chemical composition can be significantly influenced by various factors both of natural and anthropogenic characters. One of such factors is emerging adverse meteorological and environmental phenomena.

During the sampling period the most adverse me-

teorological phenomena were observed in June 2010, when the air on the shores of Lake Baikal warmed up to $25\text{--}30^\circ\text{C}$, and especially in 2012, when the territory of Irkutsk region was anomalously dry, the weather was abnormally hot in July (average monthly air temperature was $2\text{--}3^\circ$ above normal), intense dry hot winds blew and such a weather led to the air drought and then to the soil drought in June (State Reports, 2013). An important role in air pollution above Lake Baikal is assigned to forest and peat fires which deliver to the atmosphere some suspended particles, ammonium and potassium compounds, chlorides, organic components, and many others (Recommendation..., 2015). The most intense forest fires in the region including the fires in the immediate vicinity of Lake Baikal happened in 2015-2016 when the region was experiencing the 5th class emergency of fire hazard of forests. (State Reports, 2008-2017). To detect common similarities and differences in the ionic composition of the aerosol in the period of 2010-2017 the data was averaged separately for each of the lake basins. Statistical characteristics (mean - and mean-square deviations - MSD) were determined from the average results calculated for each year. The results of the study showed that despite the influence of forest fires the tendency earlier revealed persisted: the total content of the ions in the aerosol composition is the highest in the atmosphere of Southern Baikal ($1.56 \pm 1.36\ \mu\text{g}\cdot\text{m}^{-3}$), and the lowest one is in the atmosphere of Northern Baikal ($1.13 \pm 1.00\ \mu\text{g}\cdot\text{m}^{-3}$) (Table 1). Differences in the ratio of the ions and their concentrations between the lake basins are connected primarily with differences in the environmental conditions of the coasts (Khodhzer et al., 1994; Van Malderen et al., 1996; Golobokova et al., 2011). Besides, the BPPM, which was closed in 2013, operated for a long time in Southern basin.

Comparison of the sum of the ion concentrations in the aerosol in 2010-2017 with similar data of 2005-2008 showed an increase of the concentrations of 1.5 times in the composition of the aerosol above Southern Baikal, 1.7 times higher above Central Baikal and 1.9 times higher above Northern Baikal. The highest increase in the concentrations of 3-7 times was observed for NH_4^+ , K^+ , NH_4^+ , Cl^- , NO_3^- ions in the aerosol of Northern and Central Baikal where intense forest fires happened in 2015-2016. The mean concen-

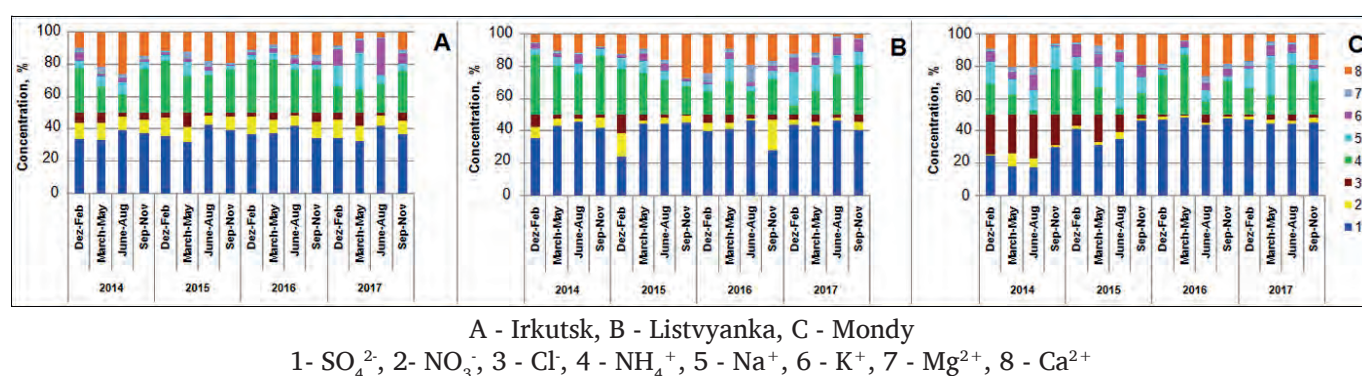


Fig. 2. Seasonal variability of ion concentrations in atmospheric aerosol composition at monitoring sites of Lake Baikal region in 2014-2017, %

Table 1. Average concentrations of ions (\bar{x}), meansquare deviations of concentrations (MSD), total of ions (Σ_{ion}) in the aerosol over Lake Baikal water area in 2010-2017, $\mu\text{g}\cdot\text{m}^{-3}$

Component	Southern Baikal		Central Baikal		Northern Baikal	
	\bar{x}	$\pm \text{MSD}$	\bar{x}	$\pm \text{MSD}$	\bar{x}	$\pm \text{MSD}$
Na^+	0.11	0.08	0.12	0.12	0.16	0.30
NH_4^+	0.26	0.44	0.15	0.21	0.09	0.15
K^+	0.20	0.41	0.15	0.26	0.15	0.33
Mg^{2+}	0.01	0.01	0.01	0.01	0.01	0.01
Ca^{2+}	0.12	0.09	0.07	0.06	0.07	0.07
Cl^-	0.20	0.33	0.14	0.20	0.12	0.23
NO_3^-	0.08	0.05	0.12	0.16	0.13	0.18
SO_4^{2-}	0.58	0.49	0.41	0.35	0.40	0.49
Σ_{ion}	1.56	1.36	1.17	0.78	1.13	1.00

trations of Ca_2^+ and SO_4^{2-} did not change. The growth of ion concentrations in the atmospheric aerosol was influenced not only by forest fires, but also by the meteorological conditions of 2010 and 2012. Figure 3 clearly demonstrates the results of the ion content in the aerosol during different observation periods, including seasons with adverse meteorological conditions. High concentrations of ions were observed in spring 2012 during the period of abnormally dry weather with its maximum concentration in Southern Baikal ($2.3\text{--}5.2 \mu\text{g}\cdot\text{m}^{-3}$) and during forest fires in spring 2016 when the mean content of ions over the entire Baikal water area was $2.2\text{--}3.2 \mu\text{g}\cdot\text{m}^{-3}$. The minimum total concentrations of ions were determined in the spring periods of 2014 and 2015 when their mean concentrations varied from 0.33 to $0.78 \mu\text{g}\cdot\text{m}^{-3}$.

Among the microelements in the composition of the soluble fraction of the aerosol, elements of mainly soil-erosion origin were predominant: Al, Fe, Zn, Sr, Mo, and Ba. Above the water area of the southern part

of Lake Baikal the concentrations of all analysis elements were higher. The concentrations of elements in the aerosol, as well as the concentration of ions, are influenced by the combustion products of forest fires. Table 2 shows a comparison of the concentrations of some microelements in the aerosol sampled in August 2015 compared with the mean concentrations in the aerosol sampled in 2008-2010, 2015 and 2017. The increase in concentration occurred from 1.4 to 6 times. The greatest increase in the concentrations of elements was observed in the aerosol of Central and Northern Baikal.

The soil and the earth's crust are the main sources of aerosol of the continental regions, and as a result, the ratio of elements in the aerosol from different regions of the world is very close and corresponds to that one for the earth's crust's (Rahn, 1976; Lowenthal et al., 1985; Swietlicki, 1989). In this regard, against the background of absolute concentrations of elements, it is rather difficult to estimate the contribution of the an-

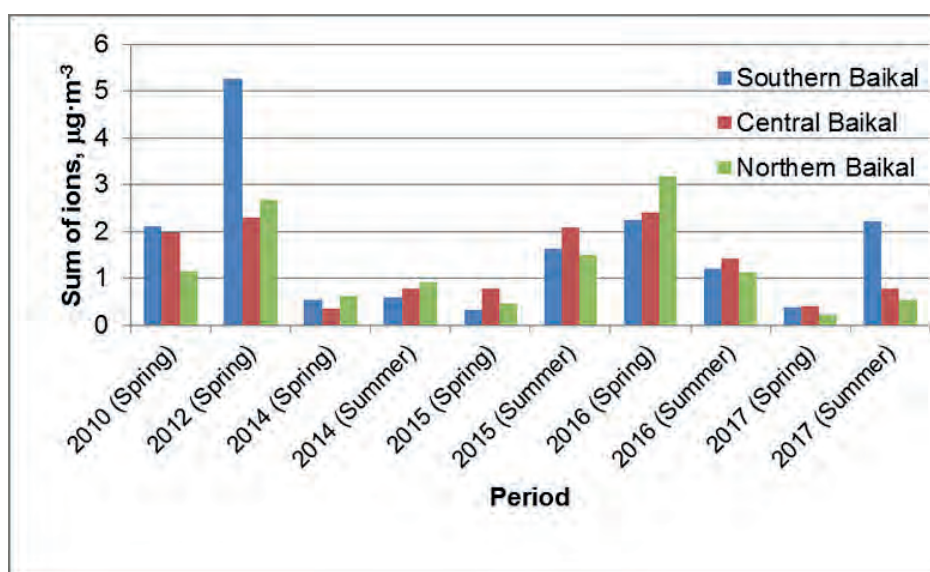
**Fig. 3.** Dynamics of the interannual total concentrations of ions in the atmospheric aerosol in the near-water layer over Lake Baikal water area, 2010-2017, $\mu\text{g}\cdot\text{m}^{-3}$

Table 2. Comparison of mean concentrations of elements in the composition of the soluble fraction of the aerosol of the near-water layer over Lake Baikal water area in different periods, ng/m³

Element	Southern Baikal	Central Baikal	Northern Baikal	Southern Baikal	Central Baikal	Northern Baikal
	mean value in 2008-2010; 2015; 2017			August 2015		
Al	87.8	77.5	36.8	448.2	408.5	176.2
V	0.23	0.11	0.07	0.32	0.23	0.15
Zn	110.2	123.0	59.9	443.3	662.9	318.4
As	0.17	0.08	0.05	0.23	0.25	0.18
Sr	4.1	4.2	2.5	20.2	23.7	14.5
Ba	178.7	204.1	121.1	1045.9	1216.0	713.8
W	2.8	2.3	1.5	10.5	10.9	6.8
Pb	0.50	0.48	0.26	1.47	1.59	0.87

thropogenic component. We calculated the enrichment factors of the elements relative to Al (Fig. 4). According to the factors it was revealed that the greatest enrichment of aerosol particles comes by the elements Zn, Se, Mo and As. Comparison of mean factors of enrichment of chemical elements in Baikal aerosol with factors of enrichment of three global types of aerosols - «urban», «background continental», «sea» (Rahn, 1976) – indicates that factors of enrichment of most of the elements of Baikal aerosol are closer in size to «background continental» type of aerosol.

3.3. Vertical fluxes S and N with dry deposition

One of the environmental criteria for the Baikal region is the assessment of sulfur and nitrogen inputs on the underlying surface. The most likely adverse effect of the transfer of sulfur and nitrogen to Lake Baikal was the acidification of atmospheric precipitations,

which led to a shift in the balance of the main ions in the waters of Southern Baikal inflows (Sorokovikova et al., 2004; Obolkin et al., 2016). The flows of sulfur and nitrogen coming from the atmosphere with an aerosol were recalculated for nitrogen from nitrates and ammonium ions, and for sulfur - from sulfates. The input of nitrogen with gases was calculated from the sum of gaseous impurities HNO₃ and NH₃, sulfur from SO₂. The following formula was used for calculations: $D = CV\Delta t$, where D is the input of substances, C is the mean concentration over the period Δt , V is the speed of dry depositions. To compare the data dry deposition rates were used taking into account the type of surface and certain climatic conditions previously used (Paramonov et al., 1999; Golobokova et al., 2007). Emission of impurities from the atmosphere to the underlying surface at the monitoring sites was considered for the period of 2000-2017. Sulfur and gas flows were higher at all the sites. This distribution of substances was also noted earlier

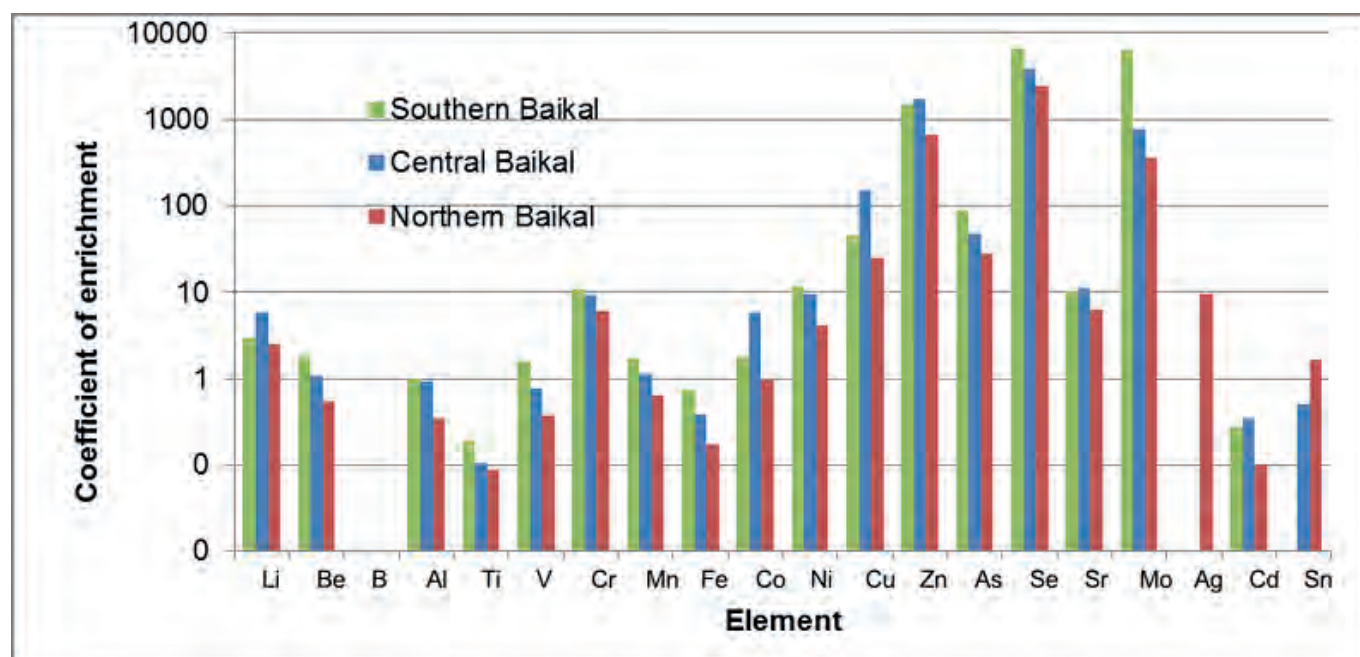
**Fig. 4.** Factors of enrichment of Baikal aerosol

Table 3. Sulfur and nitrogen flows at monitoring sites, ton km⁻² year⁻¹

Year	Irkutsk		Listvyanka		Mondy	
	N	S	N	S	N	S
2000	132	511	147	223	30	26
2001	134	522	67	191	27	45
2002	114	163	63	256	39	38
2003	128	232	138	258	38	63
2004	68	249	77	147	17	113
2005	84	1004	37	918	41	279
2006	128	506	163	736	65	78
2007	182	642	96	234	26	42
2008	140	526	69	264	26	64
2009	121	563	87	528	5	15
2010	95	892	72	507	10	79
2011	72	851	40	334	10	48
2012	71	926	32	680	16	127
2013	65	599	42	356	14	40
2014	72	332	69	256	7	40
2015	134	621	100	419	12	59
2016	153	761	147	500	59	72
2017	134	830	115	499	42	86

(Golobokova et al., 2007; Obolkin et al., 2016). The nitrogen flows with aerosol at Irkutsk site were higher in 2001, 2012 and 2015–2017, at Listvyanka site the flow was higher in 2001–2002 and 2012, at Mondy site the flows with gases were prevalent during all year round. The deposition of sulfur with gases was maximum for all the sites in 2005–2006 and 2009–2013 at average, the deposition of nitrogen in Irkutsk and Listvyanka was maximum in 2005–2011, at the site of Mondy in 2004–2008. The change in the sulfur and nitrogen flows at the sites has a similar interannual variation, despite the fact that at Mondy site the emission of nitrogen is 4 times less upon average and the emission of sulfur is 8 times less, at Listvyanka site the level of nitrogen is 1.3 times less, sulfur is 1.5 times less than in Irkutsk (Table 3). Over the last years (2015–2017) in Irkutsk, the flows of sulfur and nitrogen with aerosol decreased, but the flows with gases increased. In Listvyanka, on the contrary, the input of nitrogen with aerosol increased, the input with gases decreased, the flow of sulfur in 2016–2017 almost remained unchanged. At Mondy site the sulfur flows with aerosol decreased and increased with gases similarly as in Irkutsk. As in Listvyanka the flow of nitrogen with aerosol increased and decreased with gases.

An intake of sulfur and nitrogen all round Lake Baikal was analyzed. The intake of these substances to Baikal varies as well as at monitoring sites year by year and depends on the synoptic situation, meteorological conditions and forest fires whose frequency has increased in recent years. The highest flows of sulfur

and nitrogen were determined over the water area of Southern Baikal, the flows over Central Baikal were 1.4–1.5 times lower, over Northern Baikal - 1.8 times lower. Over the water area of South Baikal nitrogen and sulfur flows increased in 2010–2017 in comparison with the period of 2005–2008 by 2.2 and 1.1 times correspondingly (Table 4). Above Central and Northern Baikal there is an increase in nitrogen-only flows by 1.7 and 1.5 times and a decrease in sulfur flows by 1.1 and 1.5 times.

In general, the increase in nitrogen flows over Lake Baikal water area was observed in 2007, 2012, 2015 and 2016, in sulfur flows - in 2007, 2010 and 2016. The minimum intake of these substances was noted in 2014.

4. Conclusions

Long-term (1995–2017) studies of the chemical composition of atmospheric aerosol in Baikal region were carried out at the monitoring sites of Irkutsk, Listvyanka and Mondy. The quantitative estimates of spatial heterogeneity, interannual variability and seasonal dynamics of its composition are established. A gradual decrease in the sum of the concentrations of the main ions in the aerosol was noted: in Irkutsk by 2.1 times, in Listvyanka by 4.0 times, at the background site of Mondy by 3.5 times by 2006–2017 in comparison with the period of 1998.

In the present period (2014–2017), the difference in total concentrations in the aerosol between Irkutsk

Table 4. Monthly average nitrogen and sulfur flows over Lake Baikal, ton km⁻²

Period	Southern Baikal		Central Baikal		Northern Baikal	
	N	S	N	S	N	S
2005-2008	66.4	181.2	64.0	144.0	53.3	156.7
2010-2017	149.1	196.0	108.3	126.8	81.2	106.4

and Mondy sites averages 7.6 times, between Listvyanka and Irkutsk – 2.6 times. The total content of ions in 2014-2017 at Irkutsk site varied within 4.5-5.0 $\mu\text{g}\cdot\text{m}^{-3}$, at Listvyanka site – 1.3-2.0 $\mu\text{g}\cdot\text{m}^{-3}$, and at the Mondy site an increase in the total concentration of ions was noted – from 0.17 $\mu\text{g}\cdot\text{m}^{-3}$ to 1.04 $\mu\text{g}\cdot\text{m}^{-3}$ by 2017. Dissimilarity of the chemical composition is determined by the diversity of physiographic conditions of the region and by the degree of anthropogenic factors influence. The dominant in the composition of the aerosol of the Baikal region were ions of continental, both of natural and anthropogenic origin, NH_4^+ , Ca^{2+} , NO_3^- , SO_4^{2-} .

Against the background of changes in the quantitative characteristics of the aerosol, a qualitative change in the composition of the predominant characteristics took place at the sites. In the aerosol of Mondy site, the proportion of sulfates increased in 2016 and the role of chlorides decreased, the proportion of ammonium increased noticeably, in 2017 there were no noticeable changes among anions, and the proportion of sodium among cations increased especially in spring. At Irkutsk site during 2014-2016 special changes were not observed. In 2017 in the aerosol of Irkutsk and Listvyanka, there was a decrease in the proportion of ammonium and an increase in sodium and potassium.

In the near-water layer of Lake Baikal the average perennial (2010–2017) total ion concentrations were the highest in the aerosol of Southern Baikal ($1.56 \pm 1.36 \mu\text{g}\cdot\text{m}^{-3}$), the lowest – in the atmosphere of Northern Baikal ($1.13 \pm 1.00 \mu\text{g}\cdot\text{m}^{-3}$). The inter-annual and spatial heterogeneity of the chemical composition of aerosol of Lake Baikal was greatly influenced by adverse meteorological conditions of 2010 and 2012 and forest fires of 2015-2016.

High concentrations of ions were detected during the periods of abnormally dry weather (2.3-5.2 $\mu\text{g}\cdot\text{m}^{-3}$) and forest fires (2.2-3.2 $\mu\text{g}\cdot\text{m}^{-3}$). The minimum total concentrations of ions were observed in the absence of external influences (0.33–0.78 $\mu\text{g}\cdot\text{m}^{-3}$). The elemental composition of the aerosol is dominated by components of soil-erosion origin (Al, Fe, Zn, Cr, Ba), the concentration of which increases during fire danger periods by an average of 1.4-6 times.

The flows of sulfur and nitrogen, the main acidifying components, differ in different years of observations both at the monitoring sites and over the surface of Lake Baikal. The determining factors, which influence on the chemical composition of the aerosol and the flows of substances, are the orographic heterogeneities of the underlying surface, production emissions, meteorological phenomena and forest fires.

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Preliminary results of bio-optical investigations at Lake Baikal

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ABSTRACT. The preliminary results of complex bio-optical investigations carried out at Lake Baikal in July 2018 showed high variability in the light absorption properties of all optically active components, as well as their relation with hydrophysical characteristics. Vertical distribution of chlorophyll *a* was characterized by the presence of a maximum near the bottom of the euphotic zone. In this deep chlorophyll maximum layer, there were specific features of phytoplankton light absorption spectra reflecting the abundance of phycobilin-containing blue-green algae in the phytoplankton community.

