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EXPERIMENTAL ARTICLES

Ecology of Purple Sulfur Bacteria in the Highly Stratified Meromictic Lake Shunet (Siberia, Khakassia) in 2002–2009

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Abstract—Phototrophic sulfur bacteria form dense accumulations in the chemocline zones of stratified lakes where light reaches the sulfide-containing layers of water. Many works are dedicated to the ecophysiology of these microorganisms in meromictic lakes. However, the role of these microorganisms in the trophic network of these ecosystems, the ways of biomass utilization, and the contribution to the turnover of biogenic elements have so far been insufficiently understood. This work deals with the analysis of many years' seasonal dynamics of the biomass of purple sulfur bacteria and the physicochemical conditions of their environment in Lake Shunet (Siberia, Khakassia, Russia), unraveling the causes of their anomalous development in the chemocline of this lake, as well as the comparative analysis of such type of ecosystems. Lake Shunet is characterized by markedly pronounced stratification and the high density of purple sulfur bacteria (PSB) in the chemocline, which is comparable to that of Lake Mahoney (Canada) where the number of PSB is the greatest among those known in the world. It was shown that, in the period 2002–2009, the total amount of bacteriochlorophyll *a* in the water column of Lake Shunet increased and did not correlate with the seasonal variations in temperature and illumination in the chemocline. It was established that PSB cells in the purple layer experienced the effect of self-shading. The sedimentation rate of purple sulfur bacteria in Lake Shunet was low due to the pronounced density gradient in the chemocline zone. Thus, the high number of PSB in the chemocline was due to the combination of strong illumination, a high sulfide concentration, and a high water density gradient, which was responsible for stable stratification and contributed to the accumulation of the cells in a narrow layer. The data obtained could be useful for the paleoreconstruction of climatically determined changes in the level of the lake and its periods of meromixis by the presence of carotenoids and bacteriochlorophylls in the bottom sediments.

Keywords: purple sulfur bacteria, chemocline, seasonal dynamics, meromictic lake, bacteriochlorophyll *a*
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The ecophysiology of phototrophic sulfur bacteria is sufficiently well-studied in a number of freshwater and saline reservoirs [1–3]. Phototrophic sulfur bacteria are especially important as ecosystem components in meromictic reservoirs, where the dense populations of these microorganisms in the chemocline often survive even under the ice. The importance of investigations of this group of bacteria in aquatic ecosystems stems from the fact that the pathways of utilization of their biomass and its role in the trophic network are insufficiently understood [3]. Moreover, the molecular remnants of anaerobic phototrophic organisms (pigments and DNA) buried in the bottom sediments serve as an indicator of the past stratified states of the lakes [4, 5] and, consequently, may be useful for the reconstruction of the history of these lakes and the paleoclimate. However, such reconstruction of the history of a reservoir using the bottom sediments requires the knowledge of the state of its phototrophic community based on many years' observations and analysis of the conditions of habitation of the relevant groups of phototrophic organisms.

Meromictic lakes are relatively few, and only three reservoirs of this type are known in the vast Asian part of Russia: Lake Shira and Lake Shunet (Khakassia), as well as Lake Doroninskoe (Transbaikal krai). This small number may result from the fact that this territory remains insufficiently explored.

Lake Shunet merits special attention as an ecosystem in which the number of anoxygenic phototrophic bacteria in the chemocline is extremely high (over 10^8 cells mL^{-1}) and the purple layer consisting of purple sulfur bacteria (PSB) is formed [5]. This lake is characterized by a sharp salinity gradient providing for stable meromixis [6].