Keywords: chlorophyll, light absorption, optically active components, blue-green algae, downwelling radiance, Lake Baikal

1. Introduction

Remote sensing provides unique opportunity in the observation of the World Ocean due to high spatial and temporal resolution of optical scanner data. In fact, it allows developing a system for operative (real-time) ecological monitoring, which cannot be realized by traditional approach to in situ measurements. Operative remote assessment of the water quality and productivity indicators in Lake Baikal could be used for solving the current ecological problems (Fietz et al., 2005; Hampton et al., 2008; Bondarenko et al., 2012; Belykh et al., 2016; Bondarenko and Logacheva, 2016; Timoshkin et al., 2016). Correct transformation of remote sensing data to the ecological indicators requires an application of regional algorithms developed on the basis of bio-optical properties of a particular water body. The light absorption by phytoplankton ($a_{ph}(\lambda)$), by non-algal particles ($a_{NAP}(\lambda)$) and by colored dissolved organic matter ($a_{CDOM}(\lambda)$) are the most significant bio-optical properties for the algorithms (Suslin and Churilova, 2016). To adapt three-band algorithm for assessment of chlorophyll *a* concentration and colored detrital matter ($a_{CDM}(\lambda) = a_{NAP}(\lambda) + a_{CDOM}(\lambda)$) in the upper layer of Lake Baikal (Case 2 waters), it is necessary to investigate spatial-temporal variability in $a_{ph}(\lambda)$, $a_{NAP}(\lambda)$ and $a_{CDOM}(\lambda)$, to parameterize light absorption by all in-water optically active components and to assess relative contribution of each components to total

light absorption.

The aim of the paper is to present preliminary results of complex bio-optical investigations carried out for the first time in Lake Baikal in August 2018.

2. Methods

Bio-optical investigations were carried in Lake Baikal during the scientific cruise onboard RV "G.Yu. Vereshchagin" from 24 to 31 July 2018. The SBE-911plus (Sea Bird Electronics) conductivity, temperature, depth (CTD) probe provided temperature and salinity profiles, and the rosette included 24 × 5-liter Niskin bottles for water sampling. Downwelling radiance ($E_d(\lambda)$) was measured at 7 spectral bands within visible domain (within photosynthetically active radiation, PAR) with spectrometer designed and made in Marine Hydrophysical Institute (Sevastopol, Russia). Water samples were collected at several depths chosen based on the temperature and $E_d(\lambda)$ profiles.

Samples for pigment and particulate light absorption analysis were gently (vacuum < 25 kPa) filtered through 25-mm GF/F (Whatman) filters and stored in liquid nitrogen for measurements in the laboratory. Samples were extracted in 90% acetone overnight in refrigerator, and then samples were treated with a vibration mixer (FALK Falc instruments, Italy) and centrifuged. Chlorophyll and phaeopigment concentrations were determined spectrophotometrically (Jeffrey and Humphry, 1975; Lorenzen, 1967) with dual-

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beam spectrophotometer Lambda 35 (Perkin Elmer).

Light absorption by particles and by CDOM were measured in line with the NASA ocean color protocols (Mitchell et al., 2002), which was described in details in (Churilova et al., 2017). For particulate absorption, water samples were filtered under low vacuum (<25 kPa) on GF/F (Whatman) filters immediately after water sampling. The filters with samples were immediately frozen in liquid nitrogen and stored in a dewar until analysis in the laboratory. Optical density was measured with dual-beam spectrophotometer Lambda 35 (Perkin Elmer) equipped with an integrating sphere. Pigments were extracted with hot methanol (Kishino et al., 1985; Churilova et al., 2018). The b-correction was performed as described in (Mitchell, 1990).

To measure CDOM light absorption, water samples were pre-filtered and then filtered under dim light through 0.2 μm Sartorius membrane filter rinsed previously with ~ 100 ml of deionized water. The samples were stored at -20°C until analysis in the laboratory. The measurements were conducted in 0.1 m quartz cell from 300 to 750 nm with dual-beam spectrophotometer Lambda 35 (Perkin Elmer). Spectral distribution of $a_{\text{NAP}}(\lambda)$ and $a_{\text{CDOM}}(\lambda)$ coefficients were described with exponential function (Babin et al., 2003). Spectral slopes (S_{NAP} and S_{CDOM}) were calculated by fitting a nonlinear model to data from the wavelength domain of 400 – 700 and 350 – 500 nm, respectively.

Phytoplankton species composition, abundance and biomass (micro- and nano- phytoplankton) were determined with the deposition method (Kiselev, 1969; Guide to hydrobiological monitoring ..., 1992) using light microscope Axiovert 200 (ZEISS, Germany) with Pixera Penguin 600CL camera. Samples were preserved with solution of Lugol and sodium acetate (Sadchikov, 2003). Cell volume and biomass were calculated according to (Makarova and Pichkily, 1970; Belykh et al., 2011).

3. Results and discussion

For the first time in Lake Baikal, complex bio-optical investigations were carried out from July 24 to July 31 2018. Preliminary results were obtained for the surface layer (17 stations) and for a layer of 0–75 m at the station 32 located in the middle of Maloye More Strait (mMM) of Lake Baikal. In the surface layer, chlorophyll *a* in sum with phaeopigments (Chl-*a*) varied significantly (0.58–5.3 mg m^{-3}). Bio-optical properties and species composition of phytoplankton community changed significantly from the surface to ~ 75 m depth (Fig. 1–5). At the mMM station, thermocline was observed within 7–20 m layer with the maximum temperature gradient (MTG) located at 10 m (Fig. 1). Maximum values of Chl-*a* were recorded below MTG and near the bottom of euphotic layer ($\sim 1\%$ PAR) (Fig. 1).

Phytoplankton was analyzed at two depths (0 and 20 m) of the mMM station. In the surface layer, phytoplankton biomass was generally represented by *Synedra acus subsp. radians* (Kützinger) Skabitshevsky (Bacillariophyceae) and *Gyrodinium helveticum*

(Dinophyceae) (Fig. 2), which contributed 55% and 35% to total biomass (micro- and nanofractions), respectively (Fig. 3). The phytoplankton biomass decreased slightly from surface layer (280 mg m^{-3}) to 20 m (230 mg m^{-3}). However, at 20 m phytoplankton was mainly represented by another taxon compared to the surface phytoplankton. Below MTG (at 20 m), *Monoraphidium griffithii* (Chlorophyceae) contributed $\sim 60\%$ to total biomass.

Shape of spectrum and magnitude of $a_{\text{ph}}(\lambda)$ coefficients was depth-dependent (Fig. 4). In the layer above MTG, the $a_{\text{ph}}(\lambda)$ spectra were characterized by two main peaks: in blue (~ 438 nm) and red (~ 678 nm) parts of the spectrum. The values of the chlorophyll *a* specific absorption coefficient decreased with depth. The decrease was most significant in the blue maximum

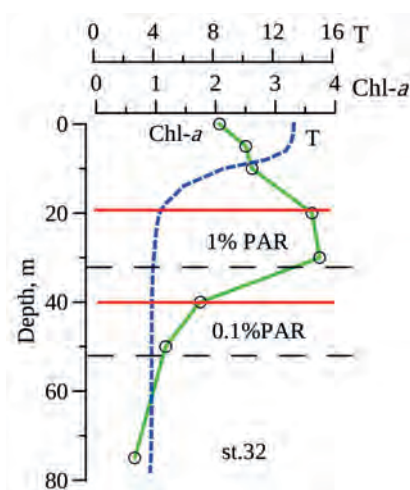


Fig. 1. Vertical distribution of chlorophyll *a* concentration in sum with phaeopigments (Chl-*a*, mg m^{-3}) (circles, green line), temperature (*T*, $^\circ\text{C}$) (blue dashed line) in the middle of Maloye More Strait of Lake Baikal (st. 32); black dashed lines indicate 1 and 0.1% level of photosynthetically active radiation (PAR); red lines – the layer, where phytoplankton light absorbance was characterized by the local maximum at ~ 560 nm.

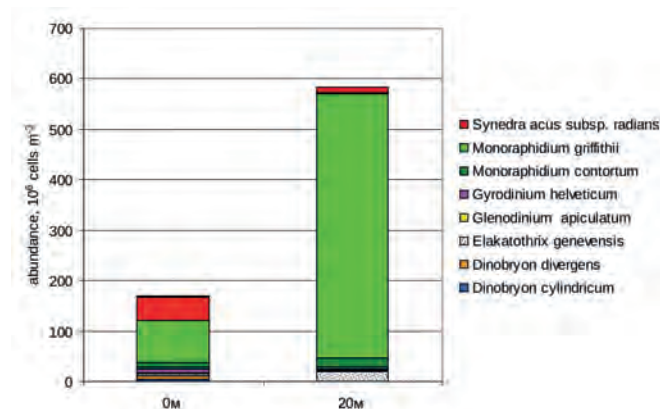


Fig. 2. The abundance of phytoplankton species at the station in the middle of Maloye More Strait (mMM) of Lake Baikal.

of the spectrum (from 0.060 to 0.033 $\text{m}^2 \text{mg}^{-1}$) than in the red (from 0.020 to 0.017 $\text{m}^2 \text{mg}^{-1}$). In the layer below the thermocline shape of $a_{ph}(\lambda)$, spectrum changed: a local absorption maximum was at ~ 560 nm. The local peak was most obvious at ~ 30 m depth, where PAR was approximately 1% of PAR incidence on the lake surface. This peak disappeared below 50 m. The local peak at 560 nm was complementary to the blue-green irradiance (510-560 nm) penetrated the bottom of the euphotic zone (1% PAR) (Fig. 5). The absorbance at ~ 560 nm is likely to be related with phycobillins, in particular, with phycoerythrin (Jeffrey et al., 1997; Six et al., 2007). Observed changes in the $a_{ph}(\lambda)$ shape reflected the changes in the share of phycoerythrin in the total amount of the pigments (accessory pigments). Therefore, the relative abundance of phycoerythrin containing microalgae (cyanobacteria) changed with depth. At depths of 20–40 m, where the local absorption peak at ~ 560 nm was obvious in absorption spectrum, the share of blue-green algae in the total biomass was sufficiently large; thus, the absorption of phycobilins was not masked by other pigments. Investigations in the Black Sea showed that the contribution of blue-green algae to the total biomass of the phytoplankton community in deep chlorophyll maximum ($\sim 1\%$ PAR) reached $\sim 50\%$ (Churilova et al., 2018). In the present research, pico-fraction of phytoplankton was not estimated. However, phycobillins are likely to be contained in pigment complex of blue-green algae (picocyanobacteria). Analysis of the micro- and nanofractions of phytoplankton showed that dominating taxon (in biomass) changed from diatoms in the surface layer to green algae below the thermocline (20m). Although the phytoplankton structure changed, the shape of $a_{ph}(\lambda)$ spectra practically did not changed not taking into account local maximum at ~ 560 nm (Fig. 4). Absence of the notable effect of shift in dominating taxons on spectrum shape is due to the fact that absorbance bands of accessory pigments of these taxonomic groups are close (Jeffrey et al., 1997).

Spectral distribution of the $a_{NAP}(\lambda)$ was described by the exponential function with the slope coefficient (S_{NAP}) equal to $\sim 0.009 \text{ nm}^{-1}$. The contribution of NAP to the total particulate absorption at ~ 438 nm increased with depth from $\sim 10\text{--}15\%$ to $\sim 30\text{--}40\%$. The $a_{CDOM}(\lambda)$ spectral distribution was described by an exponential function with the slope coefficient (S_{CDOM}) equal to $0.012\text{--}0.016 \text{ nm}^{-1}$. The $a_{CDOM}(\lambda)$ values at ~ 438 nm were $0.090\text{--}0.26 \text{ m}^{-1}$.

4. Conclusion

The preliminary results of complex bio-optical investigations carried out at Lake Baikal in July 2018 showed high variability in the light absorption properties of all optically active components, their relation with hydrophysical characteristics and phytoplankton species composition. These results underlined a necessity of detailed investigations of spatial variability in bio-optical properties at particular seasons accompanied by shift in dominating species in the phytoplankton community. Revealed regularities in

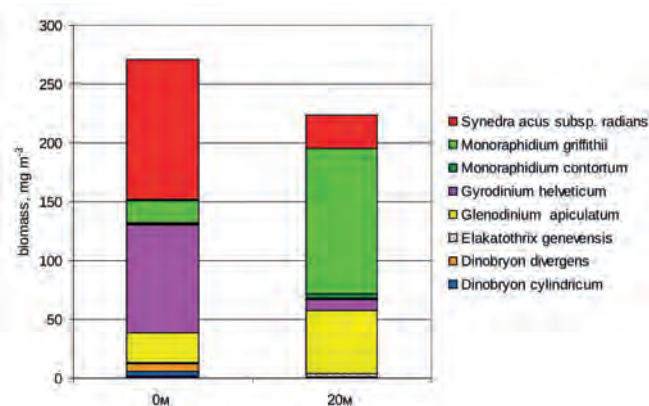


Fig. 3. The biomass of phytoplankton species at the station in the middle of Maloye More Strait (mMM) of Lake Baikal.

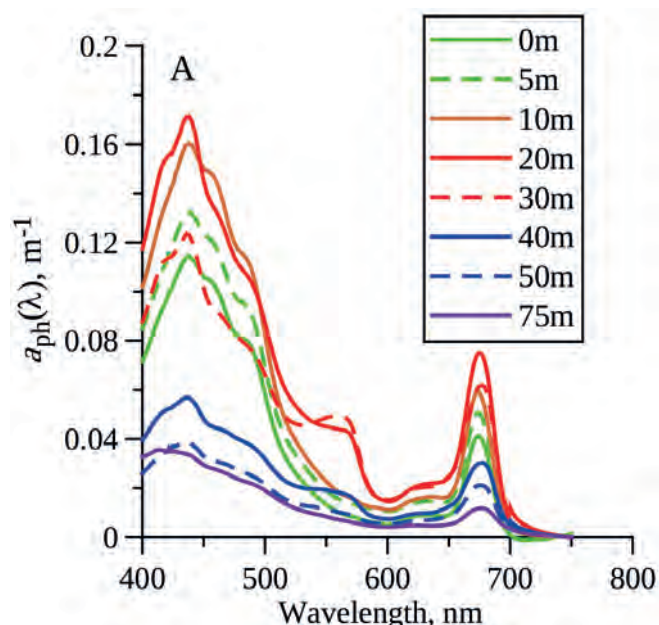


Fig. 4. Light absorption coefficients ($a_{ph}(\lambda)$) at different depths in the middle of Maloye More Strait of Lake Baikal (st.32).

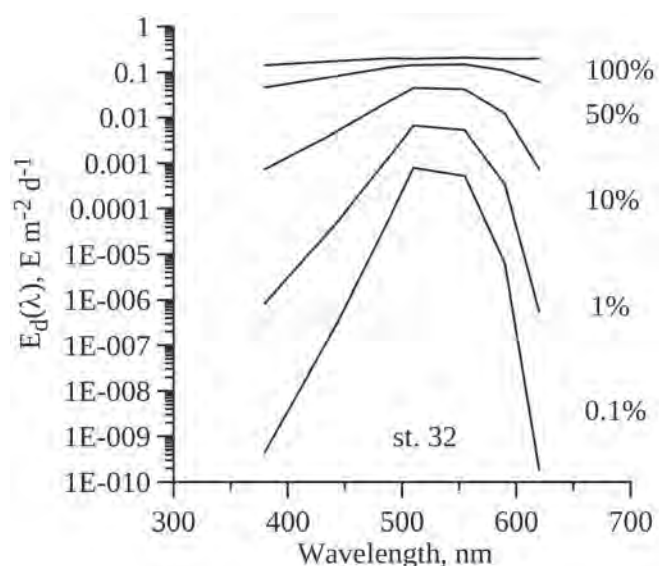


Fig. 5. Downwelling radiance ($E_d(\lambda)$) at depths with different level (100; 50; 10; 1; 0,1 %) of photosynthetically available radiation in the middle of Maloye More Strait of Lake Baikal (st. 32).

temporal and spatial variability in bio-optical properties will be used for development of regional algorithms, which are required for operative ecological monitoring of Lake Baikal based on remote sensing data.

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Lake bottom sediments as archives of paleo-climate changes on decade and millennium time scales of the Holocene-Pleistocene

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ABSTRACT. Undoubtedly, the study of the Planet's climate is one of the fundamental topics for elaboration of strategies for life support of the Earth's population. The following questions arise: i) what is the spatial pattern of intensity of these changes; ii) what is the response of the regions to climatic changes; iii) what is the critical line when changes in the ecosystem are irreversible; iv) what are causes and mechanisms of these changes; v) what is the human role in these processes; and vi) which tendencies should be expected in climate changes in the nearest future? After answering these questions, a logical question is put forward: how unique is "the climatic landscape" of the recent decades compared to the previous epochs, and are all regions similarly sensitive to climatic changes? The challenge is how to describe these changes and which methods to use in order to obtain more reliable data. More generally and despite some problems, the studies indicate the value of combining limnological and palaeolimnological records in reconstructing lake history and in disentangling the changing role of different pressures on lake ecosystems.

Keywords: Paleolakes, Climate changes, bottom sediments, the Holocene-Pleistocene

1. Discussion

Climate change has become one of the most important environmental concerns facing modern society (IPCC, 2007). The extent of natural climate variability is not yet fully understood, though understanding of this variability is crucial for predicting future trends.

Lacustrine sediment sequences can provide valuable archives of past environmental and climatological variability in terrestrial realms. To unravel the history of a lake and of lake's catchment, a profound understanding of the sedimentary processes is required. This encompasses the supply of allochthonous organic matter, nutrients and clastic material to the lake and the subsequent redistribution within the lake, as well as autochthonous organic matter and mineral deposition (Battarbee and Bennion, 2012).

Paleoclimate reconstruction can be qualitative (e.g. pollen or diatom records) or quantitative with a low, medium and high time-resolution (Fig. 1). Mechanisms of long-period, contrast and large-scale changes of the Earth's climate in the Pleistocene have been well studied. For the past decades, due to the studies of oceanic, marine and glacial cores, significant progress has been achieved in understanding of cause-effect factors that govern long-period (400, 100, 40, and 21 ka)

changes of the climatic image of the Earth with a time interval of several millennia. Thus, the global climate of the Earth during the last several Ma was studied with a step in 0.5-1 ka (e.g. Berg et al., 1991; Lourens et al., 1996; Larrasoana et al., 2003), a low time-resolution. The longest in time records were obtained from the sediment covers of the lakes Baikal (Russia), Khubsugul/Hovsgol (Mongolia) and Elgygytgyn (Russia). For example, Baikal and Khubsugul drill cores contain climate records for the past 8 and 1.3 Ma (BDP Members..., 2000; BDP-99..., 2005), respectively.

According to the Baikal records, it is supposed that weakly weathered material was delivered into the lake during the ice ages, perhaps from the nearest mountain glaciers in the northern or northeastern part of the catchment. In contrast, the mature and chemically weathered terrigenous material was transported from the river valleys during warm periods. The significant decrease of the relative contribution of the largest river input, the Selenga River, to the water budget of the paleo Lake Baikal (or possibly even its complete disappearance) during the global ice ages and deep regional cooling. As a direct result, a sharp decrease in the delivery of nutrients into Lake Baikal and the consequent decrease of its bioproductivity had to occur almost simultaneously (Grachev et al., 1997; Goldberg et al., 2008; 2010).