The community of anoxygenic phototrophic bacteria (APB) inhabiting the chemocline of this lake was characterized in a number of works. It was shown that two morphotypes of PSB were present in Lake Shunet. The first morphotype was morphologically and phylogenetically close to *Lamprocystis purpurea* [7, 8]. The

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second morphotype was morphologically close to *Lamprobacter modestohalophilus* and phylogenetically close to the strains of the genus *Halochromatium* [7]. Both species contained bacteriochlorophyll *a* and carotenoids of the okenone series as the main pigments. Only one strain of green sulfur bacteria (GSB) was isolated; it was phylogenetically most closely related to the type strain of *Prosthecochloris vibrioformis* and contained bacteriochlorophyll *d* and the carotenoid chlorobactene as the main photosynthetic pigments [7]. However, PCR/DHHE revealed that, in particular, from May to September 2005, another phylotype having only 97% of similarity to the above-mentioned one dominated in the chemocline of Lake Shunet [8]. The spatial distribution of APB was described for some dates [6, 7, 9, 10, 12]. In addition, the rates of anoxygenic photosynthesis and sulfate reduction rates were assessed in winter and summer of 2002–2003 [9, 10].

This work is dedicated to the analysis of the many years' seasonal dynamics of the biomass of purple sulfur bacteria and the physicochemical conditions of their habitat in Lake Shunet, unraveling the causes of their anomalous development in the chemocline of this lake, as well as to comparative analysis of the ecosystems of such type.

MATERIALS AND METHODS

Description of the lake. Lake Shunet (54°25′10″N, 90°13′48″E) is situated in the Republic of Khakassia (South Siberia) 19 km away from the settlement of Shira and 8 km to the southeast of Lake Shira. The maximal length is 1.2 km; the maximal width, about 0.4 km; the surface area, 0.47 km^2; the maximal depth in the 2003–2009 was 6.2 m. In 2002, the level of the lake was 0.3–0.4 m lower as follows from the visual assessment of the shoreline. The lake is without drainage and has a markedly pronounced density stratification in all the seasons, which determines its meromictic properties. Mineralization in the mixolimnion is about 17–20 g L^{-1}. Beginning from the depth of about 4.5 m, mineralization uniformly increases towards the bottom reaching ~100 g L^{-1} near the bottom. The chemocline defined as the zone where the redox potential sign changes from positive to negative, is located at the depth of about 5 m. The sulfide content in the bottom layers is as high as 450 mg L^{-1} [12]. Lake Shunet freezes at the beginning of November and gets free of ice in April. A detailed description of the lake is given in a number of works [7, 8, 11].

Sampling. Sampling was carried out in the central part of the lake in the region of its maximum depth, at the point with the coordinates 54°25′156″N, 90°13′853″E in windless weather. The investigations were carried out from 2002 to 2009, except for 2006, when field works were not carried out. In the remaining years, sampling and the concomitant measurements of the physicochemical characteristics of the water were carried out every season four times a year. In order to assess the daily dynamics in summer 2005, sampling from the chemocline of Lake Shunet was carried out five times over 24 h at 6 h intervals. In the under-the-ice period in 2008, the investigations on both lakes were carried out twice (in February and in March).

In the winter season, sampling and the concomitant measurements were carried out through a hole drilled in the ice. Sampling from the chemocline zones was carried out at an interval of 5 cm using a multisyringe stratification sampler [13] as described in [8]. Water from other depths was sampled with the standard 1.5-L bathometer.

Physicochemical characteristics. Prior to sampling, the vertical profiles of temperature, turbidity, conductivity, the redox potential, and dissolved oxygen were measured using submerged multichannel probes Data-Sonde 4a (Austin, Texas, United States) and YSI 6600 (Yellow Spring, Ohio, United States).

The sulfide concentration was determined using the Microquant colorimetric test kit (Merck, Germany). At higher concentrations, the samples were fixed with zinc subcarbonate, and the sulfide concentration was determined by the iodometric method [14]. The vertical profile of underwater illumination was measured with an LI-193 spherical submersible sensor of photosynthetically active radiation (PAR) (LI-COR Ltd., Nevada, United States). All the illumination values were normalized to the standard surface illumination 1500 μE m^{-2} s^{-1} [12]. The water density was measured with an areometer in thermostatically controlled water samples at different temperatures in the laboratory.

Microbial numbers. The bacteria were counted by staining with the DAPI fluorochrome on membrane filters according to the standard method described earlier [8]. The green sulfur bacteria were counted on the same filters by bright-field microscopy in reflected light [8, 15].

Analysis of the pigments was carried out spectrophotometrically in the acetone extracts according to the standard method described in [8, 16]. Bacteriochlorophyll *a* was identified by the presence of the absorption peak at 772 nm [17]. In those cases when bacteriochlorophyll *a* was not measured, its concentration was assessed by the number of PSB using the coefficient $(2.65 \pm 0.66) \times 10^{-14}$ g Bchl *a* $cell^{-1}$ derived fromour data on the number of PSB and the Bchl *a* concentration for the samples of 2008 (February, July, and October) and 2007 (March).

Sedimentation rate. The sedimentation flow of PSB was assessed in July 2009 with sedimentation traps, which are plastic cylinders with a sectional area of 12.6 cm^2 and a volume of 201 cm^3, open at the upper end, submerged at the depths of 5.2, 5.5, and 6 m in a rigid metal framework. The traps were arranged so that the upper cylinders did not overlap the lower ones.

Four traps were exposed at each depth for 23 days. After exposure, the PSB cell concentration in each trap was calculated by two methods: (1) from direct counts of the DAPI-stained cells on polycarbonate filters under a fluorescence microscope and (2) from the bacteriochlorophyll *a* concentration in a trap. The number and the concentration values were compared with the corresponding values in the water on the corresponding horizons. The cell sedimentation rate (cm day^{-1}) was calculated with the formula:

$$v = \frac{\Delta X}{X^0}\left(\frac{V}{S}\right)\frac{1}{\Delta t},$$

where ΔX is the bacterial concentration in a trap (cells mL^{-1}), X^0 is the bacterial concentration in the water at the same depth (cells mL^{-1}), V is the trap volume (cm^3); S is the trap area (cm^2); and Δt is the exposure time of the traps in the lake (days) [18]. The X^0 value was measured twice: at the beginning and at the end of exposure. Since the values were close, the average values were used in the calculations.

Rate of anoxygenic photosynthesis. In 2004, the rate of inorganic carbon assimilation in the dark and in the light was measured with the radioisotope method using $NaH^{14}CO_3$ according to the standard method [7].

Calculations. The surface solar radiation values were calculated for the geographical coordinates of Lake Shunet using the Solrad.xls calculator (G. Pelletier, Washington State Department of Ecology, Olympia, WA) considering the altitude above sea level and assuming that the weather was always fine. The calculated values correlated well with those measured earlier in fine weather for the corresponding dates. The daily dynamics of surface solar radiation was averaged every eight days; the share of PAR was taken to be 48% of the total solar radiation; the share of reflected radiation was taken as 1% [19]. The under-the-ice period was assumed to last from November 9 to May 1 according to the averaged results of field observation. The vertical attenuation coefficients K_d for the water column were calculated in our previous work using the PAR profiles measured for Lake Shunet at different time with an LI-193SA submerged sensor (LI-COR Ltd., Lincoln, Nevada, United States) [12]. The vertical attenuation coefficients for ice were calculated based on the same in situ observations and the ice thickness values measured during field expeditions. The dynamics of the ice cover thickness was calculated using the one-dimensional mathematical model developed for Lake Shira [20]. The calculated ice thickness values correlated well with those measured for Lake Shunet at the corresponding dates; therefore, we applied this calculation for Lake Shunet as well. The specific carbon content value in the cell volume 1.21×10^{-13} g C μm^{-3} was used to convert the number of PSB cells to carbon [21], and the average cell diameter was taken as 2 μm; the volume was calculated using the formula for a sphere. The vertical sulfide flow into the chemocline zone was calculated according to Fick's law proceeding from the concentration gradient using the coefficient of molecular diffusion for sulfide $D = 1.52 \times 10^{-5}$ cm^2 s^{-1} [22].