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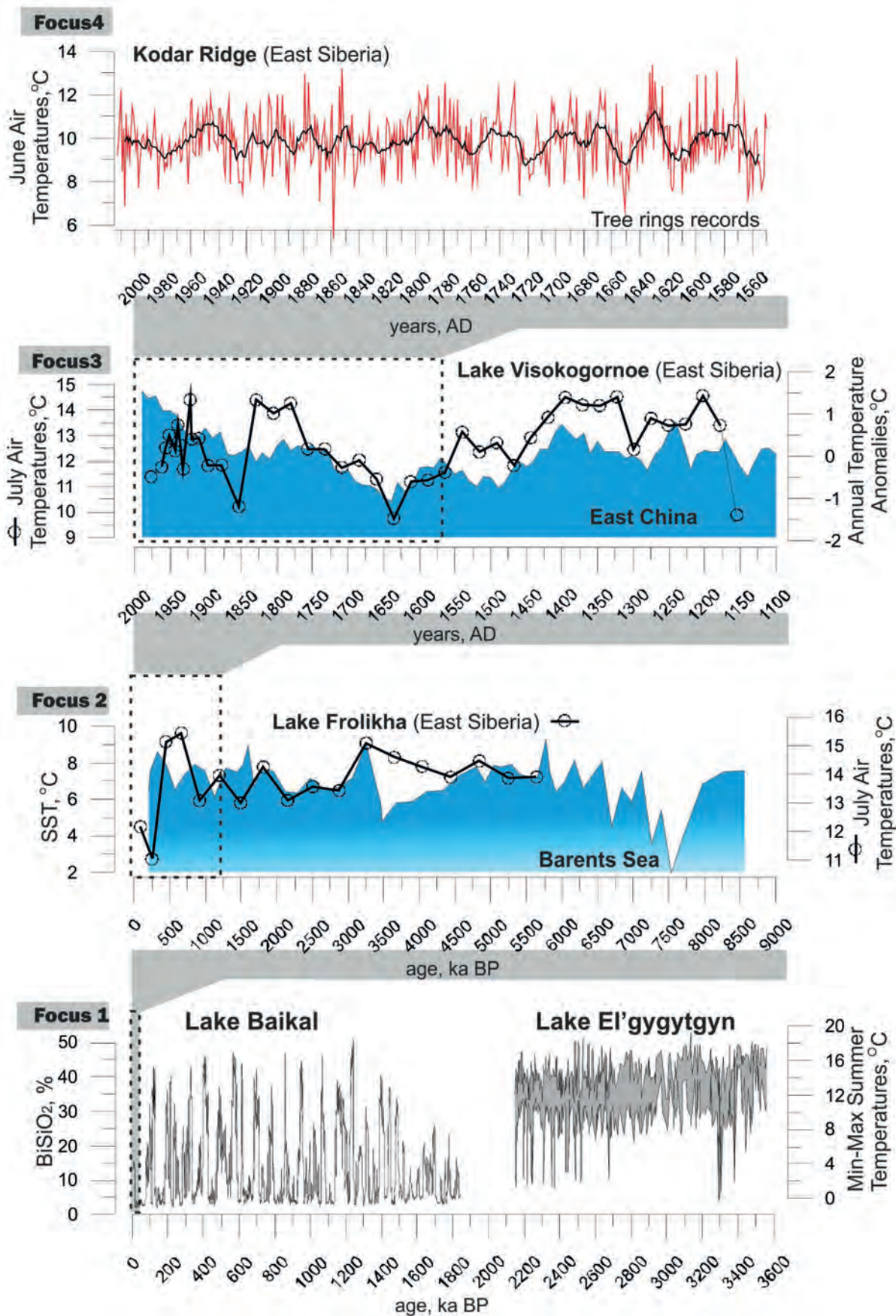


Fig. 1. Paleoclimate reconstruction at different time-frames. *Focus 1* – low-resolution records from bottom sediments of “old” lakes, Lake Baikal according to Prokopenko et al. (2006) (IGBP PAGES/World Data Center for Paleoclimatology), Lake Elgygytyn according to Brigham-Grette et al. (2013) (IGBP PAGES/World Data Center for Paleoclimatology); *Focus 2* – middle-resolution records of the Holocene, July air temperature was obtained base on chironomid analyses (Lake Frolikha, East Siberia), SST - Reconstruction of summer sea-surface temperatures of the Barents Sea (Voronina et al., 2001 (IGBP PAGES/World Data Center for Paleoclimatology)); *Focus 3* – high-resolution records of the last Holocene, Lake Visokogornoe according to Fedotov et al. (2015), Temperature variation in China according to Yang et al. (2002); *Focus 4* – high-resolution June temperatures inferred from tree-rings reconstruction (the Kodar Ridge, East Siberia)

In contrast to the Baikal records, a geochemical signal is main in Khubsugul pale-records (Fedotov et al., 2004). The formation of the Khubsugul isotopic and geochemical records depended on the change of the regional aridity level. The high aridity is determinate on abrupt increasing of values these records. During the aridity periods, the lake level was low, the lake water was of high salinity and the evaporation enriches determined $\delta^{18}\text{O}$ composition of the lake waters. It assumes that the Khubsugul level was stable on ~ 100 m below what the modern level during ca. MIS-2 and the second half of MIS-3, at the evaporate/inflow ratio 2.7 (Fedotov et al., 2015).

Records from Lake Elgygytyn (NE Arctic Russia) show that 3.6-3.4 million years ago, summer temperatures were $\sim 8^\circ\text{C}$ warmer than today when pCO_2 was ~ 400 ppm. Multiproxy evidence suggests extreme warmth and polar amplification during the Middle Pliocene, sudden stepped cooling events during the Pliocene-Pleistocene transition, and warmer than the present Arctic summers until ~ 2.2 Ma, after the onset of the Northern Hemispheric glaciation (Brigham-Grette et al., 2013).

Meanwhile, investigations of the European Holocene sections with high time resolution visualized that long-term climatic phases were extremely heterogeneous (e.g. Jones and Mann, 2004; Luterbacher et al., 2004; Osborn and Briffa, 2006; Mann et al., 2009). In addition, the Holocene climate changes were some triggers for socio-economic developments. For example, climate cooling in Central Asia can be seen as a triggering for the actions of peoples such as the Huns and Mongols in the history of the vast parts of Eurasia from China to Central Europe (Schlütz and Lehmkuhl, 2007). Therefore, it is currently important to obtain: i) information on climate changes with a time interval of a year-decade and ii) their quantitative parameters.

Fossils of chironomid larvae (non-biting midges) preserved in lake sediments are well-established palaeotemperature indicators, which can provide quantitative estimates of past temperature change using numerical chironomid-based inference models (transfer functions). This approach to temperature reconstruction relies on the strong relationship between air and lake surface water temperatures and the distribution of individual chironomid taxa (species, species groups and genera) that has been observed in different climate regions (arctic, subarctic, temperate and tropical) in both the Northern and Southern Hemispheres (Brooks and Birks, 2001; Brooks, 2006).

Climate changes in East Siberia for the past 5.5 ka can be described by four episodes based on chironomid analyses. Thus, maximum in regional moisture occurred at ca. 5.5-3.5 cal. ka BP and mean July temperatures was ca. 14°C . The following episode of 3.5-1.7 ka BP was characterized by a tendency to dry conditions and to temperature drop by 1°C . During the third episode (1.7-0.8 ka BP), dry conditions decreased, but the temperature change was negligible. In the fourth episode (0.8 ka BP to present), the climate changed significantly associated with the Medieval Warm Period. This event was characterized by warm and dry condi-

tions, when the dry component was maximal for the past 5.5 ka.

More detailed chironomid, biogenic and geochemical lake records showed a clear decrease in summer temperatures occurred in East Siberia after ca. 1400. It was linked with the beginning of the Little Ice Age (Fedotov et al., 2015). The coldest summer occurred about ca. 1570-1700 and 1830-1900. It assumed that the most significant changes of the lake bio-productivity and the catchment area occurred about ca. 1160-1350, 1350-1590, 1590-1730, 1730-1900 and 1940 to the present. The most dramatic period with unfavorable climate conditions for the lake biota was during 1590-1730.

However, many questions about climate changes in Northern Eurasia are still open. If to compile a scheme on which the Holocene section of the Northern Hemisphere will be marked with a high precision, Europe and North America are the best studied regions, North and Central China are less studied areas, and only several regions have been sporadically investigated in Mongolia, Transbaikalye, East and Western Siberia. On the other hand, high contrast landscape zones (tundra, taiga, steppe, high-mountain areas and deserts) are located in Northern Eurasia, and regional features of climate changes can be well illuminated. In particular, why climatic regimes of various landscape zones were different under similar global conditions (orbital parameters, insolation level, evaporation from the oceans and movement of humid fronts). Comparing the obtained new records with those from Europe and Inter-Continental Asia, we can determine control factors and feedback inside the Eurasian climatic system, as well as its interaction with the global system at the inter-regional level. It is expected to widen information on how ecosystems of different landscape zones respond to climatic changes and which elements of these systems are more resistant or, vice versa, sensitive to short-period climatic fluctuations. In addition, the results of this study will enlarge information on the effect of global anthropogenic factor of the industrial period on the stability of regional ecosystems.

2. Conclusion

As future climate changes are expected to have a major impact on freshwater lake ecosystems, it is important to assess the extent to which changes taking place in freshwater lakes can be attributed to the degree of climate change that has already taken place. It is necessary to examine evidence spanning many decades by combining long-term observational data sets and palaeolimnological records. However, there is also evidence of climate influence related in some cases to natural variability in the climate system, and in others to the trend to higher temperatures over recent decades attributed to anthropogenic warming.

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Gas hydrates in Lake Baikal



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ABSTRACT. Subsurface gas hydrates over all the area of their potential occurrence under the floor of Lake Baikal, the only freshwater body where they occur, have been sought and studied since 2000. Two of three known gas hydrates cubic structures (structure 1 biogenic methane hydrates and structure 2 biogenic methane and thermogenic ethane hydrates) have been found in the lake sediments. Large autogenic carbonaceous formations atypical for the lake have been discovered in the areas of gas hydrates occurrence. A new so-called “Baikal” mud volcanoes formation mechanism with shallow roots previously unknown in the seas is described. This mechanism is related to destruction of gas hydrates under their stability zone due to a tectonic activity and warm fluid income. The focus and source of the gas-saturated fluid are determined to be buried depositions of delta fronts, depocenters in the middle of the basins and subsurface ancient sedimentation masses at the eastern flank. The 2018 integrated geological and geophysical survey allowed to discover 54 hydrate-bearing structures represented by 26 mud volcanoes, 18 hydrate mounds, 9 seeps and 1 pockmark. Not only sedimentation masses of various age and many kilometers thick, but also the tectonic dislocation grid determine the distribution of these structures on the floor of Lake Baikal. The fluid pathways are formed through impaired vertical and gently inclined zones of the main rift faults and secondary faults as well as along permeable lithological sedimentation boundaries when the layers rise from the depocenters in the center of the basin to its flanks.

Keywords: Baikal, gas hydrates, mud volcanoes, carbonates

1. Introduction

History of study of sedimentary cover in the Lake Baikal includes several key points related to considerable achievement in investigation of the structure and geological development of the Baikal depression. It resulted in the changes of conceptions about the age, stages of development and processes in sediments. Discovery of gas hydrates in subsurface bottom sediments, substantiation of particular Baikal type of mud volcanoes activity as well as creation of high-resolution digital model of bottom topography (DMT) can be referred as turning-point in the Lake Baikal study. DMT was created based on new high-resolution survey of the lake bottom by multi-beam echo-sounders Seabeam 1050 in 2009 (Cuylaerts et al., 2012) and Kongsberg 710S (2015-2017) (Khlystov et al., 2016a; 2016b; 2017). Due to DMT, up to 90% of hydrate-bearing structures were discovered, the bottom topography was defined more exactly, and the history of Baikal depression and underwater elevations in the past was reconstructed.

Phenomenon of formation of gas hydrates with different composition in the unique freshwater basin has a fundamental scientific significance. Results of hydrates investigations are also used for reconstruction of biogeochemical methane cycle in the bottom sediments and water column, as well as for understanding of geological reasons for occurrence of the hydrate-bearing structures in different bottom areas of Baikal.

2. Results

54 structures containing gas hydrates in subsurface sediments have been discovered since 2000 up to the present time (Fig. 1, Table 1). It has become possible not only due to the new DMT, but also due to results of other type of geophysical investigations, as well as visual observation from the MIR submersible. Generally, mud volcanoes represent gas hydrate-bearing structures (26 objects). Some of them (18 objects) are, probably buried (Fig. 1). In the sections of the latter, the mud breccias were not observed, whereas upper layer contained contemporary diatom ooze. However, based

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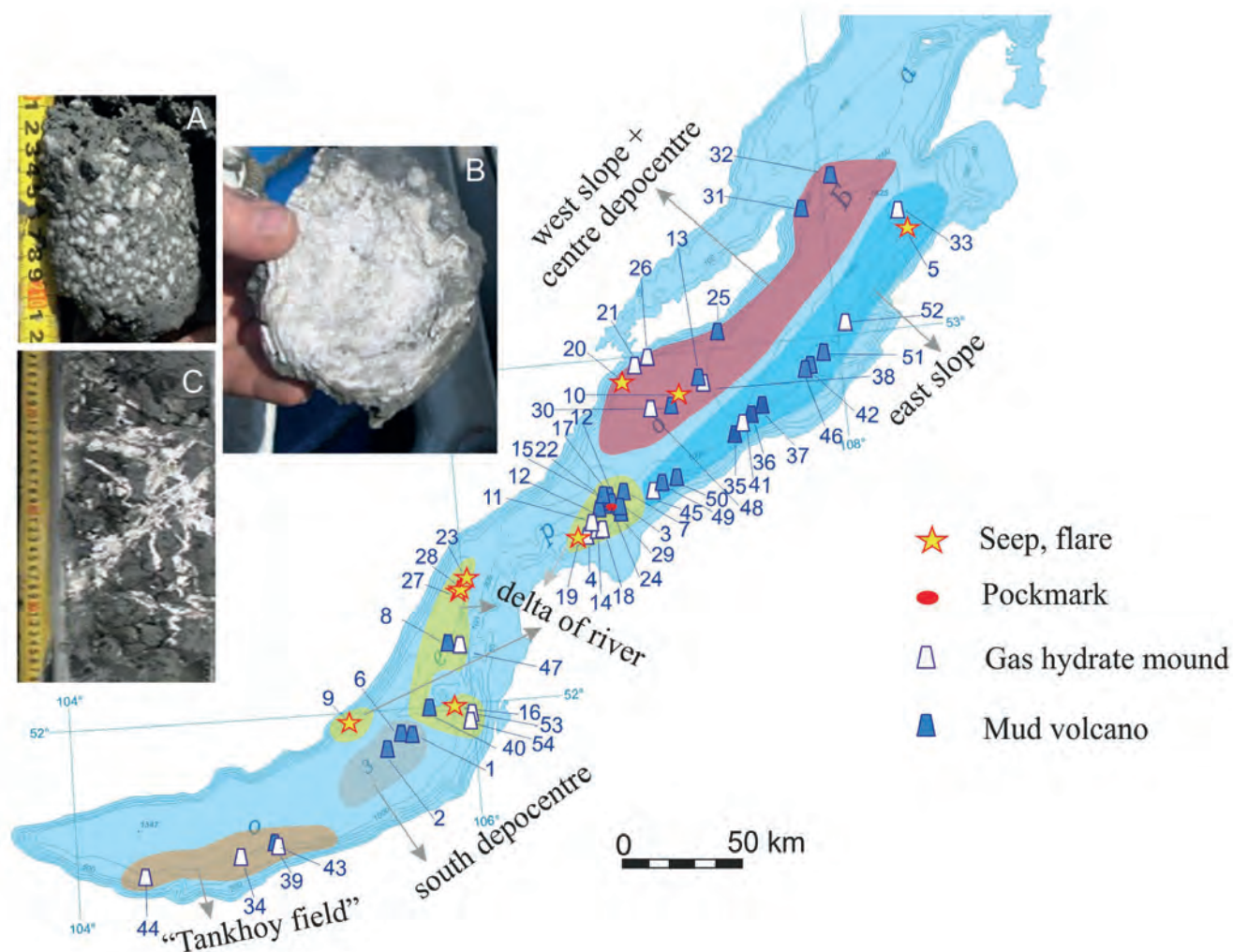


Fig. 1. Geographical distribution of the gas hydrate-bearing structures discovered in the South and Central Basins of Lake Baikal. Different types of the gas hydrate-bearing structures according to Table 1 are marked with the numerals. The structure distribution areas depending on the focus of the fluid are semitransparent. The picture shows different types and textures of gas hydrates: A – granules; B – massive; C – veins and streaks of different occurrence.

on morphological (positive topography) and geophysical data, they were similar to other mud volcanoes. This structure can be referred to gas hydrate mound of type “A” described by Matsumoto at Joetsu Basin (Matsumoto et al., 2011).

Mud volcanoes and gas hydrate mounds were mostly represented by single structures with a diameter from several hundreds meters to 1.5 km, but at the three areas they form groups and can merge into the large ridge or group of elevations (Cuylaerts et al., 2012; Khlystov et al., 2013; Akhmanov et al., 2018). Nine structures were represented by seeps, i.e. zones of focused gas or oil and gas discharge at the lake bottom without transformation of topography. Only one gas hydrate-bearing structure are associated with pockmark. Gas hydrates in the lake extend to the Southern and Central Basins, as well as over the underwater Akademicheskii Ridge. This phenomenon influences considerably biodiversity and the processes of relief formation in various bottom areas.

Gas hydrates are usually observed in bottom sediments in the form of layers, veins and strings with different orientation, but sometimes they consist of “clinker” granules (see Fig.1). The latter are hydrates of crystallographic structure 2 and consist of the mix-

ture between microbial methane and thermogenic ethane. The content of ethane in the hydrate-bound gas exceeds 13% (Kida et al., 2006). Other hydrates have structure 1 and include mainly microbial methane. Gas hydrates containing the thermogenic methane and ethane were obtained from the area of oil and gas seep “Gorevoy Utes”, gas seep “PosolBank”, and mud volcanoes “Kukuy” and “Kedr” (Kalmychikov et al., 2006; Hachikubo et al., 2010; 2017).

In addition to gas hydrates, relatively large (up to 5-6 cm) authigenic carbonates (siderites and rhodochrosites) were formed in the sediments of gas hydrates-bearing structures. Previously, it has been considered for a long time that the sediments of Lake Baikal are carbonate-free due to low alkalinity of the lake water and undersaturation of pore waters with respect to Ca by one-two orders relative to values required for calcite crystallization (Mizandrontsev, 1975). Soft and firm authigenic siderites were considerably enriched with heavy ^{13}C isotope and had positive $\delta^{13}\text{C}$ values sometimes exceeding +30‰ VPDB. It indicates the close relation between the mechanism of their formation and processes of methane generation (Krylov et al., 2008a; 2008b; 2010). Subsurface authigenic carbonates enriched with ^{13}C isotope to such degree were not ob-

Table 1. Gas Hydrate-bearing structures of Lake Baikal (2000-2018)

№	Name	Type	Year of discovery	Depth, m	№	Name	Type	Year of discovery	Depth, m
1	Malenky	mv	2000	1306	28	KrasniyYar-3 (KrY3)	seep	2013	776
2	Bolshoy	mv	2003	1352	29	K-5	mv	2014	895
3	K-2	mv	2005	875	30	St. Petersburg-2 (SPb2)	mv	2014	1412
4	K-0	gm	2006	419	31	Khoboy	mv	2014	451
5	Gorevoy Utes	seep	2006	860	32	AcademRidge (AR)	mv	2014	691
6	Malyutka	mv	2006	1322	33	Barguzin	gm	2014	1278
7	K-6	mv	2007	1033	34	Mamay	gm	2015	520
8	P-2	mv	2007	821	35	Kamenny	mv	2015	937
9	Goloustnoe	seep	2008	396	36	Tonky	mv	2015	1019
10	St. Petersburg (SPb)	seep	2009	1410	37	Talanka	mv	2015	999
11	K-10	gm	2009	542	38	Novosibirsk-2 (N2)	gm	2015	1508
12	K-1	mv	2010	668	39	Kedr	mv	2015	593
13	Novosibirsk (N)	mv	2010	1391	40	PosolBank-2 (PB2)	mv	2016	732
14	K-8	gm	2010	432	41	Ostrov	gm	2016	953
15	K-9	mv	2010	763	42	Turka	mv	2016	678
16	PosolBank (PB)	seep	2010	514	43	Kedr-2	gm	2016	565
17	K-4	mv	2011	828	44	Solzan	gm	2016	706
18	K-11	gm	2011	424	45	Oblom	gm	2017	781
19	K-12	seep	2011	502	46	Turka-2	mv	2017	711
20	Seep13	seep	2011	1258	47	P-3	gm	2017	584
21	Krest	gm	2011	1288	48	MSU	gm	2018	1380
22	K-3	mv	2012	773	49	Enkhaluk	mv	2018	682
23	KrasniyYar (KrY)	seep	2012	740	50	Sukhaya	mv	2018	890
24	K-P	pock-mark	2012	941	51	Kitami (KIT)	mv	2018	810
25	Ukhan	mv	2012	1392	52	LIN SB RAS (LIN)	gm	2018	422
26	Unshuy	gm	2012	1323	53	PosolCanyon (PC)	gm	2018	416
27	KrasniyYar-2 (KrY2)	seep	2013	759	54	PosolCanyon-2 (PC2)	gm	2018	520

mv – mud volcano; **gm** – gas hydrates mound

served in the other regions. Other process responsible for crystallization of the carbonates in hydrate-bearing structures of the lake is destruction of the organic matter. In this case, the rhodochrosites with negative values of $\delta^{13}\text{C}$ are formed (Krylov et al., 2018).

Analysis of geological composition of all types of hydrate-bearing structures and adjoining areas of Lake Baikal, especially those discovered during the 2014-2017 expeditions, allow us to confirm that all studied structures are related to two principal ways of gas-saturated fluids migration. First, fracture zones of different rank, which dissect a part or entire sedimentary body of the lake, located directly at the source of gas-containing

fluid (zones with elevated sedimentation rates, modern and buried delta systems, group ridges (remnants), and outcrops of the “old” sedimentary layers “Tankhoy field”). Second, the permeable inclined sedimentary layers ascended from the basin center to its flank at the lake slope. Fluid discharge and structure formation occurred there either in the secondary fracture zone, or in the places, where these layers outcropped at the lake bottom.

Now, several mechanisms explaining formation of gas hydrate-bearing structures are considered. Minami et al. reported potential deep-rooted fluid at two certain gas hydrate-bearing mud volcanoes in Lake Bai-

kal (Minami et al., 2014; 2018). Another mechanism based on the model of gas hydrates dissociation at the zone of lower boundary of their stability due to ascending the warm fluids from the deeper layers can be adapted to formation of both mud volcanoes and seeps. Transformation of hydrates into gas and water lead to the pressure increase that result in penetration of gas saturated fluid, including deeper located fluid, to the lake bottom forming seeps and mud volcanoes. If this mechanism is accompanied by destruction of deeper “old” layers, then the underwater elevation consisting of mud breccias, mud volcano, is formed. Depth of the roots of these mud volcanoes determined by the level of lower boundary of gas hydrates stability and for Lake Baikal does not exceed 450 m. At the moment, we have only this evidence of relatively shallow position of mud volcanoes sources in the lake. Thus, the main reason for the formation of mud volcanoes is not the compression of sediments, as it occurred in all known onshore and offshore mud volcanoes, but the overpressure due to destabilization of gas hydrates. Therefore, this kind of mud volcanoes can be called “Baikal type” (Khlystov et al., 2018 in press).