RESULTS

Vertical structure and meromixis. Throughout the period of investigation, the water column of the lake retained stable chemical stratification (Fig. 1). In the bottom layers, beginning from a depth of about 4.5 m (August 2003–2011), the concentration of dissolved salts sharply increased, attaining at the bottom the maximal values about 90–100 g L^{-1}. The vertical distribution of salinity in the depth range from the surface to 4.5 m varied depending on the season, and the concentration varied between 10 and 25 g L^{-1} (Fig. 1). The salinity in the lower part of the water column hardly changed during the seasons indicating the absence of full circulation of water in Lake Shunet. The sharply heterogeneous distribution of dissolved salts combined with the invariably high sulfide concentration in the bottom layers indicated the meromictic character of Lake Shunet during the period of observation. Thus, the water column of the lake was divided into the aerobic mixolimnion and the anaerobic monimolimnion.

The seasonal dynamics of salinity and temperature in the mixolimnion was significantly affected by the processes of formation and thawing of the ice cover. The salinity in the mixolimnion in the under-the-ice period was at the peak due to the partial release of dissolved salts from the freezing water. At this time, the uniform distribution of salinity (conductivity) and temperature was observed from the surface to the depth of 4 m (depth of the halocline location), which suggests convection during the period of autumnal mixing and freezing over (Fig. 1). In spring, the salinity in the upper 1–2 m noticeably decreased due to ice thawing. Later, upon heating, the warm epilimnion was formed, and its depth gradually increased in summer due to turbulent mixing. Salinity in the epilimnion increased gradually during summer due to mixing with the underlying, more saline layers (Fig. 1). By midsummer, a weakly pronounced thermocline was established at the depth of about 3 m. In autumn, upon cooling, mixing continued until the profiles from the surface to a depth of 4 m became absolutely uniform.

Thus, the mixolimnion of Lake Shunet was monomictic during the period of investigation and underwent full circulation in autumn. No circulation was observed in spring due to the desalination of the upper water layers resulting from the thawing of the ice crust. A similar dynamics of vertical distribution of temperatures and salinity was described for Lake Shira [23].

The redox zone (the boundary between the oxygen- and sulfide-containing water layers) determined from