3. Conclusion

In summary, the following factors serve as the sources for fluid material and play the dominant role in the distribution of the focused fluid discharging structures at the bottom of Lake Baikal: river deposits of the buried delta-fronts, areas with high sedimentation rates at the central parts of the depressions, and the shallow “old” sedimentary layers located at the eastern slope of the lake (“Tankhoy field”). Ways of fluid migration can be related to weak vertical and slightly inclined zones in the major rift-forming and secondary faults, as well as along permeable lithological boundaries, when the layers ascend from the centers with high sedimentation rates in the central part of the depression to its flanks. Destabilization of gas hydrates at the lower boundary of their stability is the main reason for the formation of mud volcanoes with shallow roots at the lake bottom, in spite of the fact that the Baikal rift system is the tension zone without wide areas having abnormally high strata pressure inside sedimentary section. Baikal model of the formation of mud volcanoes allow re-evaluation of the importance of mud volcanoes during the prospecting of oil and gas deposits in water basins, as well as estimation of the geological risks in the tectonically active and hydrate-bearing areas of the seas.

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Environmental DNA as a new tool for assessing the biodiversity of Lake Baikal

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ABSTRACT. Environmental DNA (eDNA) analysis is a powerful tool for detecting aquatic animals. Numerous studies have shown the high efficiency of the eDNA methods for the detection of species that are difficult to identify using traditional approaches. Other studies have demonstrated the high usefulness of eDNA for studies of species communities and species abundance. Here, we briefly describe the advantages of this method in relation to studying the biodiversity of the ecosystem of Lake Baikal. Traditional methods of biodiversity monitoring have a number of limitations here due to a great depth of the lake and a large number of rare endemic species, which occupy limited habitats, often in the deep-water zone. Using eDNA will overcome this limitation. It can fundamentally change the understanding and assessment of the biodiversity of Lake Baikal and solve other scientific and environmental problems in the region.

Keywords: eDNA, biodiversity, Baikal

Development of systems for assessing and controlling the state of biological complexity of wildlife is an important issue of preserving the diversity of living organisms. Attention to this problem is growing due to the increased anthropogenic impact on the environment. With the growth of industrial and agricultural production, as well as mining, man has increasingly destructively intervened natural ecosystems. As a result, the planet's climate is changing, habitats are being destroyed, the number of endangered and invasive species is growing. The extinction rate of species today may be 1,000 or more times higher than the natural (De Vos et al., 2015). Effective biodiversity assessment systems are required to assess and predict the dynamics of these processes. Development of such systems is the most urgent task with respect to rare and endangered species, endemic species, as well as groups of species that can serve as indicators of the state of the ecosystem. One of the most powerful approaches used for these purposes is the environmental DNA analysis (eDNA).

eDNA is a DNA extracted from samples of water, soil, air, and other natural substrates. The source of eukaryotic eDNA is feces, urine and epidermal cells, as well as damaged or decaying organisms. Once appearing in the external environment, eDNA gradually degrades remaining in a state suitable for analysis, from several hours to hundreds of thousands of years, depending on the environment (Willerslev et al., 2004). eDNA analysis was first used to study extinct animal and plant species in ancient sediments (Willerslev et al., 2003). Since then, this area of research has been

developing dynamically: methods are being improved, and the range of tasks is expanding. This is mainly due to the development of new methods for large-scale DNA sequencing (NGS) and quantitative PCR (qPCR). Currently, eDNA analysis technique is used to identify terrestrial and soil organisms, but this approach has proven to be the most effective for solving the problems of biodiversity analysis in water bodies. Numerous studies have shown the high efficiency of the eDNA for the detection of species that are difficult to identify using traditional approaches (Taberlet et al., 2012), for non-invasive registration of endangered species (Sigsgaard et al., 2015) and for identification of invasive species (Tréguier et al., 2014).

Compared to traditional biodiversity research approaches, eDNA analysis has several advantages and disadvantages. The first and most important advantage of the method is high sensitivity. eDNA isolated from a small amount of substrate is sufficient for detecting a species, including its low density. Consequently, this method is more cost effective compared to traditional approaches. A number of experiments, as well as research in laboratories and under natural conditions showed a positive relationship between the population density of a species and the amount of eDNA (Thomsen et al., 2016; Yamamoto et al., 2016). However, using eDNA it is still impossible to estimate biomass and population size, as well as such characteristics as the ratio of groups by sex and age, their morphological, behavioral and other features.

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Lake Baikal occupies a special place among the problems of assessing and preserving the diversity of living organisms. Approximately 1000 plant species and 2.5 thousand animal species have been described for Baikal; more than 2/3 of them are endemic (Timoshkin, 1997). Endemic species of animals and plants of the lake are deeply adapted to the conditions of this cold-water oligotrophic reservoir and, therefore, can be extremely sensitive even to minor changes in the ecosystem. The actual number of species living in the lake considered to be much higher than it is known today. Difficulties in studying biodiversity at Lake Baikal are associated with a large depth of the lake, as well as with the fact that many endemic species are rare and occupy limited habitats, often in the deep-water zone.

What issues of biological complexity at Lake Baikal can be solved using eDNA?

eDNA analysis will significantly expand, speed up and simplify the **qualitative assessment** of Baikal biodiversity. The technical implementation of research in this area is called “metabarcoding”, two-technology approach, which are taxonomic identification of organisms using DNA analysis and high-performance parallel sequencing. To implement this approach, a ready-made database is required, in which DNA sequences of specific genes are tied to specific species. Indeed, metabarcoding is applicable to known Baikal species, for which such DNA sequences were determined. The range of issues that can be solved using this approach is rather wide: from the research of animal and plant communities to monitoring of the distribution of individual taxonomic groups, both temporally and spatially (Deiner et al., 2017). In this respect, the studies of species from the deep-water zone of the lake are particularly relevant. Until recently, the study of the diversity of

deep-sea benthic organisms in Baikal has been carried out using deep-sea trawling on relatively flat horizontal platforms. The collection of hydrobionts in the zones with deep-water slopes was carried out more or less fragmentarily. The use of the eDNA will allow covering the entire lake for the research, despite the relief and depth. Concerning the species that have not yet been described, in perspective, metabarcoding will allow us to map unknown genetic groups of Baikal organisms, thus, bringing us closer to an assessment of the scale of our knowledge about Baikal biodiversity.

eDNA analysis in combination with an allele-specific quantitative PCR is an effective tool for identifying and monitoring of **individual species**. The targets of such research at Baikal are mainly deep-water fish species and the most abundant species, which play an important role in the ecosystem of the lake or have a commercial value. Using eDNA, we will be able to study and regularly monitor the seasonal migration and spawning behavior of these species. Rare endangered species of Baikal, such as Baikal sturgeon, Arctic char (Lake Frolikha) and others (<http://oopt.aari.ru/rbda-ta/900>), also require constant monitoring. Monitoring of the spread of invasive species is another very important area in the investigations of the lake ecosystem. In this regard, the spread of the Amur sleeper in the lake is the greatest danger.

The use of eDNA can be the only effective tool for assessing the **abundance of some Baikal hydrobionts** — potential indicators of the state of the lake ecosystem. Baikal oilfishes may be one of such indicators (Fig.1). Two species of the Baikal oilfish, big and little, are the dominant Baikal fish by biomass. They are common in the pelagic zone of the lake and are found at all depths. In addition, these species are deeply adapted to this cold-water oligotrophic reservoir and, therefore, can be very sensitive to even minor changes



Fig.1. (A) Big Baikal oilfish (*Comephorus baicalensis* Pallas, 1776), (B) Little Baikal oilfish (*C. dybowskii* Korotneff, 1905). From (Teterina et al., 2010).

in the ecosystem (Taliev, 1955; Teterina et al., 2010). To date, there are no cheap and easy ways to monitor the biomass of these species. Since oilfishes are bubble-free fishes, hydroacoustic methods cannot be used for this purpose. At present, it is impossible to estimate the biomass of species using eDNA analysis; however, this approach may be the only effective tool for assessing and monitoring of the abundance of Baikal oilfish. The same approach can be used as an additional tool for assessing the abundance of Baikal commercial fish, in particular, Baikal omul.

eDNA analysis is a powerful and cost-effective method for investigating the biological complexity of wildlife. Using this approach can fundamentally change the understanding and assessment of biodiversity of Lake Baikal, as well as solve other scientific and environmental problems in the region.

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Vital fluorescent dyes for study of silicifying organisms



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ABSTRACT. The mini-review describes application of vital fluorescent dyes to study silica biosynthesis in diatom algae and siliceous sponges. Mechanisms of these processes are not clear and study of growing siliceous constructions in living organisms is complicated with silica transparency and presence of mature elements (siliceous valves of diatoms and spicules of sponges). Specific fluorescence dyes stain growing siliceous elements of skeletons and also primary siliceous particles which are formed during silicon capture from the environment. Vital fluorescent dyes based on 7-nitro-2,1,3-benzoxadiazole, pyridyl oxazole and coumarin fluorophores are described as well as examples of application of these dyes to study diatom algae and siliceous sponges.

Keywords: fluorescent dyes, diatom algae, siliceous sponges, biosilicification

1. Introduction

A lot of living organisms use siliceous constructions for building of important elements of their bodies. The most known are diatom algae (>20% of primary oxygen and organic carbon production (Treguer et al., 1995), siliceous sponges (water filterers and a source of biological active compounds), rise and horsetail. For many decades scientists try to answer question: how these organisms build highly ordered constructions from silica at ambient temperatures? This question is intriguing and important because:

- biosilica shows a great number of sophisticated constructions which are species specific (Fig. 1);
- biosilica properties are closer rather to amorphous quartz than to silica gel from aqueous medium (Grachev et al., 2008);
- living organisms synthesize siliceous constructions at ambient conditions, without high temperatures and aggressive chemicals;
- understanding of molecular mechanisms of biosilicification gives not only valuable information of these organisms but also provides new knowledge in silicon and organic chemistry including approaches to new materials.

Siliceous particles and constructions are colorless and transparent which complicates their study with optical methods. These objects are electron dense and

so are visible with transmittance electron microscopy but this method requires complex sampling and is not applicable for living specimens. Staining with vital fluorescent dyes is a powerful approach in biology which becomes more actual in last decades with dissemination of confocal microscopes. Rhodamines where the first dyes applied to staining growing cultures of diatoms (Li et al., 1989). These substances were accumulated in growing siliceous valves and girdle bands but the mature elements of siliceous frustules were colorless. The such behavior of rhodamines is explained with formation of silica in Silica Deposition Vesicles (SDVs) of diatoms. These intracellular organelles are close to lysosomes and their content is acidic (pH = 5.5) (Vrieling et al., 1999) which stimulate accumulation of amines in these vesicles. Application of rhodamines allowed to obtain interesting data on diatom physiology (Safonova et al., 2007) and to synthesize new fluorescent materials by biotechnological approach (Kucki and Fuhrmann-Lieker, 2012). Unfortunately, rhodamines are not the convenient instrument because of low gap between staining and toxic concentrations. On the other hand, quantum yield of rhodamines in aqueous medium is high (>70% (Arbeloa et al., 1988) which results in high fluorescence from the cultivation medium.

A set of new dyes for vital staining of silicifying organisms was elaborated and this mini-review describes compounds based on 7-nitro-2,1,3-benzoxadiazole (NBD) fluorophore and more specific dyes which change fluorescent spectrum after incorporation into silica (PDMPO and coumarin derivatives).

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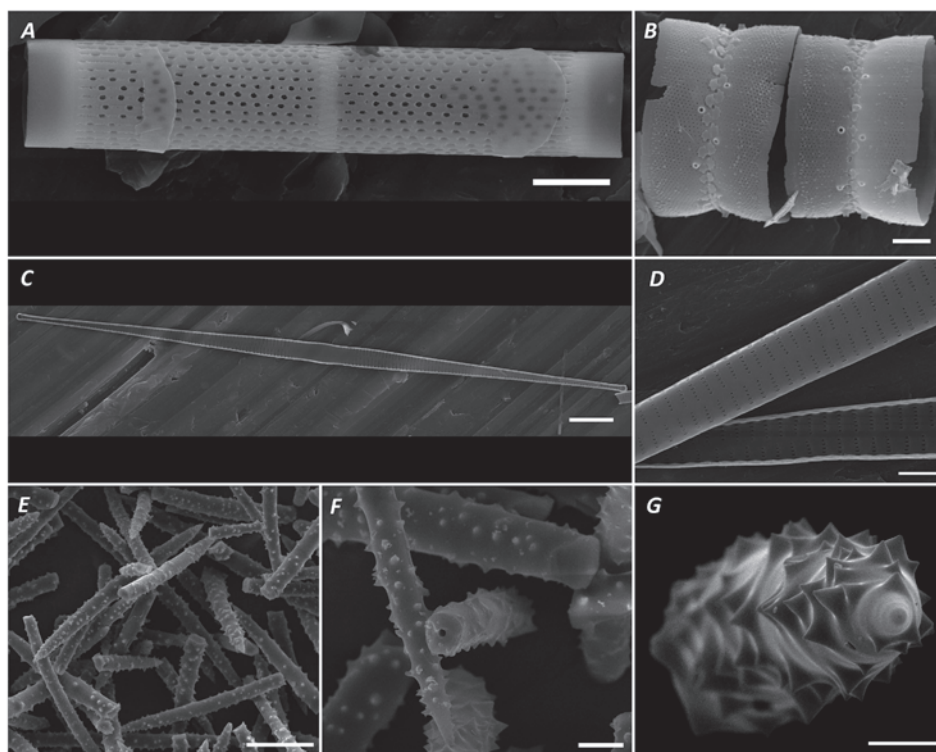


Fig. 1. Scanning electron microscopy images of diatom siliceous valves (A – *Aulacoseira baikalensis* (K. Meyer) Simonsen 1979; B – *Stephanodiscus meyeri* Genkal & Popovskaya; C and D – *Ulnaria ferefusiformis* M. Kulikovskiy & H. Lange-Bertalot); E-G – sponge spicules of *Lubomirskia baicalensis* (Pallas, 1773). Scale bar represents 50 (E), 10 (A, C and F), 5 (G) and 2 (B, D) μm .

2. NBD based dyes for vital staining

The first NBD derivative for staining the growing siliceous frustules of diatoms was HCK-123 (Fig. 2) (Descl  s et al., 2008). This dye contains a basic substituent which provides incorporation of the dye into acidic SDVs. NBD moiety is often applied for labeling of amine containing compounds (proteins, carbohydrates and etc.) with the use of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (Cl-NBD). This reagent is relatively cheap and readily reacts with primary and secondary amines.

Frustules of the diatom algae contain an interesting type of polyamines, so called long-chain polyamines (LCPAs). These compounds consist of amine groups separated by trimethylene fragments, with the first segment frequently containing four methylene groups (Kr  ger et al., 2000). Some amine groups are methylated, the LCPAs chains contain from several to

> 20 nitrogen atoms. These substances are available in very limited amounts from the natural sources which complicates study of their properties and role in biosilicification. We have elaborated (Annenkov et al., 2006; 2009) a stepwise approach to synthesis of LCPAs, including individual compounds with 2-7 nitrogen atoms and oligomeric mixture containing polyamines with > 20 nitrogen atoms.

The reaction of NBD-Cl with short polyamines (2-3 nitrogens) resulted in new dyes NBD-N2 and NBD-N3 (Fig. 2) (Annenkov et al., 2010). NBD-N3 as well as HCK-123 stains acidic organelles of eukaryotic cells but NBD-N2 is more selective and penetrates into growing siliceous structures only. This selectivity is explainable with shorter basic chain of NBD-N2 (one amine nitrogen only). NBD-N2 was successfully applied in study of initial stages of the diatom valve biosynthesis (Annenkov et al., 2013) and spiculogenesis

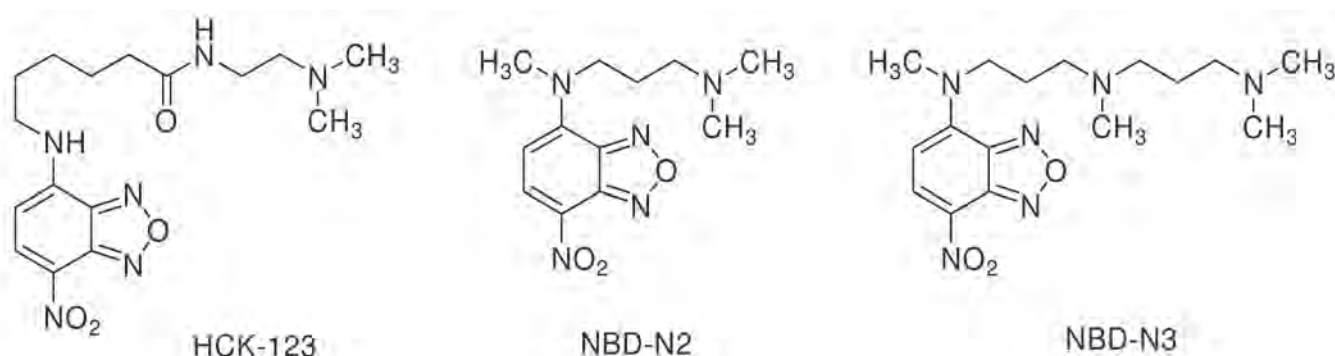


Fig. 2. Structures of NBD based dyes: HCK-123, NBD-N2 and NBD-N3.

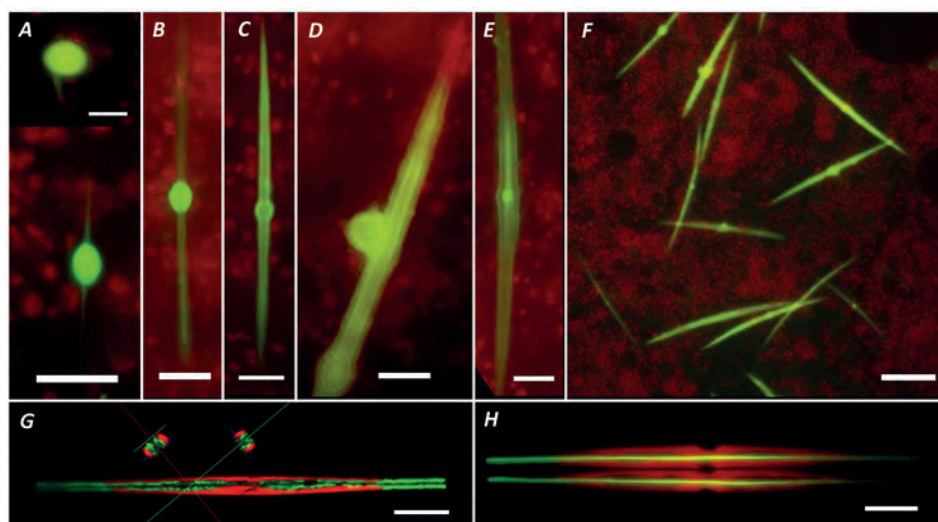


Fig. 3. Initial stages of sponge spicule (A-F, *L. baicalensis*) and diatom valve (G and H, *U. ferefusiformis*) growth visualized by cultivation in the presence on NBD-N2 dye. The main stages of spiculogenesis in *L. baicalensis* are: silicon accumulation in sclerocyte (A, top); formation of organic filament and protrusion of the new spicule from the cell (A, bottom and B); further elongation of the filament, sclerocyte capture silicic acid and organic substances from the extracellular space which allows further growth of the spicule (C); new sclerocytes merge with the growing spicule (D and E) and a mature spicules (F) are obtained. Submicrometer siliceous particles are formed in the diatom cytoplasm after 30-60 s after silicon addition to silicon free cultural medium (G) and after 10-15 min new siliceous valve is visible (H). Scale bar represents: 20 (F) and 10 (A-E, G and F) μm .

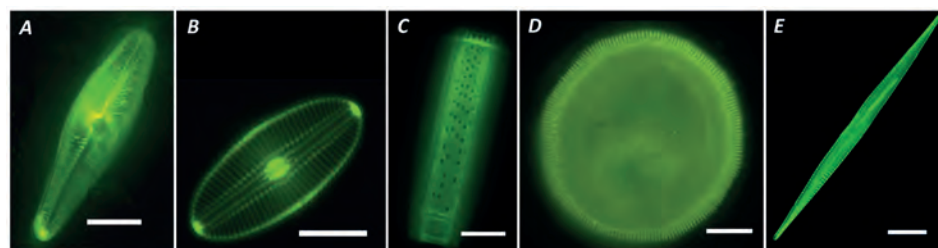
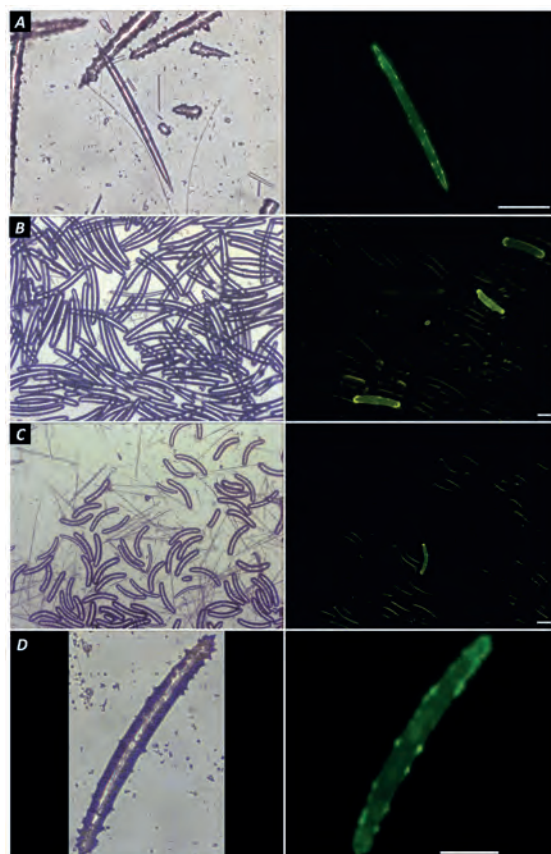


Fig. 4. Fluorescent images of diatom siliceous valves after cultivation in the presence of NBD-N2 dye. A-D - natural samples obtained from Lake Baikal (Russia), E - siliceous valve of *U. ferefusiformis*. Diatoms were cultivated for 3 days in the presence of 0.5 μM NBD-N2 and the biomass was treated with $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (1:1) mixture. Scale bar represents 10 μm .

in sponge *L. baicalensis* (Annenkov and Danilovtseva, 2016). Fluorescent and confocal microscopy allowed to visualize primary submicrometer silicon-containing particles formed during capture of silicic acid with diatoms and growing spicules starting from single sponge cell (Fig. 3).