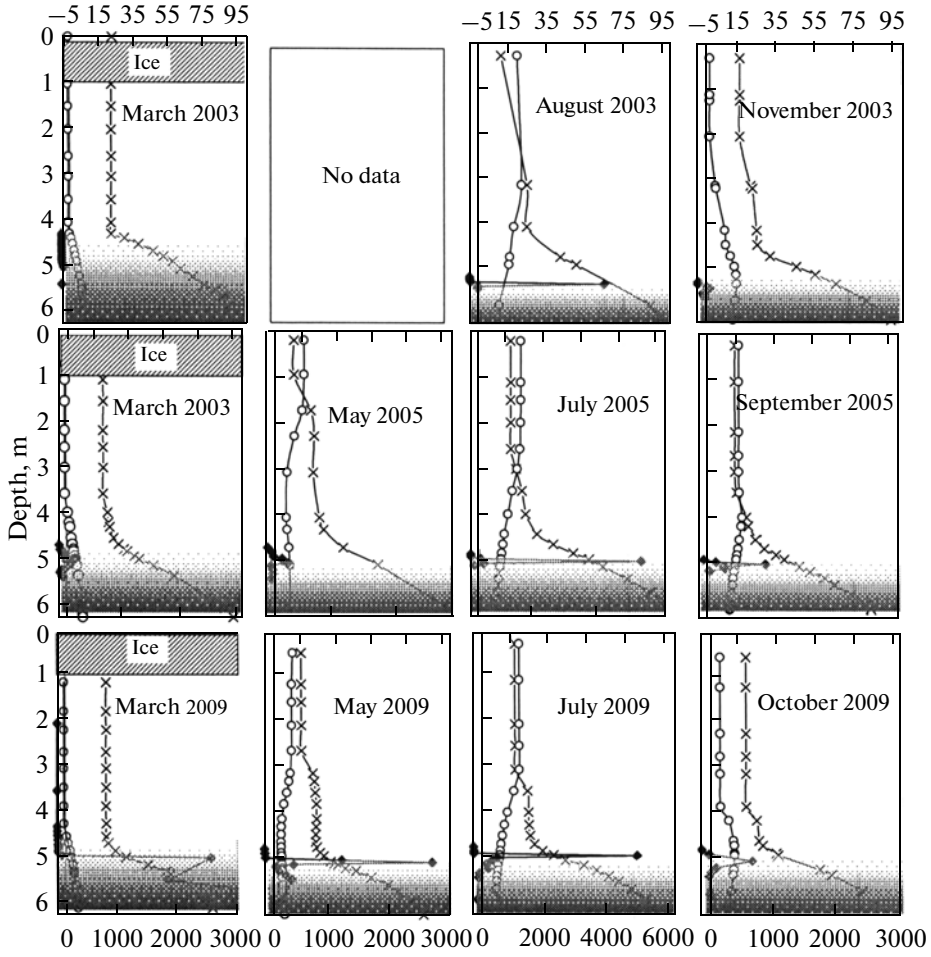

Fig. 1. Seasonal dynamics of vertical distribution of the physicochemical characteristics of Lake Shunet and the bacteriochlorophyll *a* concentrations in different years. The anaerobic zone is designated by gray shading. Temperature, °C (○); conductivity, mS cm^{-1} (×); and Bchl *a*, μg L^{-1} (◆).

the change in the redox potential sign from positive to negative was localized in summer at the depth of 4.8–5.2 m. In March 2003, the redox zone boundary was recorded to rise to 4.3 m; in March and October 2007, it was located at 4.5 m. In most cases, the position of the redox zone coincided with the point of the maximal water density gradient (Fig. 1). However, the redox zone never rose to the lower boundary of the mixolimnion determined as the lower boundary of the uniform distribution of salinity and temperature. The depth of the location of the redox zone in Lake Shunet was therefore determined not only by mixing of the mixolimnion but also by the balance of the redox processes occurring in this zone. These processes include bacterial photo- and chemosynthesis, as well as chemical oxidation by the oxidizing agents dissolved in water (oxygen, iron, etc.).

The dynamics of the PSB biomass. The PSB number peaked in the chemocline of Lake Shunet, in all the seasons forming the so-called purple layer (Fig. 2). This layer reached the highest development during the open water periods and was situated in the redox zone within a depth range of about 5 cm. The water samples from this layer had intense purple coloration. While the multisyringe sampler was not used in July 2002, a sharp turbidity peak at the redox boundary (not shown in the figures) recorded by the submerged multichannel probe indicated the presence of the purple layer at the depth of about 5 m in 2002.

During under-the-ice periods of 2002–2003 and 2003–2004, the PSB number in the chemocline decreased below the threshold visible with a naked eye, which we estimated at about 10^7 cells mL^{-1} (Figs. 1, 2). In March 2003, the number of PSB in the chemocline was about $0.5–1 \times 10^6$ cells mL^{-1}; however, the multisyringe sampler positioned in the redox zone probably did not sample the lower PSB maximum, since the redox zone rose to 4.3 m, i.e., 0.7 m higher than in the previous summer (Fig. 1) (see Discussion).

Beginning from 2004, the purple layer persisted during the under-the-ice periods, although it was dis-