NBD-N2 and NBD-N3 penetrates into siliceous frustules of diatoms and sponge spicules (Fig. 4-6). The dyes are deeply buried in the biogenic silica and retain fluorescent activity after treatment with strong acids and oxidizing agents during cleaning of organic substances. The stained valves and spicules are suitable for study with confocal microscopy (Fig. 6) which allows to obtain additional information about internal structure of the material without real slicing.

Fig. 5. Fluorescent images of diatom siliceous valves and sponge spicules after cultivation in the presence of NBD-N2 dye. A - *L. baicalensis*, B - *Baikalospongia bacillifera* (Dybowsky, 1880), C - *Swartschewska papyracea* (Dybowsky, 1880) and D - *Baikalospongia intermedia* (Dybowsky, 1880). Sponges were cultivated for a month in the presence of 0.5 μM NBD-N2 and the biomass was treated with bleach. Fluorescence allows to distinguish spicules grown during the experiment from old spicules. Scale bar represents 50 μm .



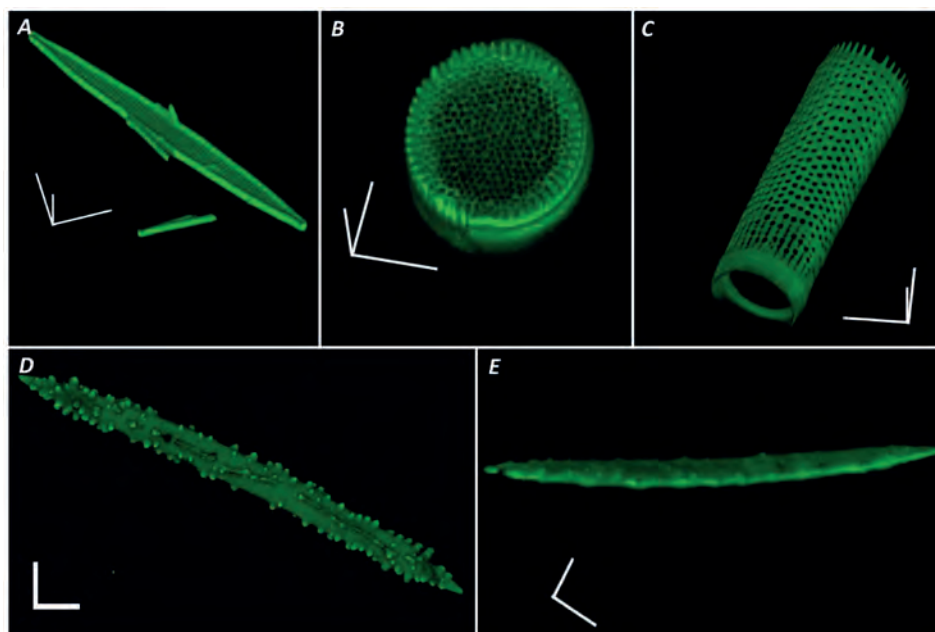


Fig. 6. Confocal microscopy 3D images of diatom siliceous valves and sponge spicules after cultivation in the presence of NBD-N2 dye. A - siliceous valve of *U. fereusiformis*, B and C - siliceous valve of diatom *Aulacoseira* sp., D and E - siliceous spicules of *L. baicalensis* sponge. Scale bar represents 10 (A-C) and 20 (D and E) μm .

The vital fluorescent staining is especially valuable in the case of sponges. These multicellular invertebrates have a skeleton from needle-like spicules which are fastened with organic compounds. Sponge cells and a lot of symbionts live inside this skeleton. The spicule shape and peculiarities of the spicule growth (spiculogenesis) are important for the sponge classification and as a marker of sponge growth and health in various experiments. Our works (Annenkov et al., 2014; 2016) give new powerful tool for study of the siliceous sponges. The siliceous spicules are colorless and transparent which decrease efficiency of light microscopy in their study but fluorescent spicules are a good object for confocal microscopy (Fig. 6). Staining with NBD-N2 dye allowed us to see growing spicules from the single silicon-enriched cells (Fig. 3) (Annenkov et al., 2016). This study was done with the whole sponges or sponge cultures (primmorphs) without isolation of the growing spicules because the fluorescent staining allows to observe these objects surrounded by other cells and mature spicules.

Study of the sponge physiology and influence of various ecological conditions on the sponges requires methods of the spiculogenesis control. This is not an

ordinary task because any sponge and primmorph contain old spicules and how to distinguish between old spicules and new ones, formed during experiment? The vital staining with the fluorescent nontoxic dye is the easy and accurate method to do this (Fig. 5).

3. Fluorescent derivatives of long chain polyamines

Study of NBD-Cl interaction with LCPAs (more than 4 nitrogen atoms) resulted in unexpected reaction of NBD-Cl with tertiary nitrogen atoms giving rise to NBD derivatives and unsaturated compounds (Annenkov et al., 2015). A set of new dyes containing relatively long polyamine substituents (≥ 3 nitrogen atoms) was prepared in this work (Fig. 7, Annenkov et al., 2018). The longer polyamine chain in these dyes increase activity in association with silica due to coordination with surface silanol groups. This property of the new dyes is useful in fluorescent staining of diatom frustules, siliceous spicules and silica containing natural sediments (Fig. 8). In the latter case the siliceous particles become easy distinguishable from non-stained terrigenous admixtures.

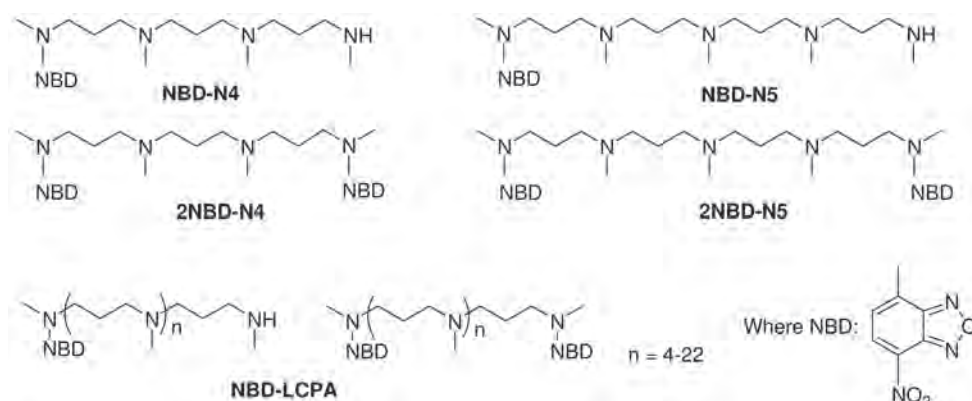


Fig. 7. Structures of fluorescent dyes based on long chain polyamines.

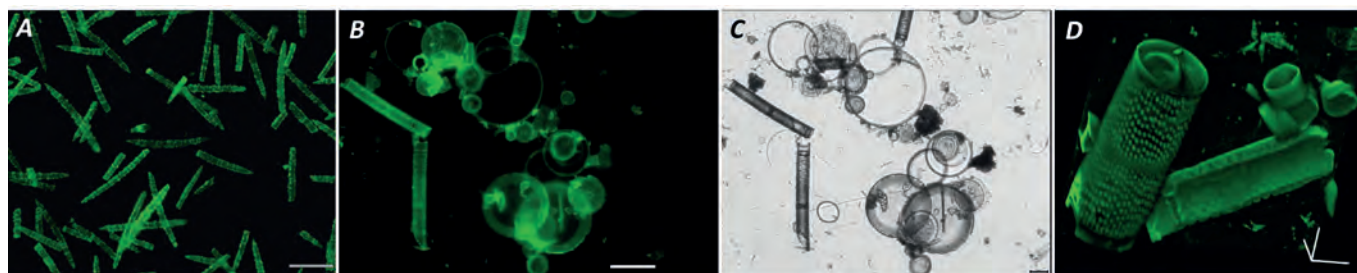


Fig. 8. Siliceous materials stained with fluorescent dyes based on long chain polyamines. A - *L. baicalensis* spicules, B-D - Baikalean sediment. Scale bar represents 100 (A), 20 (B and C) and 10 (D) μm .

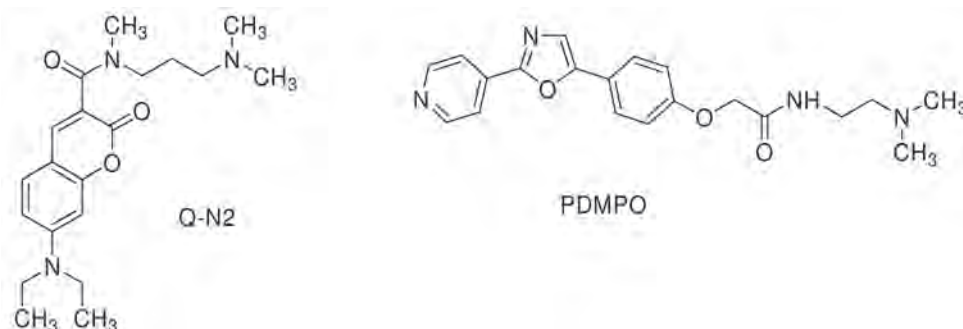


Fig. 9. Structure of Q-N2 and PDMPO dyes.

4. Dyes with variable fluorescent properties

As discussed above, NBD based dyes stain acidic cell vesicles and SDV due to specific structure of the substituent at fluorophore group. The next generation of fluorescent dyes for vital staining of siliceous structures in living cells changes fluorescent spectrum under association with silica. The first representative of these dyes is 2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbonyl)methoxy)-phenyl)oxazole (PDMPO, Fig. 9) (Shimizu et al., 2001; Parambath et al., 2016). Fluorescence of PDMPO depends on pH and the emission spectrum changes under association with silanol groups or involving into acidic vesicles. This behavior is useful in study of silicifying organisms as well as in study of silicification reactions *in vitro*.

Recently we have synthesized a new coumarin based fluorescent dye Q-N2 (Fig. 9) which contain amine group in the substituent (Annenkov et al., 2019). In contrast to PDMPO, fluorescence spectrum of this dye in aqueous medium does not depend on pH value. Aggregation of Q-N2 with silica or entrapping into siliceous materials results in enhancing of blue fluorescence and appearance of green emission. These effects were studied with fluorescence microscopy and they are easy visible with a routine epifluorescence microscope: fluorescence color changes from blue to cyan under excitation at 350-360 nm and green fluorescence appears under excitation at 470 nm (Fig. 10).

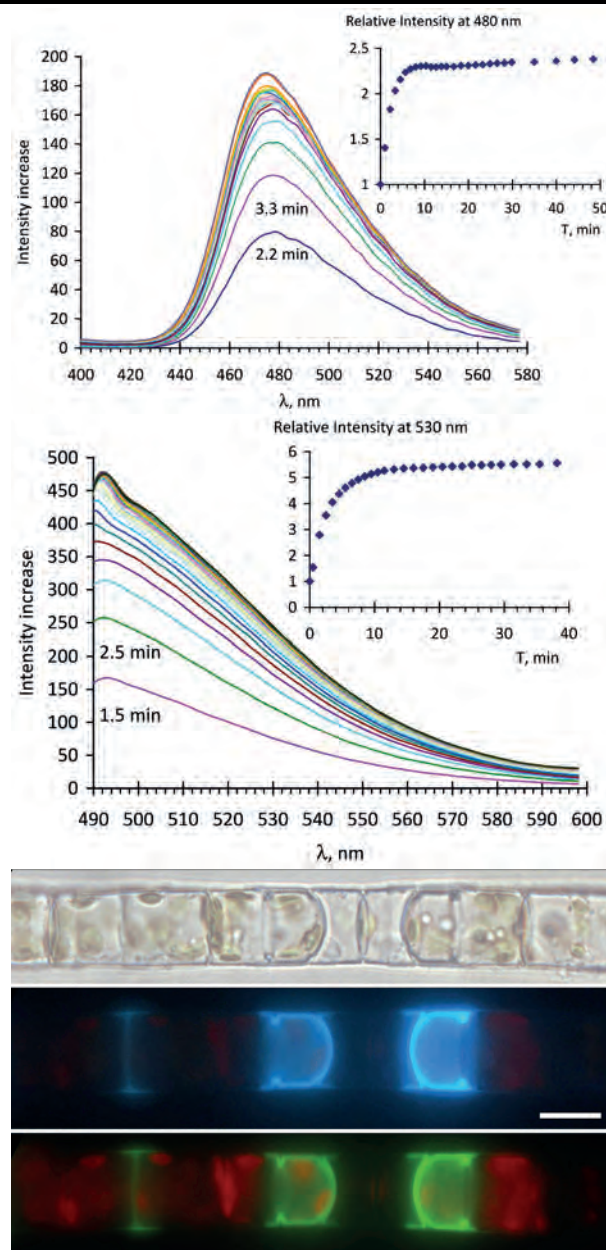


Fig. 10. Change of Q-N2 emission spectrum in the presence of 100 mM silicic acid during condensation at pH 7 (top spectrum - excitation at 365 nm, bottom spectrum - excitation at 470 nm), the data show faster growth of the fluorescence intensity in green area. The microscopy images show cells of *Aulacoseira* sp. after cultivation in the presence of 0.5 μM Q-N2, top - light image, middle - excitation at 370 nm, bottom - excitation at 470 nm. Scale bar represents 10 μm .

The absence of pH-depending changes of the Q-N2 fluorescence allows to attribute the strong cyan (green) fluorescence to siliceous structures. The other advantage of Q-N2 is the excitation maximum below 400 nm, far from excitation of chloroplasts. In combination with high quantum yield (11% comparing with <1% for NBD derivatives) this decreases damaging effect on living cells during microscopy investigation. Q-N2 shows inhibitory effect on the diatom growth in 10 μ M concentration (20-30% inhibition) which is 20 times higher its staining concentration. Q-N2 stains growing siliceous structures in diatoms and sponges (Fig. 11) and video monitoring of the silicon capture allowed us to find formation of condensed oligosilicates in several seconds after silicon capture with the diatom cell (Annenkov et al., 2019). This observation confirm hypothesis about silicon assimilation by diatoms in the form of partially condensed silicic acid.

Acknowledgments

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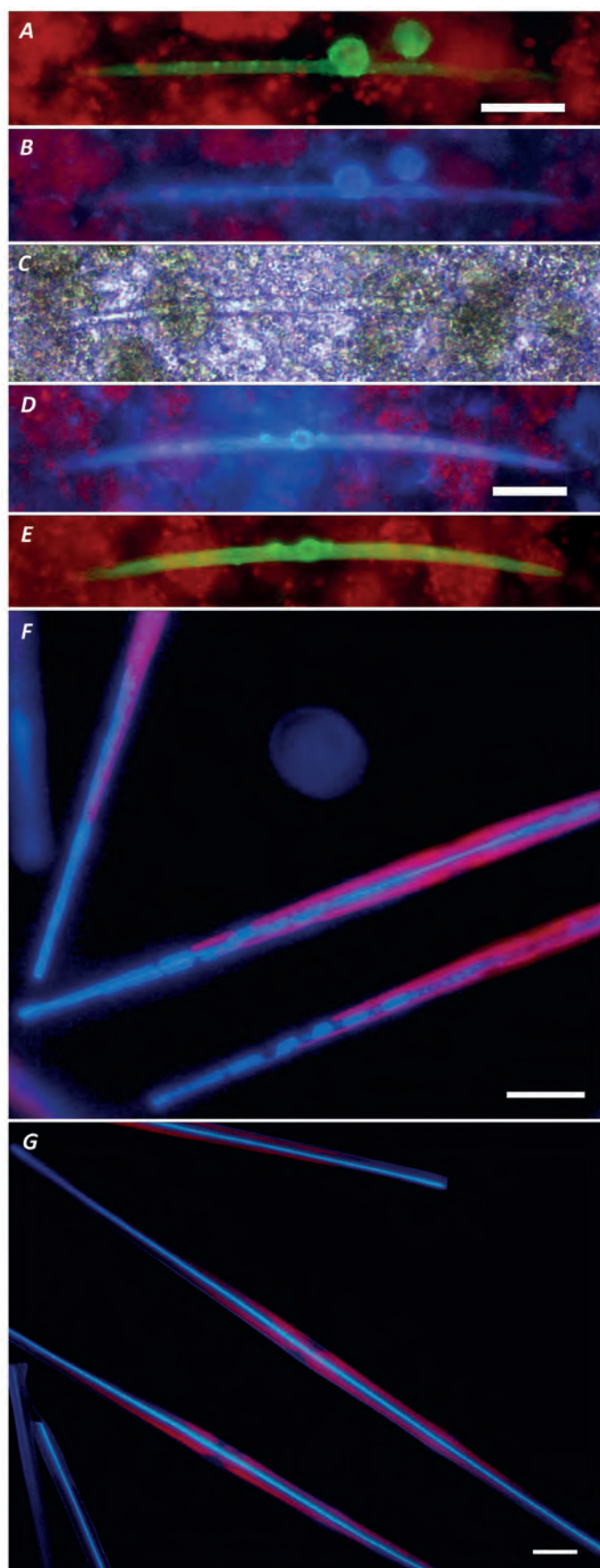


Fig. 11. Initial stages of sponge spicules (A-E, *L. baicalensis*) and diatom valve (F and G, *U. fereusiformis*) growth monitored with Q-N2 dye. The cyan color of new siliceous structures is distinguishable from blue fluorescence of free Q-N2. Scale bar represents 20 (A-E) and 10 (F and G) μ m.

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Coastal zone of the world's great lakes as a target field for interdisciplinary research and ecosystem monitoring: Lake Baikal (East Siberia)

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ABSTRACT. Limnological data on the coastal zone of the world's greatest lakes are scanty in contrast to the deep-water research that is actually the main reason for a delayed response of the researchers' and public community to evident anthropogenic changes the lake ecosystems are experiencing world-wide. The present study reports on the interdisciplinary investigations in the coastal zone of Lake Baikal during 2000–2018 and gives a brief description of the splash zone as a principal part of the lake ecosystem poorly known in other lakes of the Planet so far. Recent surveys of Lake Baikal showed a key role of the coastal zone research for the fundamental limnology and efficient monitoring of the lacustrine ecosystems. The splash zone of Baikal is briefly described due to its peculiarity. Principal reasons of significance of lake coastal zone research such as: maximal biodiversity of lacustrine ecosystems, high macrobenthos productivity in Baikal, intensive biogeochemical processes in rift and karst lakes and their reasoning are described. This is the zone that first of all colonized by invasive species and where the obvious cyclicity in plankton-benthos relationships is clearly expressed. Early warning signs for the ecological disturbance, such as blooms and wash-ups of native and/or alien algae, degradation of pelagic and benthic communities, bioaccumulation of pollutants (including organochlorine compounds) by hydrobionts, etc. are manifested in the coastal zone. A short report of the current ecological crisis in the coastal zone by 2018 is presented. The uniqueness of Lake Baikal makes the ongoing eutrophication different from all other Palearctic lakes. Therefore, the hydrochemical indicators of the water column of great lakes do not match the commonly accepted principal eutrophication criteria pool. Biological indication approach appears most appropriate for the analysis of the initial eutrophication stages. The author points out real and potential sources of excessive biogenic element supply into the lake ecosystem such as, sewage contamination of the estuarine parts of Baikal tributaries by the coastal settlements and vessels, pollution of the ground and interstitial waters of the beaches, input of biogenic elements as a result of mass mortality of sponges and other hydrobionts, secondary contamination by decaying algal wash-ups, intensive nutrient influx after numerous forest fires on the lake coasts and aerosol contamination. All these factors provide new opportunities to evaluate the initial eutrophication stages either on Baikal or in any other of the giant lakes. Special emphasis is given to an inadequate governmental lake monitoring systems focusing on the pelagial zone without taking into consideration the coastal (splash zone included) biological communities. Our landscape-ecological method served a basis for elaborating approaches for complex monitoring of Baikal coastal zone recommended as a part of the Federal Baikal Ecosystem Monitoring.

Keywords: coastal and splash zones, community degradation, *Spirogyra*, blue-greens, algal blooms, plankton and benthos interrelations, invasive species, Lubomirskiidae mortality, ecocrisis, POP bioaccumulation, monitoring, Baikal

1. Introduction: what is the problem?

Brief analysis of the world limnological literature evidences, that pelagic zone of the great lakes seems to be much more precisely and regularly investigated as compared to their coastal zone (splash zone includ-

ing). Most of the governmental monitoring schemes exclusively concentrated on the hydrochemical, hydrobiological etc. parameters and their dynamics in the pelagic zone (Zohary and Gasith, 2014; Timoshkin et al., 2015; 2018). If we will compare the data on the multiyear dynamics of the plankton and macrozoobenthos communities in the great lakes of the globe at the

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species level, it became evident, that available information on the successions of the benthonic communities, as distinct of planktonic ones, is very limited, scarce or even lacking (ILEC..., 1999–2018; Kravtsova et al., 2009). From this viewpoint Lake Baikal offers exemplary illustration. Indeed, the current ecological condition of Baikal ecosystem is interpreted in a diametrically opposed manner. Scientists, investigating the pelagic zone, evidence on the main hydrochemical parameters, remaining more or less stable during last 50 years of investigations (Khodzher et al., 2017); POP concentration in zooplankton and fish are quite compatible with that of the fishes from high mountain Alpine lakes of Europe (Gorshkov et al., 2017), changes in zooplankton during last 60 years are correlated with global warming¹ (Hamton et al., 2008; Moore et al., 2009; Izmayeva et al., 2016). Alternatively, limnologists, dialing with coastal zone, warn of profound changes in benthonic communities, related to anthropogenic pressure; high POP concentrations in some benthonic algae and invertebrates, hydrochemical and microbiological pollution of stretches of the rivers within the settlements and the near-shore zone, etc. Moreover, according to their viewpoint, some of these ecological changes might already be irreversible (for short characteristics of the ecological crisis in the coastal zone and main References, see below).