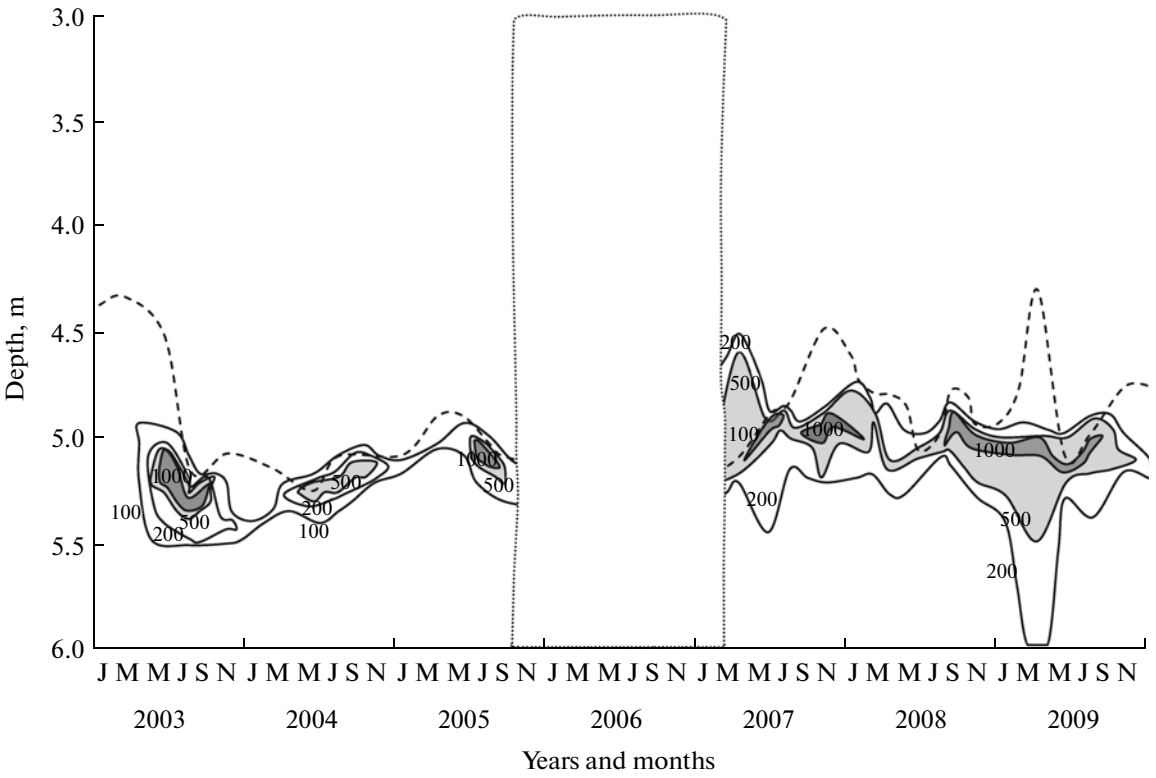

Fig. 2. Dynamics of the PSB abundance in Lake Shunet. The isopleths are expressed in μg Bchl *a* L^{-1}; the broken line shows the position of the redox zone.

tributed within a broader depth interval than in the preceding summer periods and the peak number of PSB was about an order of magnitude lower than in summer (Fig. 2). In some years, the integral amount of Bchl *a* under 1 m^2 assessed for the interval from the upper boundary of the chemocline to 5.5 m did not undergo the regular seasonal dynamics (Fig. 3). In 2002 and 2003, the summer values of the integral amount of Bchl *a* were significantly higher than the winter values (Fig. 3), which was also confirmed by the visual assessment of the color of the samples. Thus, until 2004, the standard seasonal dynamics with a summer maximum and a winter minimum was observed (Fig. 3). From 2004 until now, the winter value of the integral amount of Bchl *a* under 1 m^2 was never significantly lower than the summer value, which was also supported by visual observations. Moreover, no correlation was observed between the integral amount of Bchl *a* under 1 m^2 and either temperature or illumination. Thus, despite significant errors, we can assert that the regular seasonal dynamics tended to disappear from 2003 to 2009 and there was a trend for the integral amount of Bchl *a* to increase in the depth range studied (Fig. 3).

Diurnal dynamics of PSB. In order to reveal the diurnal dynamics of APB, sampling was carried out with a multisyringe sampler on June 26–27, 2005. No significant changes in the vertical distribution of PSB were recorded.