In the common sense, a formerly polluted (eutrophied, for example) lacustrine ecosystem is usually considered “enough recovered”, if its hydrochemical parameters as well as the qualitative and quantitative characteristics of the plankton became close to initial ones. Such conclusions, based only on offshore sampling, are often false. Normal hydrochemical parameters in the giant or – “hydrochemically recovered” after eutrophication smaller lakes may “coexist” with deeply depressed and modified benthonic communities.

“Interestingly, governmental monitoring in many countries also focuses on the offshore pelagic zone while mostly ignoring the nearshore zone. For example, a deficit of coastal monitoring in the Laurentian Great Lakes caused the USA and Canada, in their latest revision of the Great Lakes Water Quality Agreement (2012), to call for a “Nearshore Framework” that includes enhanced study and monitoring of coastal environments throughout the Great Lakes” (cited from Timoshkin et al., 2016).

The aim of this paper is to summarize the main results of the interdisciplinary investigations of Baikal coastal zone (2000–2018) and to show their importance in terms of both: for theoretical limnology and for the establishment of the optimal schemes of the great lakes monitoring. I just wish to draw the attention of the scientists to this extraordinarily important field of limnology.

The investigation methods were published earlier (Timoshkin et al., 2011; 2012a; 2012b; Kulikova et al., 2012; Tomberg et al., 2012). All photos are taken by the author (if not indicated differently); some of

them are taken from the scientific archives of the projects, supervised by the author.

2. What is the coastal zone in the lakes?

Similarly to marine and oceanic ecosystems, the coastal zone of the lakes includes shallow water, splash zone and the area between the upper border of the splash zone and the slope foot (or – constructions like parapets, etc.). Due to the specific hydrological conditions and Irkutsk hydroelectric power station activity, the term “splash zone” of Lake Baikal should be considered separately.

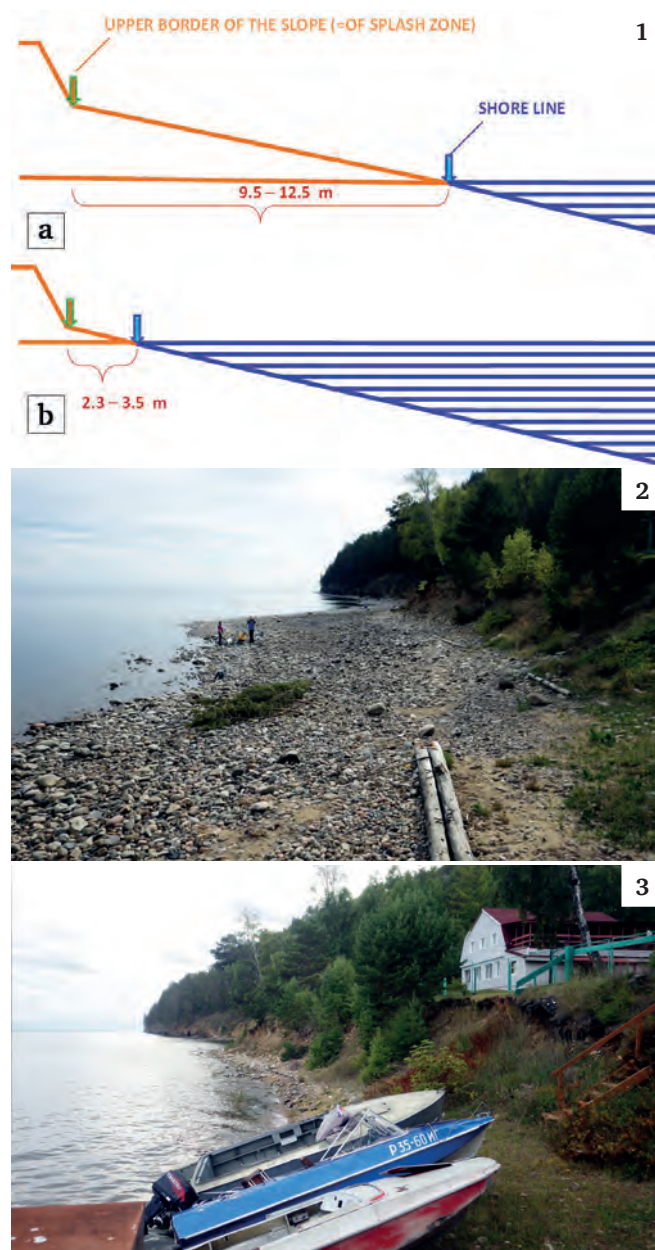


Fig. 1. Intra-annual fluctuations of the water level (the upper border of the splash zone) along the standard transect, opposite of the field station of Limnological Institute SD RAS, Bol'shye Koty Bay; western coast of South Baikal in 2011–2018. **a** – May–June. **b** – September (after Timoshkin et al., 2011; modified). **2, 3** – intra-annual fluctuation of the water level, the same area and the standard photograph position: low water level, May 25, 2016; typical for the spring season (2); high water level, September 4, 2018; typical for the autumn season (3).

1 Strictly speaking, correlation does not automatically mean, that two (or more) correlating processes have cause-and-effect relationships.

2.1. Baikal splash zone: definition; importance for interdisciplinary research and monitoring

The term “splash zone” is commonly used by marine biologists in intertidal zonation, denoting a part of the littoral zone subjected to water splashes. Evidently, a similar zone with a variety of environmental gradients, defined by abiotic factors also exists in all the great lakes. Splash zone of the most Eurasian lakes remains a white spot for limnologists.

E.B. Karabanov (1990) was the first, who introduced the term “splash zone” into limnology of Lake Baikal. It was characterized as analog of the supralittoral zone in marine ecosystems, which occupies the coastal area between the shore line and 2 m above the water level. However, after our interdisciplinary investigations of Baikal splash zone (Timoshkin et al., 2011; 2012a-c), the definition was significantly changed. Baikal splash zone is a mobile zone, the borders of which are constantly migrating throughout the year as a result of the spring-summer rise of the lake level. Its upper border migrates 10–14 m (western shore, e.g., Bolshye Koty Bay) or up to 15–20 m (eastern shore, bays) landward (Fig. 1), and in winter-spring period it moves significantly backward. The Lake water level varies within ca. 1 m.

As the intermediate zone, the splash zone represents a combination of abiotic and biotic factors, typical for both – terrestrial and lacustrine ecosystems. “Terrestrial ecologists” consider the splash zone as a part of the limnic ecosystem, their standard transects usually ended at the lake’s slope. Vice versa, the standard limnological transects usually begin at the shore line of the lakes. The splash zone remained “owner-less, or – abandoned”. This is the main reason why our knowledge on the limnic splash zones has been so poor. Meanwhile, Baikal splash zone has been found out to be rich in feeding resources: high concentrations of detritus and domestic waste providing a favourable biotope for specific communities and affecting hydrochemistry and microbiology of interstitial and coastal waters. Four invertebrate faunistic complexes of different origin were described in this zone: Palearctic, cosmopolitan and other species (immigrants from Eurasian or Siberian water bodies) (1); common or endemic Baikal hydrobionts (2); as well as soil infauna migrants, such as earthworms (3) and representatives of terrestrial biocenoses – beetles, spiders, etc. (4) (Timoshkin et al., 2011; 2012a-c). Most of the endemic “hydrobionts”² dwelling the splash zone of Baikal were found to be evolutionary young and can be considered as a perfect model objects for interdisciplinary investigations of the initial stages of the endemic speciation. Recent observations showed that this zone is subject to most heavy anthropogenic load: its grounds serve a special buffer filtering household waters discharged from the neighboring settlements; maximal concentration of domestic waste is registered in the splash zone. Hence, the anthropogenic load on the lake can be easily recorded

2 Literally, the permanent residents of this zone are already not hydrobionts, but – “splash zone inhabitants”.

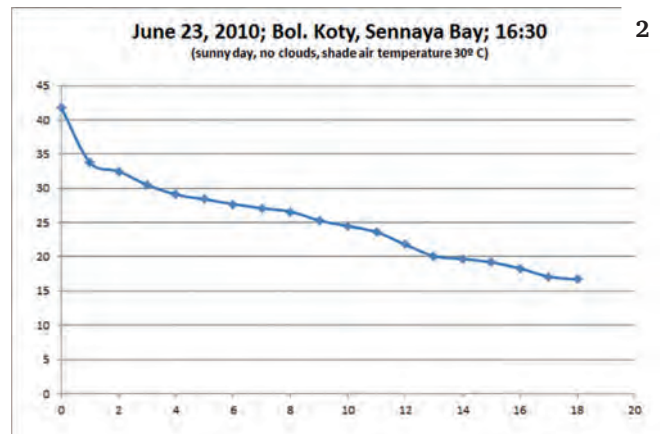


Fig. 2. Example of the surface temperature (°C, Check-temp Co.) (1) and its variation (°C, Y axis) within the soil profile (2) (cm; X axis) in the splash zone. Measurements taken ca. 1 m above the shoreline.

in the splash zone, a sensitive “indicator” of the changes in Baikal ecosystem. While the animal diversity in splash zone is comparatively poor and the macrozoobenthos communities, as a rule, are “monodominant”³, the simple monitoring scheme has been elaborated and proposed (ob. cit.). Monitoring of the great lakes splash zone must be viewed as an indispensable integral part of the effective monitoring scheme of their ecosystems (Timoshkin et al., 2011; 2012a).

3. Why coastal zone is so important for interdisciplinary investigations and ecosystem monitoring?

3.1. Zone of the maximum biodiversity

The maximum abundance and number of species in 14 largest lakes of the globe dwell the coastal (shallow water) zone, even if it occupies comparatively small area of the lake (Vadeboncoeur et al., 2011). The same is true for many ancient lakes (Kostoski et al., 2010; von Rintelen et al., 2012), including Lake Baikal (Index of animal..., 2001–2011; Timoshkin, 2001; Timoshkin et al., 2004; Semernoy, 2007): their highest biodiversity concentrated in the coastal zone. The greatest species diversity in Lake Baikal occurs on the substrate in shallow waters ranging in depth from 1 to

3 Means, that 1–2 species of turbellarians, oligochaetes, gammarids, etc. are dominating in the communities.

50 m. Zohary and Gasith (2014) state, that most fish and macro-invertebrates of Lake Kinneret use littoral habitats for at least part of their life cycle. Many hydrobionts use littoral resources (substrate for attachment, refuge, food) for reproduction and growth.

3.2. Zone of high benthos productivity

From the common viewpoint, Baikal water has low mineralization and potentially cannot support the high productivity rates of benthonic communities. Nonetheless, as stated earlier (Kravtsova et al., 2009; Timoshkin et al., 2009), the biomass and number of macrozoobenthos of the stony bottom of Lake Baikal in 2000–2003 was high enough: $59 \pm 5.1 \text{ g/m}^2$ and 11 ± 0.7 thousand specimens/ m^2 respectively⁴. These parameters are substantially higher than that in other lakes of the globe, for example, Khubsugul (4.8 g/m^2), Issyk-Kul' ($5\text{--}7 \text{ g/m}^2$), etc. (Kravtsova et al., 2009). As distinct, the productivity of marine zoobenthos may be several times higher and runs, for example, in Caspian Sea, up to 240.7 g/m^2 (Guseinov, 2005). So, from this viewpoint, Lake Baikal occupies intermediate position between ordinary Palearctic lakes and marine ecosystems. Which factors may provide possibility for abundant zoobenthos development in Baikal?

3.3. Zone of intensive biogeochemical processes

Coastal zone (and – in particular – the near shore and splash subzones) is the area of maximum concentration of the autochthonous and allochthonous organic matter, strongest intermixing processes (wave activity) and rather contrast environmental parameters. In combination with decomposition of wash ups⁵, high surface and inside the sandy bottom temperatures of the splash zone, vital activity of algal belts, they may significantly intensify the rates of biogeochemical processes, rock hydrolysis, and, indirectly the benthos productivity. Both – experimental and in situ observations support these conclusions. During the calm sunny day the surface sandy bottom temperature in the splash zone may exceed 40°C ; at 5 cm below bottom surface it varies within $27\text{--}30^\circ\text{C}$ (Fig. 2; original data). As evidenced by series of papers, summarizing results of our interdisciplinary research (2008–2018), an important role in the biogeochemical cycles of the coastal zone belongs to attached organisms – lichens, macrophytes and sponges. **First**, in the process of the vital activity they may significantly influence and change the hydrochemical parameters of the near shore and near the bottom water layers of the coastal zone. For example, the main hydro-

chemical parameters, measured in June–August within the near shore zone, occupied by typical filamentous alga *Ulothrix zonata*, may be as high as pH 9–9.5; dissolved oxygen – up to 150% of the saturation limits (Volkova et al., 2012; original data). The same parameters, measured in the mesocosm experiments with *U. zonata*, show much higher ranges (day time): pH 11.6; O_2 saturation limits up to 260% (ob. cit.). Kulikova et al. (2008) found that the underwater lichens of *Verrucaria* genus cover 60–70% of the rock debris and widely distributed along the west coast of South Baikal (in particular – Berezovy test-site, Bol'shye Koty areas) at 1–14 m depth, with maximum abundance at 1–3.5 m. Depending on the rock type, the projective area on the bottom of *Baikalospongia* encrusting sponges, dwelling at the same depth range, vary within 14–100% (Kulikova et al., 2009a; see below).

Second, they are able to synthesize and extract a wide spectrum of macro- and microelements, organic compounds (including environmentally aggressive organic acids), which literally destroy the minerals of the stony bottom and perform significant vital element contribution to the ecosystem. At least three indirect data sets support this conclusion: 1) significant abundance and huge biomass of sponges⁶ simply cannot be determined by nutrients (especially – of silica, for example) taken from the water column only; 2) the same is true with regard to the elemental composition of the dominant groups of hydrobionts (Kulikova et al., 2007; 2008a; 2008b; 2009b; 2011; 2013; 2017; 2018); 3) selectiveness of biofilms, algae and invertebrates to the petrographic composition of the rocks on stony bottoms of the coastal zone (Timoshkin, 2001–2011; Suturin et al., 2005; Timoshkin et al., 2005; 2009; Parfenova et al., 2008).

In situ experiments with the artificial sterile plates made of 3 dominant rock types, distributed on the stony bottom of the Berezovy test-site and exposed at 3–10 m depth for 1–18 months on the Lake's bottom, evidences, that microorganisms, microphytobenthos and meiozoobenthos organisms strictly prefer the marble and granitoid substrates rather than amphibolite plates (ob. cit). Similar results obtained with encrusting sponge and gastropod mollusks investigations. Analysis of underwater rocky bottom photographs and the petrographic composition of the rocks with attached invertebrates evidences, that both invertebrate groups demonstrate selectiveness of the rock types to inhabit. They both prefer to dwell the destroyed granitoids rather than amphibolites. Rather high density of gastropods was detected on granitoids with carbonate and mica rockstreaks, while sponges prefer to inhabit the granitoids with microcline and garnet rockstreaks (Timoshkin et al., 2003). Therefore, the biodiversity and bioproduction on the stony littoral depend on the diversity of the petrographic composition of rocks and their geochemical properties. Evidently, **the geochem-**

4 These numbers are compatible with earlier data on macrozoobenthos productivity (Kozhov, 1963).

5 First classification of Baikal wash-ups of different origin and the data on their seasonal and annual dynamics; their influence on the hydrochemical regime and the elemental composition of the interstitial waters of the splash and near shore zones can be found in Kulikova et al., 2012; Timoshkin et al., 2011; 2012a-c; Tomberg et al., 2012.

6 Sponges – the most abundant macrozoobenthos group in the stony areas of the coastal zone of Lake Baikal. Their wet biomass may reach up to 3 kg/m^2 for encrusting and up to 1.5 kg/m^2 – for *Lubomirskia baikalensis* (Kozhov et al., 1969; Kaplina, 1970).

ical factor shall be considered as one of the most important factors of the ecological segregation, endemic speciation and evolution in ancient lakes of carst and rift origin. Coastal erosion and mudflows contribute up to 1550 000 m³ of the non-hydrolized stony material in the coastal zone annually (Agafonov, 2002). **Therefore, the rocky bottom of the coastal zone shall be considered as an unlimited source of the vital elements and nutrients for Baikal ecosystem.**

3.4. Coastal zone is the most sensitive and first responding to contamination

It became commonly accepted, that the coastal zone provides most accurate evidence of immediate ecosystem's response to the current anthropogenic changes.

First symptoms of phytoplankton and protozooplankton degradation of the great lakes can be traced in the coastal zone. They are especially illustrative opposite the tributaries and coastal settlements. Bondarenko and Logacheva (2016) described the degradation of coastal zone phytoplankton in 2000–2015. Algae of “Baikal complex”, such as *Aulacoseira baicalensis* (K. Meyer) Sim., *Cyclotella baicalensis* Skv., *Stephanodiscus meyeri* Genkal et Popovsk., and spore-forming *A. islandica* were absent (or present in negligible amounts)⁷ and replaced by huge amounts (up to 7 million cells/dm³) of small-cell *Chlamydomonas* species, as well as another indicators of organic pollution, euglenophytes (4–7 thousand cells/dm³). These algae did not occur in plankton earlier (1975 to 1990th), their insignificant amounts appeared in beginning of XX century. The authors believe, that these structural changes in phytoplankton and dominance of the small-cell species shall be considered as clear evidences of the eutrophication of the Lake Baikal shallow areas.

3.5. Benthos of coastal zone – among the first responders to anthropogenic contamination

Several negative ecological phenomena have been detected in Baikal coastal zone within last 5–7 years.

1. Significant changes in the macrophyte belt composition, development and stratification were described (Kravtsova et al., 2012; 2014; Timoshkin et al., 2015; 2016; 2018; Timoshkin, 2018; Volkova et al., 2018). In 2013–2018, a mass blooms of non-typical for Baikal algae – *Spirogyra* spp. were detected with maximum biomass in autumn in the shallow water zone throughout much of the lake⁸: Baikalsk, Slyudyanka, Kultuk towns; along the Old Baikalian Rail Way; List-

vyanichnoe, Obuteikha, Bolshye Koty, Peschanaya, Babushka, Aya Bays; Goloustnoe settlement (South Baikal); some areas of Maloe more Strait; Maksimikha Bay (Middle Baikal); Nizhneangarsk, Severobaikalsk towns; Senogda, Frolikha, Ayaya, Onokochanskaya and Boguchanskaya Bays (North Baikal). Also in 2014–2017, the mass development of *Spirogyra* was noted on Ol'khon Island at two localities (i.e., the ferry harbor in Perevoznaya Bay and Shamanka Bay opposite the town of Khuzhir on Ol'khon Island). By 2015, mass growth of *Spirogyra* was reported at several new localities along the west coast of South Baikal (Emelyanikha, Sennaya Bays and a coast opposite Polovinnyi Cape) as well as Maloe More Strait (i.e., coastal zone off Sakhyurte Settlement and Kargante Bay). From 2016 its remarkable autumn proliferation was detected in Aya Bay (one year after the new hotel, ca. 300 m away of the shore line was opened there), and in majority of the north-western shallows from Senogda Bay to Elokhin Cape. **In summary, *Spirogyra* spp. developed massively and even dominated the benthic macroalgal community along much of the eastern coast, and in many places along the western coast of Lake Baikal.** Interestingly, the maximum development of *spirogyra* – a comparatively thermophilic algae (optimal temperature for growth is ca. 20°C), was always detected during autumn (September–October) with water temperatures ranging within 4–8°C. At least, two sites investigated to date (i.e., Listvennichnyi Bay in South basin and Tyaa-Senogda coast in North basin) were characterized by year-round mass blooms of *Spirogyra* spp., which sometimes include other diatom and filamentous green algae species that previously were non-typical for open parts of Lake Baikal (such as *Oedogonium* spp.; or *Fragillaria* spp., which became extremely abundant in along to the west coast of North basin between Onokochanskaya and Senogda Bays; etc.). Starting from May–June 2016 mass development of *Spirogyra* has also been detected in the littoral opposite of Baikalsk City (at 3–7 m depths) and several wooden harbors (Khuzhir, Bolshye Koty settlements). It means, that **the number of areas with the all-the-year round *Spirogyra* spp. mass development is gradually increasing.** The same is true for the gradual horizontal distribution of the alga along the north-western coast – its area is increasing year by year, mostly at the depths of 0.5–2 m.

Only one of ca. 12 *Spirogyra* morphotypes, found in the Lake and its tributaries, has been dominating in these areas of the lake's rocky littoral within 2013–2017. It has simple cell walls, 4 in “young” and one homogeneous chloroplast(s) – in “adult” cell; the filaments attached to the rocks by rhizoids (see Timoshkin et al., 2018 for additional description and color Figs). As a rule, this *Spirogyra* morphotype has patchy distribution in the coastal zone (especially sharply expressed along to the western and Olkhon Island coasts), the blooms are mostly concentrated opposite of the settlements and recreation centers (with poor or without any sewage water purification systems).

Results of 2016–2018 investigations evidence, that the *Spirogyra* morphotype 1 in significant masses develop from end of August till September–October

7 At the same time, these species vegetated in open areas of Lake Baikal.

8 It is easier to indicate areas where the alga was not found: Bol'shoi Ushkani Island, most of the coastline of Ol'khon Isl. (except for Perevoznaya Bay and a coast opposite Khuzhir), and the northwestern coast from Elokhin Cape to Maloe More Strait (with several exceptions of patchy distribution in areas of recreation activity).

and creates the new for Baikal, well distinguished algal belt at the depths range 1–10 m (with maximum abundance at 2–5 m) along to the west coast from Senogda Bay to Elokhin Cape. It prefers to vegetate on the rocky bottom and avoid the cliffs. So far, the shallows of the later Cape is the most southern border of the belt distribution along to the west coast of the North Basin.

As shown by Timoshkin et al. (2018), neither global warming nor the cases of the comparatively low lake water level provide us any arguments to consider them as the key factors, influencing mass *Spirogyra* development in Baikal coastal zone. Moreover, the minor, but permanently entering the coastal zone nutrient additions from non-purified sewages provide possibility to overcome the depths of 20 (North Baikal) or even 30 (South Baikal – I.V. Khanaev, pers. comm.) meters! Evidently, *Spirogyra morphotype 1* is very sensitive indicator of the human wastes and should be considered as a perfect indicator of the sewage water contamination in oligotrophic ecosystems such as Baikal.

2. Giant amounts of the coastal accumulated material, mostly consisting of algal detritus, have been detected in the north and south tops of the Lake and opposite of Maksimikha settlement (Barguzin Bay) (Fig. 3). Intensive development of macrophytes in Maksimikha Bay was due to *Cladophora* sp. (*glomerata*?) (ca. 50% of the total wet biomass), Characeae, *Elodea* and other higher water plants. The most pronounced *Spirogyra* blooming and wash ups were regularly found in the northern basin, along the western mouth of Tyra River and Zarechnoe–Senogda coasts (Severobaikalsk City vicinities) (Fig. 4). Wet biomass of this algal wash up opposite of Zarechnoe, in the autumn of 2013 reached up to 90 kg/m² (Timoshkin et al., 2015). All mentioned regions belong to the near-shore settlements or bays with poor or none waste purification systems and natural harbors for numerous vessels. The blooms shall be considered as the clear evidence of “indirect” eutrophication in many particular regions of the coastal zone. Evidently, the natural buffer and filtrating ability of Baikal’s splash zone is limited. Abundant amount of nutrients can reach the interstitial and near-bottom water layers through the ground of the splash zone and cause the blooms. The distribution pattern of the fecal indicating bacteria (see below) strongly support this supposition and can be used as sewage benchmarks. Due to the huge size of the lake and permanent intense water-wind activity (mixing) high nutrient concentrations often cannot be detected by commonly accepted hydrochemical methods in the surface and near-bottom water layers.

3. Increase of the typical Baikalian macroalgae productivity in some areas of the shallow water zone. According to the world literature, it should be considered as early symptom of the eutrophication.

4. Mass development of the «saprophytic» (proliferating on sick sponges and died macrophytes, see below) (Figs 5, 9) and «free-living» blue-greens (Figs 6-8) in several areas of the Lake. Significant amount of the Oscillatoriales filaments have been first found by the author in the drudge benthonic samples, taken from



Fig. 3. Algal wash-ups opposite of Maximikha Settlement, Barguzin Bay, September 24, 2018.



Fig. 4. *Spirogyra* wash-ups in Senogda Bay, north basin, June 12, 2018.

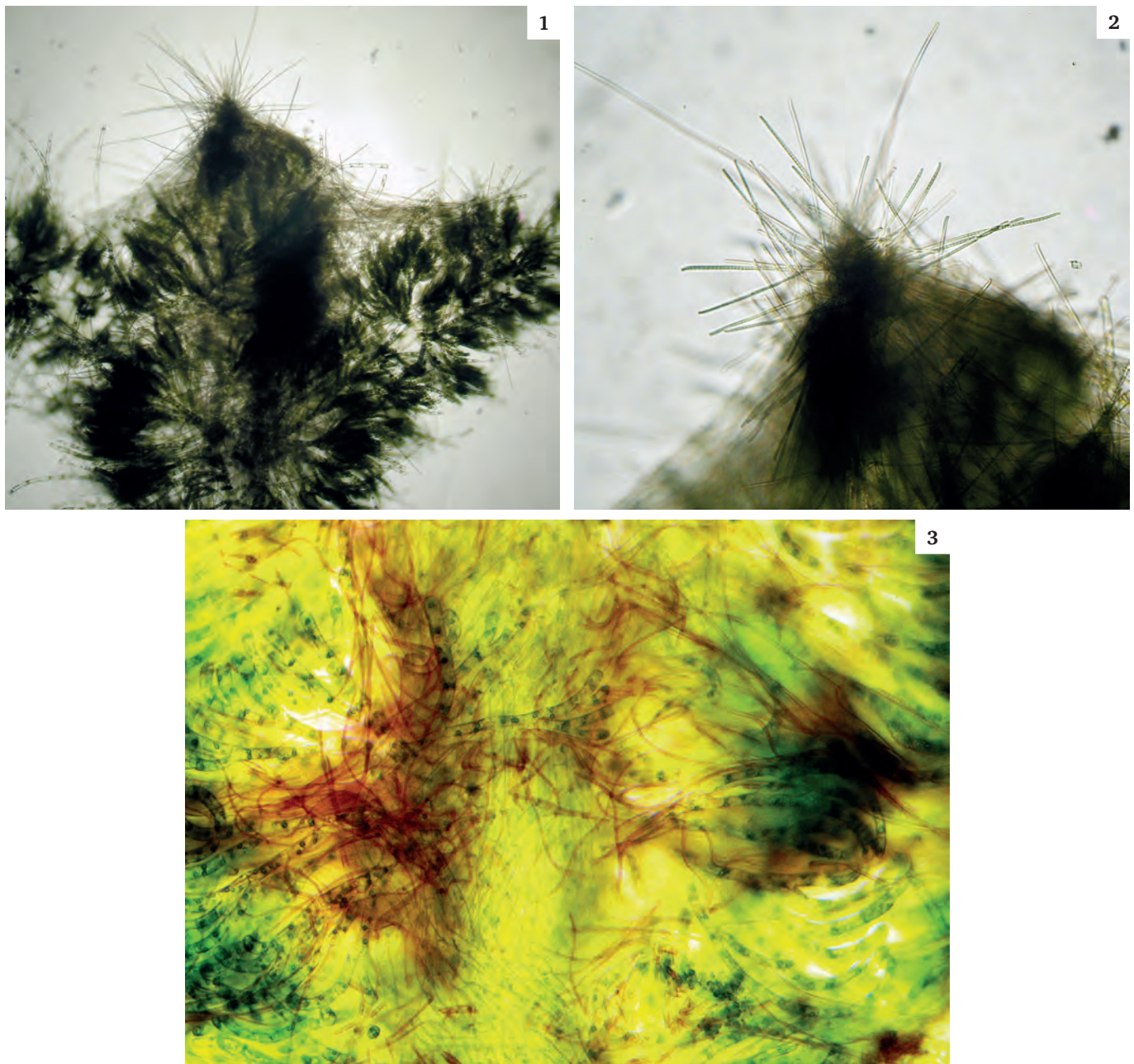


Fig. 5. Mass development of the “saprophytic” blue-greens (Oscillatoriales) on the dying thalli of *Draparnaldioides* spp. 1, 2 – opposite of the LIN SD RAS field station, Bolschye Koty Bay, August 1, 2011. Width of the algal branch on 1 – ca. 2.5 mm. 3 – The same, sample taken on July 25, 2018, the same area. Width of the branch of *Draparnaldioides* – ca. 1.1 mm.

10–15 m depth, southern of Peschanaya Bay (South Baikal), in summer of 2013 and 2014. In 2015–2016 their mass blooming found as well in the shallows of Bolshye Koty, Barguzin bays, etc. (*Phormidium*, *Oscillatoria*, *Tolypothrix* spp. and others). Most unusual is the mass penetration of the *Tolypothrix*, *Oscillatoria* and other cyanoprocaryote spp. into the first algal belt, usually created by green filamentous alga *Ulothrix zonata* (Fig. 6) (Timoshkin et al., 2016). In September–October 2015–2016 the blue-greens abundantly developed on the shore line rocks and nearby, at the depths 0.2–0.5 m, at Bolshye Koty (Fig. 7). This trend of the mass benthonic blue-green development in the coastal zone of entire Lake has continued in 2017–2018. For example, they absolutely dominated (in combination with diatoms) in the algal communities at the “Drizhenko expedition bank” (east coast of the north basin) (Fig. 8). Since the encrusting *Baikalospongia* spp., which were

dominating here did not look healthy and branched *Lubomirskia baikalensis* were found to be either sick or damaged, possibility of the mass blue-green blooms on the sick encrusting sponges cannot be ruled out. Such examples of the mass blue-green development on the completely destroyed *Baikalospongia* sp. bodies was found at the rocks near Bolschoy Cheremshanyi Cape (Fig. 9). The wet biomass of *Tolypothrix* spp. (abundantly covered by attached diatoms) on the depth 11–12 m, near Bolschoy Cheremshanyi Cape, September 29, 2018, counted by stone unit method, may exceed 240 g/m².

The «saprophytic» representatives of *Phormidium* and *Oscillatoria* genera, dwelling in Baikal, are not able to fix the atmospheric nitrogen. Their development is evidently limited by nitrogen. This limitation does not exist near the dying sponges and macrophytes. Their destroying bodies extract abundant nutrients (= special

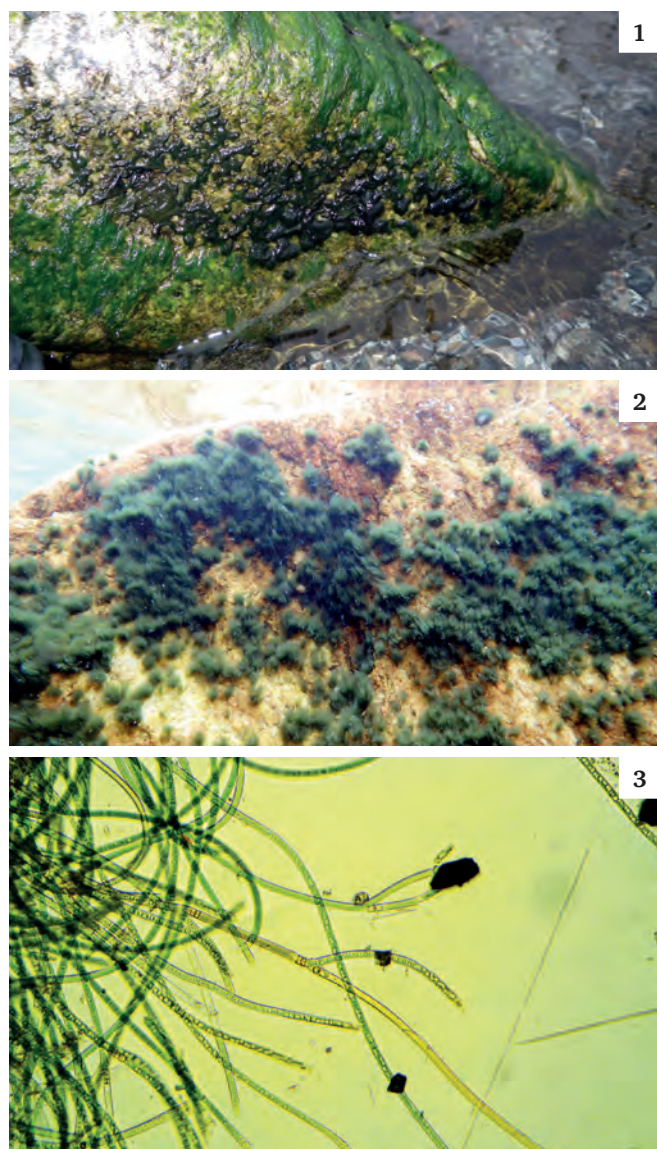


Fig. 6. Mass development of benthonic blue-greens (*Tholypothrix distorta*) within the *Ulothrix zonata* belt. Bolschye Koty Bay, August 31, 2016. **1, 2** – above-water and underwater views of the colonies respectively; length of the blue-green spot on **1** – ca. 20 cm. **3** – light microscopic image of the *Tholypothrix distorta* branch, ca 1.1 mm in the length.

type of eutrophication!) (I.V. Tomberg, O.A. Timoshkin, unpublished), which can be easily utilized by the «saprophytic» blue-greens what provides possibility for their mass proliferation. Vice versa, the most evident reason of «free-living» blue-green proliferation (which are able to fix N) is “indirect and forest fire eutrophication” first of all due to additional non-organic phosphorus input through the inlets and ground waters.

According to O.I Belykh (pers. comm.), both types of the cyanoprocaryotes are able to produce different toxins (including saxitoxins). Such abundant blooming of Oscillatoriales in general and within the first algal belt in particular has never been detected in the lake before.

5. Mass Gastropoda mortality (mostly – representatives of *Lymnaea* genus) is regularly detected in 2013–2018: billions of died shells found on the sandy beaches between Tyva River and Senogda Bay at the north top of Baikal. These “cemeteries” are located along the are-

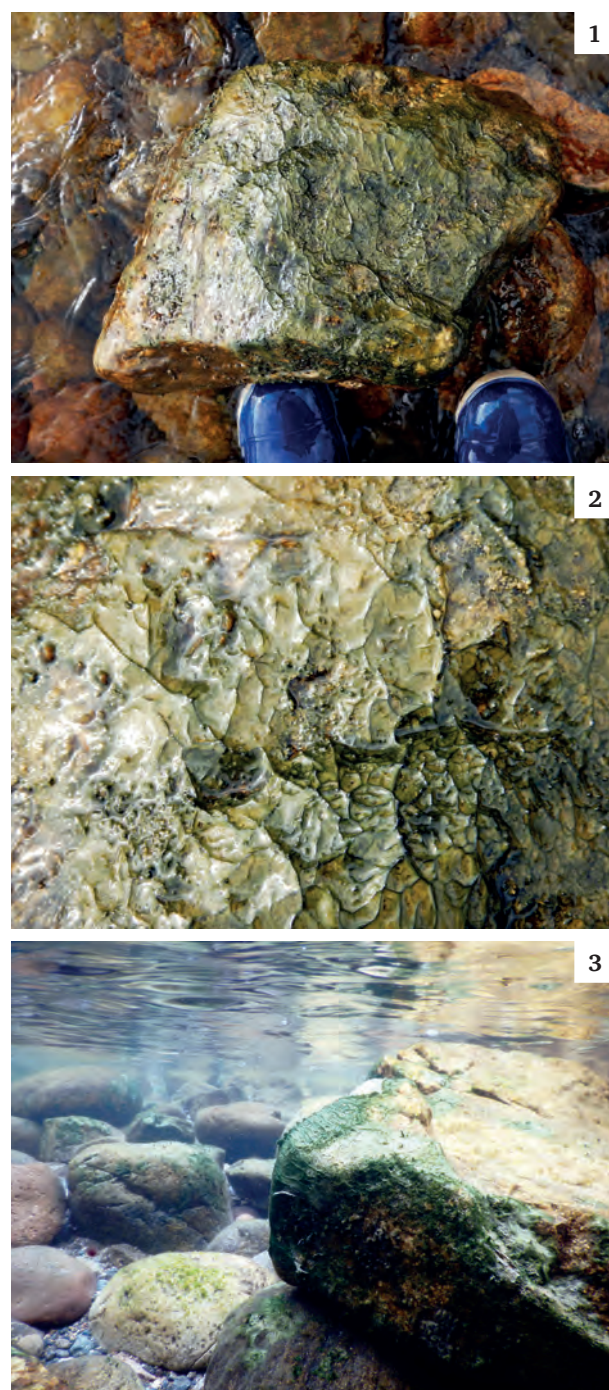


Fig. 7. Mass development of benthonic blue-greens (*Phormidium* spp.) in the near-shore phytocoenoses of Bolschye Koty Bay, October 23–24, 2016. **1–2** – aerial, **3** – underwater, **4** – light microscopic images of the biofilms and their filaments respectively. Magnification on the **4** – ca. 400 X.

as of the most abundant *Spirogyra* development, influenced by sewages from Severobaikalsk City. Less abundant *Lymnaea* shell accumulations found along the splash zone, off Maximikha settlement in Barguzin Bay (June 2015).

6. Mass mortality and several kinds of diseases of endemic Lubomirskiidae sponges at the scale of the entire lake were described in 2013–2018 (Bormotov, 2011; Timoshkin et al., 2014; 2015; Khanaev et al., 2018). All 3 ecological forms of the sponges (branched, encrusting, globular) can be sick. Over than 50 dives performed in 2014, 40 – in 2015. Depending on area, from 30 to 100% of branched *Lubomirskia baikalensis* specimens were found to be either sick or damaged and died. According to Dr. Ch. Boedecker (pers. comm.), in most of the studied areas of south basin (September 2014) the sick sponges were usually found at the depths above 15–20 m. However, deeper leaving sick specimens of the branched sponges were found already in June 2015 and later on.

It was described, that the most distributed sponge illness is accompanied by mass development of the “saprophytic” *Phormidium*-shaped blue-greens (Timoshkin et al., 2015). Their filaments are comparatively large, cherry-red and mobile. Light-microscopic analysis evidences, that each affection patch on the sponge surface consists of 1–2 dominating blue-green species (90–95%). Different deformations and damages of the external surface of the sponge body (= initial stages of their bodies destruction) in most cases happen prior to the mass blue-green development. According to preliminary data, the branched sponges, dwelling in the south basin (Listvyanichny, Bolshye Koty Bays, off Chernaya River mouth) are most of all affected by illnesses. For example, almost 100% of *Lubomirskia baikalensis* specimens, dwelling off Chernaya River mouth along the standard bottom transect (South Baikal, 1 m wide and 10 m long; at the depths 3–12 m, during 2014–2015) were either damaged, or thick and died (A.B. Kupchinsky, S. Aurich, pers. comm.). Much less damaged or even healthy *L. baikalensis* specimens were found in September 2014 around the north-western coast; area, approximately located between Elokhin Cape and Bolshye Olkhonskye Vorota.

As shown earlier, the same blue-green morphotypes used to dwell on the destroying thalli of *Draparnaldioides* macrophytes at the end of their vegetation season (Timoshkin et al., 2012). The mass development of the blue-greens began from the basal parts of the algal thalli (attached to the rock) and gradually covers all the thallus (Figs 5, 9.1). Surely, the mass proliferation of similar morphotypes of Oscillatoriales on the destroying Baikal endemic sponges and macrophytes has the same pattern and reasoning. Mass necrosis of the algal⁹ and sponge bodies may supply the “saprophytic” blue-greens by essential nutrients (nitrogen, for example), what could significantly stimulate their

9 Mass blue-green development on thalli of *Draparnaldioides baikalensis* in Bolshye Koty Bay was regularly detected in September–October 2007–2018. L.A. Izhboldina (2007) described several *Phormidium* spp., developed at the basis of *Draparnaldioides pumila*.

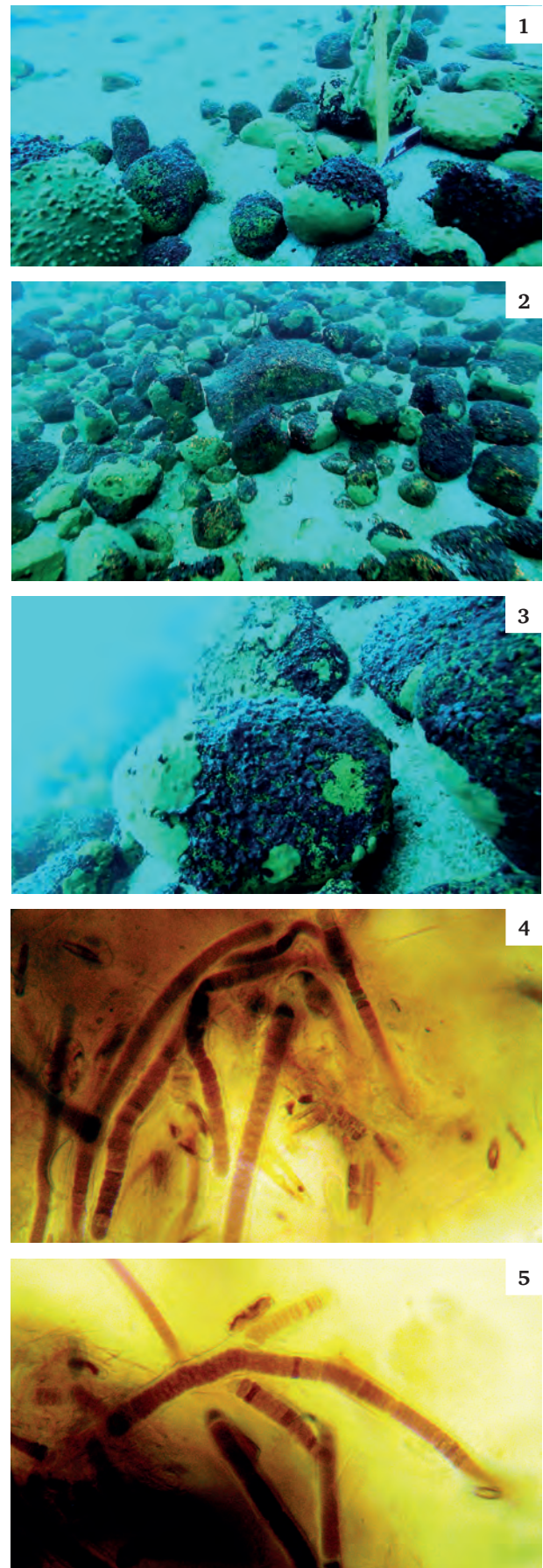


Fig. 8. “Kingdom” of blue-greens and encrusting sponges at the “Drizhenko expedition bank”, opposite Frolikha Bay (east coast of the north basin), September 28, 2018, depth 11–12 m. **1–3** – underwater views of the landscape and the rocks, covered by blue-greens and *Baikalospongia* spp. **4–5** – light-microscopic image of the blue-green filaments (*Tolypothrix* sp. ?). Magnification ca. 400 X.

mass development. Earlier, in 2003–2004, mass development of Oscillatoriales on sponges was time to time detected in south and middle basins¹⁰ on the encrusting and branched Lubomirskiidae (Izhimey Cape, 2004). Small areas of the sponge bodies (several cm²) were covered by dark-red or brown biofilms (Fig. 10). But during all period of our previous investigations (2000–2008) I have never seen the completely sick/damaged/died sponge bodies as in 2010–2018. Possibly, the blue-greens developed on the damaged (or – naturally dying) body portions of the sponges and never caused their mortality. During Lubomirskiidae mass extinction the development of the “saprophytic” oscillatoriales also became abundant.