Assessment of the sedimentation rates of purple sulfur bacteria. In order to assess the flow of the cells of phototrophic sulfur bacteria from the photic zone into the bottom horizons, the sedimentation rate of bacteria was assessed in summer 2009 at different depths by the method of sedimentation traps.

In most cases, the sedimentation rate values assessed by the Bchl *a* concentration were higher than those assessed by cell count, which was probably a result of the destructive processes occurring in the traps during their exposure. Occurrence of destructive processes was also confirmed by the fact that the main Bchl *a* absorption peak in the sedimentation material of the traps shifted from 772 to 759 nm, which indicated the presence of bacteriopheophytin *a*, the product of bacteriochlorophyll *a* degradation [24]. The low sedimentation rate of PSB in Lake Shunet (about 0.1 cm day^{-1}) may probably be explained by a high water density in the monimolimnion.

Assessment of the production characteristics of PSB in Lake Shunet. Proceeding from the previously determined rates of CO_2 assimilation in the purple layer of Lake Shunet in the light (1600 mg C L^{-1} day^{-1}) [6, 7], the specific growth rate of PSB would be ~0.02 day^{-1}, meaning the duplication time of 33 days.

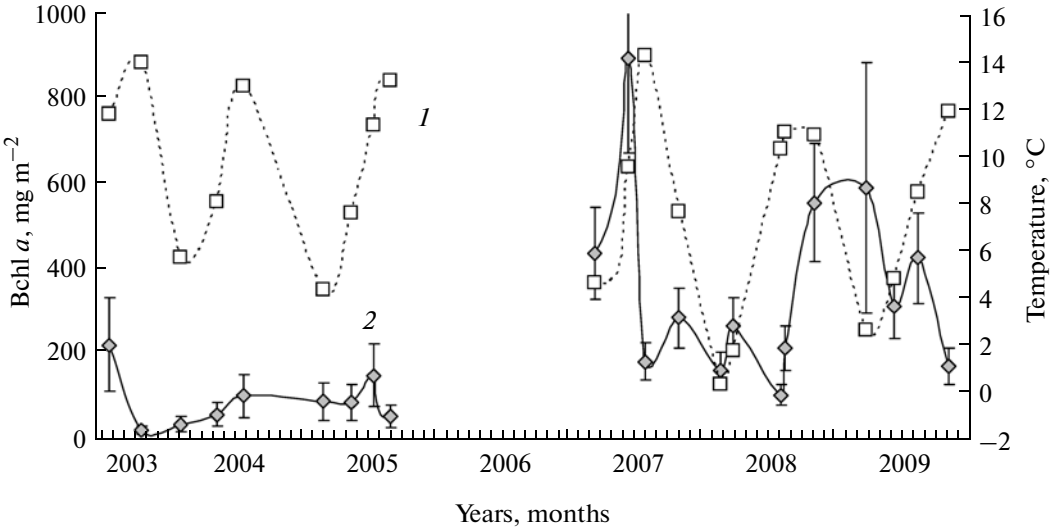

Fig. 3. Dynamics of the integral amount of bacteriochlorophyll *a* and temperature in the chemocline of Lake Shunet. Bchl *a* (*1*) and temperature (*2*).

This estimate was made assuming that all PSB cells in the purple layer divided at the same rate.

In August 2004, in situ incubation of the vials was carried out not at the sampling horizons, but 0.3 m closer to the surface, thus increasing PAR intensity by approximately 20% compared to the intensity at the upper boundary of the purple layer, assuming the vertical light attenuation coefficient value determined for the water column of Lake Shunet ($K_d = 0.28$ m^{-1}) [12]. In this case, the carbon assimilation rate in the light increased to 2240 mg C L^{-1} day^{-1}. The number of PSB in the chemocline was, however, approximately 5 times lower than in 2003. Accordingly, the specific growth rate in the purple layer estimated by carbon increased to 0.14 day^{-1} and the duplication time decreased to five days, i.e., 7-fold compared to 2003. This indicates that PSB in the purple layer of Lake Shunet were limited in light energy, although the PAR intensity at the upper boundary of the purple layer exceeded 50 μE m^{-2} s^{-1}, which exceeded the saturating value for this PSB species [21]. Consequently, the PSB present in the purple layer in August 2003 probably experienced the self-shading effect. At the same time, the cells at the upper boundary of the layer were probably limited by sulfide deficiency as was shown for Lake Mahoney by Overmann et al. [21].