The reasoning of the mass development of the “free-living” blue-greens in the benthos needs a special analysis, what is currently in the progress and will be published elsewhere.

The precise reasons of the mass Lubomirskiidae mortality are to be clarified. To my mind, one of many suppositions made earlier deserve the special attention. As hypothesized earlier (Timoshkin et al., 2015), the most probable reason of this phenomenon might be the physiological abnormalities in the relations between the endosymbionts (such as green *Zoochlorella*) and the sponge proper. Some precise processes and nutrient exchange mechanisms between algal symbionts and the sponge cells, elaborated during the long coevolution and coexistence in the oligotrophic waters, may be easily broken due to miserable but permanent addition of the sewage nutrients. It may cause the destruction of the sponge bodies, which has been detected so frequently. Analogous processes, causing the mass death of another sedentary lower Metazoan group – corals (which as well coexist with endosymbiotic blue-green algae), also inhabiting oligotrophic ecosystem of the ocean, are happen due to miserable but permanent eutrophication process (Yamamuro et al., 2003; Bell et al., 2014). For another potential reason - see section 5.

7. Numerous dead bodies of the endemic gammarid *Macrohectopus branickii*, exclusively dwelling in pelagic zone deeper than 150 m, were found in the splash zone and near shore algae aggregations of Senogda Bay in September 2018 (Fig. 11). Most of the crustaceans belonged to the adult females (ca 2 cm long), which normally create the aggregations and avoid the shallow water zone. The reasons of their mortality are not clarified yet.

8. High concentrations of the fecal indicating bacteria have been determined in the surface and near-the-bottom water layers along the coasts opposite the settlements. The same is true for the interstitial waters (especially – under the coastal accumulated algae) of the splash zone. For example, the enterococci concentrations often exceed 2000 colony forming units per 100 ml (V.V. Malnik, pers. comm.).

3.6. Invasive and non-indigenous species

Invasive and non-indigenous species first of all appear in the coastal zones of the lakes, often evidenc-

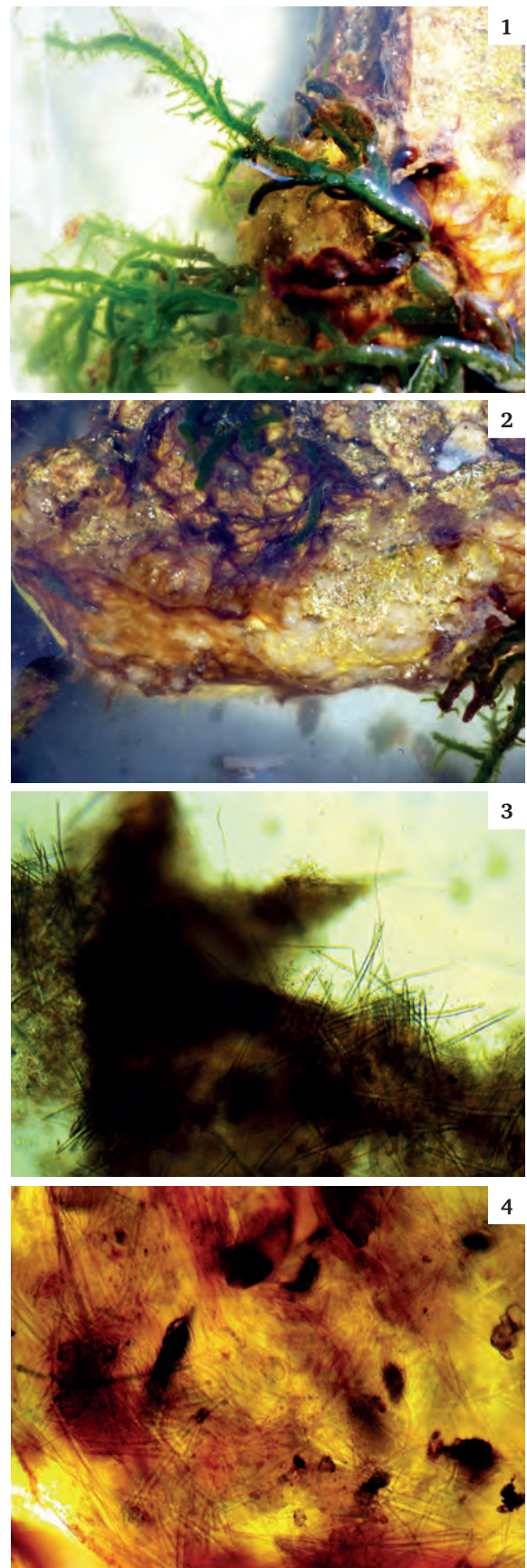


Fig. 9. Mass development of the saprophytic blue-greens. 1 – on the dying thalli of *Draparnaldioides* sp.; 2 – brown red biofilm on the completely destroyed *Baikalospongia* sp. (whitish spot from the right: the biofilm was partly removed to show the destroyed sponge body). 3, 4 – light-microscopic image of the blue-green filaments and sponge spicules. Magnification ca. 100 X. September 29, 2018; 1 mile south of Bolschoy Cheremshanyi Cape: 54035.857' N; 108043.075'E; the same stone unit.

ing on the early stages of ecological disturbances in lacustrine ecosystems. They can easily be detected if the process of regular survey (monitoring) has been performed. During last 10–15 years, the following species became abundant and even dominating the coastal zone communities of Baikal: filamentous green algae of *Spirogyra* genus, gastropod mollusks of Palearctic *Lymnaea* genus (in particular – *Lymnaea (Radix) auricularia* (L.)), riverine caddis flies *Apatania majuscula* McL., *Hydatophylax nigrovittatus* (McL.) (Fig. 12) (Sitnikova T.Ya, pers. comm., own observations; Kravtsova et al., 2012; Timoshkin et al., 2016; 2018; Rozhkova et al., 2018). Due to the multi-year monitoring of the coastal zone Trichopteran species, performed by Dr. Rozhkova N.A. and Dr. Nepokrytykh A.V., we know, that these species first penetrated the coastal zone of the south basin and currently they are spread and rapidly expanding this zone in some areas of the middle basin.

4. Interrelationships of plankton and benthos development are most apparent in the coastal zone

Well known, that water ecosystems, following the environmental cycles (solar radiation, temperature, etc.) may demonstrate the regular cycles in their development. Their graphical representations can be compared with cordial rhythm. The best known “cordial rhythm” in Baikal pelagial – the phenomenon of “*Melosira* years”, which took place every 3–4 years (Fig. 13) and characterized by intensive mass proliferation of typical for open Baikal planktonic *Aulacoseira* diatoms and other algae (Antipova and Kozhov, 1953; Evstafiev and Bondarenko, 2002a; b; etc.). Phytoplankton cycles determine the cycles in zooplankton development (for summary, see: Kozhov, 1963; Guide and key to pelagic animals..., 1995). It seems perfectly clear that the cycles in phytoplankton growth are evidently related to zooplankton life cycles and even determine them. It is well-known for Baikal researchers (ab. cit.). Several distinct abundance and biomass peaks in the phytoplankton growth pattern suggest similar fragmentary and inhomogeneous inputs of organic carbon from the pelagial zone to the lake bottom where it becomes available for primary benthic consumers. It means that quantitative characteristics of benthic Baikal communities should likewise be discrete and non-uniform in both perennial and seasonal aspects. These processes are particularly prominent in the littoral communities and most illustrative during ice cover period. We found, that the first, meroplanktonic stage of *Aulacoseira baicalensis* development clearly expressed in and beneath the ice cover in the coastal zone (Timoshkin, 2001). Mucous cords and filaments, attached to the lower side of the melting ice (March 2000, Berezovy test site), from several cm to 2.5–3 m in the length (Figs 13, 14), demonstrate unusual for this low water temperature growth rate (1–3 cm per day: Timoshkin et al., 2000; Timoshkin, 2001). The cords mostly consist of *Aulacoseira* filaments and, in combination with abundant invertebrate in- and epifauna, rep-



Fig. 10. *Phormidium*-shaped blue-greens (dark-red spots) on the surface of *Baikalospongia* sp., so-called pre-crisis period. 1–2 – photos taken along to the standard transect “T0” on Berezovy test-site, South Baikal, station 0.2, depth 2–2.4 m; October 27, 2004. Underwater photos taken by NIKONOS-V with SEA&SEA 35M-3 attached macroring; film No 1632; frame numbers 22; 25A; the total area of each of the frames: 110.5 cm².



Fig. 11. Died bodies of the pelagic amphipod *Macrohectopus branickii* along the shoreline of Senogda Bay, north basin. September 27, 2018.

resent cryophilic community No 2 (sensu Timoshkin, 2009¹¹). The period of mass sinking of these cords and filaments onto the bottom in late March–April usually correlates with migration of the giant abyssal triclads to the coastal zone (for laying cocoons), mass hutching of young endemic gastropods. Reproduction of some crustacean, mollusks and cottoid species (Taliev, 1955; Kozhov, 1963; Sideleva and Nagorny, 1985); hutching of the coregonid larvae and their downstream migrations into the lake's coastal zone (Chernyaev, 1982) are confined just to this period. Evidently, the pelagic and bottom communities in the coastal zone demonstrate a certain degree of synchronic development, which can be expressed in mutual co-adaptations of the life cycles of the dominant species, peaks of their maximum abundance and/or biomass, etc. (Timoshkin et al., 2000). For more clear correlation we need to know the multi-year “cordial rhythm” of benthos development, which currently is lacking. *Thinking of the optimal scheme of Baikal ecosystem monitoring, one should consider this possibility of synchronic development of plankton and benthos in the coastal zone.*

The current irregularity of *Melosira* years (and their almost complete absence in South Baikal since 2007: Bondarenko and Logacheva, 2016) can be explained (at least, in part) by the rapid ecological changes, which sharply expressed first of all in the coastal zone (for summary, see Timoshkin et al., 2016).

5. Bioconcentration and bioaccumulation of pollutants in coastal benthic plants and animals

As shown earlier (Timoshkin et al., 2016), the specific composition of POP in the interstitial waters of Baikal splash zone can be used as a perfect markers of sewage pollution in Severobaikalsk vicinities. Important conclusions, regarding the POP distribution and bioaccumulation in the coastal zone communities were made during the collaborative NPO TAIFUN–LIN SD RAS expeditions and research (2014–2015) (Persistent..., 2017; Samsonov et al., 2017).¹² 1) In all cases the specific POP concentrations in different coastal algae and invertebrates were significantly higher as compared to their concentrations in the water. It evidences on their high ability to accumulate and bioconcentrate the organic pollutants. 2) Entire spectrum of organochlorine pesticides, found in Baikal waters so far, were identified in the coastal algae. The concentrations of some OSP's (i.e., toxaphenes, nonachlores, DDE) in algal dry mass may exceed the analogous concentrations in water 1000–5000 fold. 3) Based on POP analyses in Baikal hydrobionths, other than fishes (Gorshkov et al., 2017) (see the footnote 12), it was confirmed that the POP concentrations in their bodies may ex-

11 In total, four types of the cryophilic communities were described depending on their dominant species. Two of them are characterized on the Figs 13–15.

12 Coastal algae (*Ulothrix zonata*, *Spirogyra* spp.), encrusting and branched sponges, gastropods, amphipods of *Acanthogammarus* genus were analyzed.



Fig. 12. Numerous larvae (1, 2) and pupae (3) of formerly rheophilic caddis fly *Hydatophylax nigrovittatus* (McL.), which currently occupied the coastal zone of open Baikal. 1, 2 – September 18, 2018, opposite of Kultuk Settlement, depth 1 m; 3 – June 13, 2013, opposite of Baikalsk cellulose plant combinat, 1.5 m. Identification of Dr. Rozhkova N.A., Dr. Nepokrytykh A.V.

ceed analogous concentrations in surrounding waters in hundreds of one times. ***In particular, we have found that the PCB concentrations in Lubomirskiidae bodies may exceed their concentrations in the water column 100–1000 fold!*** For example, the total PCB concentrations in the body of the branched sponge *Lubomirskia baikalensis* varied within 3.0–19.1 (on average, 9.16) ng/g of the dry weight; while their concentration in the surface waters vary within 1630–4860 pg/dm³ (Persistent..., 2017). Therefore, it is a reasonable working hypothesis to suggest that high concentration of organic pollutants (including POP) in Lubomirskiidae bodies potentially may cause their illnesses and mortality. Another example – endemic benthonic side-swimmers

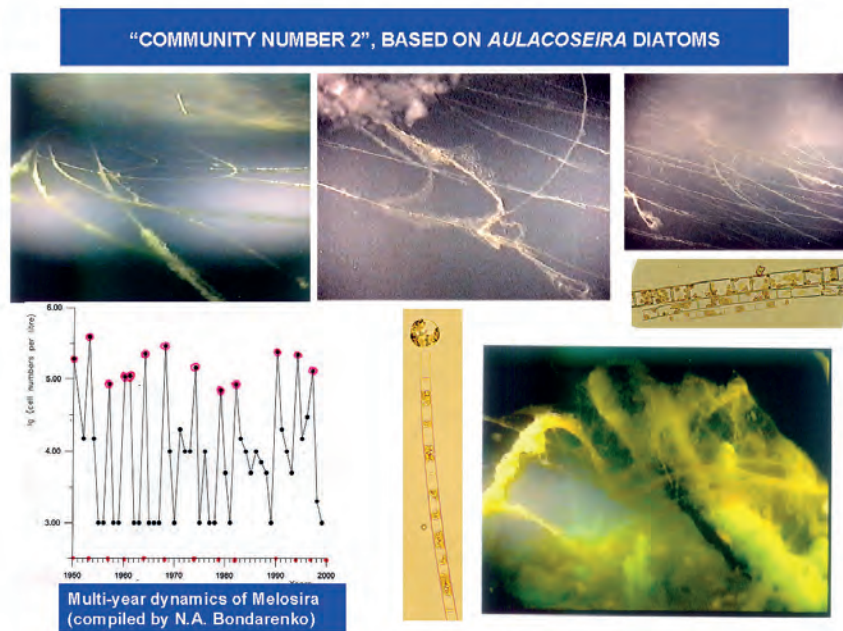


Fig. 13. “*Melosira cordial rhythm*” in Baikal pelagial; graph in the left lower corner (compiled by Dr. Bondarenko N.A.); three upper photos: “ice community No 2” – under-ice filaments, mostly consisting of *Aulacoseira baikalensis*; intermediate narrow photos – light microscopic images of *Aulacoseira* sp.; *Lubomirskia baikalensis* branches, covered by the merged down to the bottom ice algal filaments, right lower corner (after Timoshkin et al., 2009).

of *Acanthogammarus* genus. All types of organochlorine pesticides, found in Baikal waters so far, were also detected in the gammarid bodies. Some of the specimens analyzed demonstrate the highest values of pesticides ever detected in Baikal fauna: DDT metabolites – over 1200 ng/g of lipids; PCB (polychlorinated biphenyls) – over 4000 ng/g of lipids. According to our unpublished data, *Acanthogammarus* sp., which was analyzed, belongs to hunters and/or necrophages (top level in the trophic chains). This factor may determine such a high pollutant concentrations in their bodies.

Besides measurement of POP and other pollutant concentrations in the waters, soils, sediments, we have to

elaborate the list of indicative coastal Baikalian algae and invertebrates which are able to accumulate and concentrate these pollutants and promote this species list as a basement for the regular optimized monitoring scheme.

6. How the stable hydrochemical status of the water column may correlate with “casual” understanding of the term “eutrophication”?

Eutrophication of average lake, in common sense, usually characterized by increasing the trophic

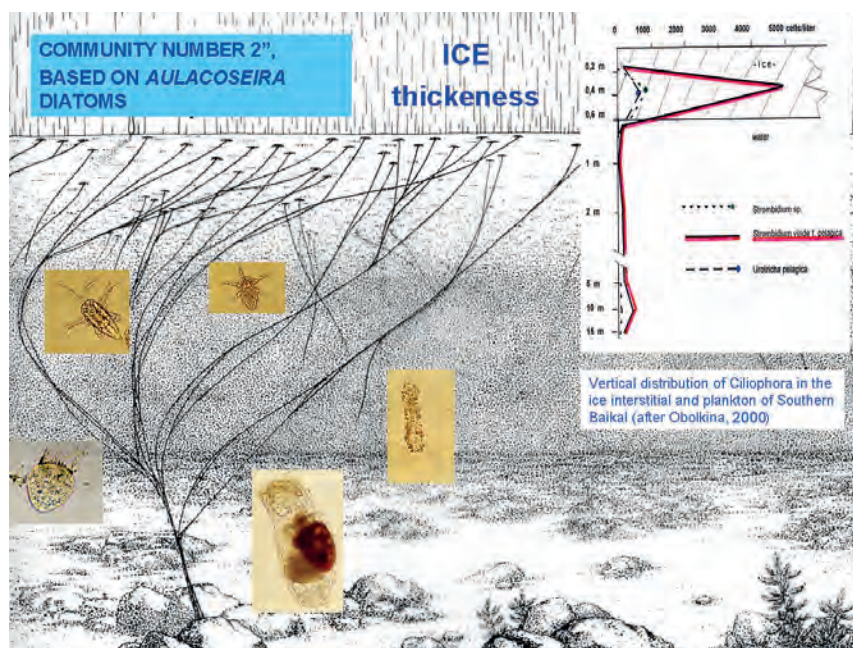


Fig. 14. “Ice community No 2” – under-ice filaments, mostly consisting of *Aulacoseira baikalensis*; reconstruction made by help of underwater video records, taken on the depth ca. 3 m, Berezovy test-site, spring 2001 (after Timoshkin et al., 2009). Upper graph from the right: vertical ciliophora distribution in the ice interstitial waters (after Obolkin et al., 2000; Obolkin, 2003).



Fig. 15. "Ice community No 1". Upper and right photos – ball- and finger-shaped cavities within the ice thickness, filled up by *Gymnodinium* cells (after Timoshkin et al., 2009). Light-microscopic images of the algal shells (photos by Bondarenko N.A. in the middle) and the *Gymnodinium* distribution inside the ice thickness, left lower corner (data of Bondarenko N.A.).

status of the water body due to nutrient enrichment, elevated nutrient concentrations in water column, lowering of transparency and dissolved oxygen concentrations due to increased abundance of phyto- and zooplankton, mass development of planktonic blue-greens, etc. Negative ecological processes observed in Lake Baikal do not fit in with the commonly accepted picture of "casual eutrophication": most hydrochemical parameters (including the nutrient concentration, dissolved oxygen, transparency) of the main water masses are stable; the trophic status, in general, has not been changed (yet). Therefore, many scientists do not believe that the eutrophication in Baikal is currently in progress – they just keep in mind the standard "eutrophication picture", described above. Nonetheless, the deep degradation of the coastal zone communities is evident (see above) and to my deep believe, mainly caused by eutrophication. *The following main "eutrophication sources" can be distinguished with regard to Baikal ecosystem:* "casual eutrophication" of the inlets and near shore settlement waters (1), contamination of the ground waters and the interstitial waters of the splash zone (2) by sewages; secondary contamination due to algal wash ups decaying (3); mass extinction of endemic Lubomirskiidae sponges (4); numerous coastal taiga fires (5); aerosol contamination (6).

7. Landscape-ecological approach and the universal scheme of the great lakes monitoring

Karabanov E.B. was the first researcher, who introduced the landscape-ecological approach into Baikal limnological survey (Karabanov et al., 1990). He elaborated the landscape-ecological zonation of entire Lake Baikal, which included 61 bottom landscapes and

11 physical-geographical regions of the water body. The most species-rich coastal zone was subdivided into eight landscapes only. This zonation provided reasonable scientific basement for establishment the set of standard test-sites and transects for regular observations. Following his ideas and considering landscapes as universal units of natural organization and as a fundamental basis for standardized monitoring schemes, Timoshkin et al. (2005) elaborated and proposed the universal scheme of the great lakes monitoring based on the landscape-ecological approach. Because the anthropogenic impacts may influence biota at all levels of organization (communities → populations → organisms → cells → molecules), biotic monitoring should cover each level. In practice, methods used commonly to investigate community and population structure, dynamics of dominant species, teratic species and genetic diversity, including chromosome aberrations, should be applied. Following this landscape zonation, we have established 8 standard transects along to the coastal zone of entire lake and performed more or less regular investigations since 2004. Detailed description of the monitoring scheme and main results are given in Timoshkin et al. (2009) and partly presented here.

8. Conclusion

Coastal zone of the large lakes surely deserves the special attention of limnologists. The gap of our knowledge on the ecology of this zone should be covered as soon as possible.

The uniqueness of Lake Baikal makes the ongoing eutrophication different from all other Palearctic lakes. Therefore, the hydrochemical and other indicators of the water column of great lakes do not match the principal eutrophication criteria pool. Biological

indication approach in the coastal zone appears more appropriate for the prompt detection and analysis of the initial eutrophication stages in the large lakes. Obviously, the pelagic zone and pelagic communities of the giant lakes are much more conservative, stable and quite resistant to anthropogenic impact as compared to the coastal zone.

The governmental monitoring schemes of the large lakes of the planet are often not effective due to “superconcentration” of the efforts exclusively on the pelagial. In order to detect, understand the reasons and properly describe the anthropogenic changes of the ecosystems at the full scale we have to pay a special attention to the monitoring of the coastal zone (the splash and near-shore zones including) and, especially – to the benthonic communities. As distinct of plankton, the precise investigations and monitoring of zoobenthos is almost “extinct direction” in limnological surveys of many countries.

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