Sedimentation rates of purple sulfur bacteria in Lake Shunet

Depth, m	Sedimentation rate, cm day^{-1}	
	By the number of PSB	By the Bchl *a* concentration
5.2	0.160 ± 0.080	0.07 ± 0.02
5.5	0.006 ± 0.006	0.10 ± 0.01
5.8	0.004 ± 0.002	0.14 ± 0.02

Using the coefficient value $K_b = 0.050$ m^2 (mg Bch *a*)$^{-1}$ of PAR attenuation for the purple bacterium *Lamprocystis purpurea* [21], it was possible to calculate the integral number of PSB under 1 m^2 required for attenuation of the quantum flow falling on the surface of the purple layer to the minimal value supporting PSB growth, i.e., to 0.4 μE m^{-2} s^{-1} [25]. According to the Beer–Lambert law,

$$I_L = I_T \exp\{-K_b B\}, \quad (1)$$

where I_L is the lower limit of PAR quantum flow (0.4 μE m^{-2} s^{-1}); I_T is the PAR quantum flow incident onto the upper boundary of the purple layer; K_b is the coefficient of PAR attenuation by *Lamprocystis purpurea* cells (m^2 mg Bchl *a*$^{-1}$); and B is the integral amount of Bchl *a* under 1 m^2 (mg Bchl *a* m^{-2}). If approximately 50 μE m^{-2} s^{-1} reaches the purple layer in the summer time [12], it then follows from (1) that $B = 96.6$ mg Bchl *a* m^{-2}. The amount of Bchl *a* measured in Lake Shunet in summer always exceeded this value, and the highest value (480 mg Bchl *a* m^{-2}) was recorded in June 2007 [12]. Hence, it may be concluded that in summer, only a portion of the PSB population lives under the conditions sufficient for generative growth; in particular, in June 2007, this part was about 20%.

Assessment of the under-the-ice production of photosynthesis by PSB. The share of under-the-ice production in the annual cycle was estimated assuming that light deficiency was the main factor limiting the growth of anoxygenic phototrophic bacteria in the lake chemocline [2, 21]. Decreasing temperature was

the second essential factor during the under-the-ice period.

The seasonal dynamics of PAR intensity at the chemocline depth during the year was estimated on the basis of seasonal dynamics of PAR at the surface and the absorptive properties of ice and the water column. For comparison, it was sufficient to consider a simplified situation when the depth of the chemocline position remained unchanged, there was no snow in winter, and the weather was cloudless all year round. The chemocline depth of 4.9 m throughout the year was accepted.

Importantly, even the minimal illumination value recorded in the chemocline of Lake Shunet (0.97 μE m^{-2} s^{-1}) with a solid snow blanket above the ice crust was sufficient for generative growth of PSB.

The calculated annual dynamics of the PAR quantum flow onto the upper chemocline boundary for the hypothetical case of an absolutely snowless winter, i.e., for the situation when under-the-ice illumination is at its maximum, is shown on Fig. 4. In Lake Shunet, the integral amount of PAR quanta throughout the under-the-ice period was only ~7% of the total annual amount; hence, the maximal share of photosynthetic production in the chemocline of Lake Shunet during the under-the-ice period must not exceed 7% of the annual value. Its real share should be still less due to a significant decrease in temperature in the chemocline of Lake Shunet, resulting in decreased rates of all microbial processes.

DISCUSSION

The dense populations of anoxygenic phototrophic bacteria in the chemocline of stratified lakes are a characteristic and sufficiently well-investigated phenomenon [1, 3, 16, 21, and many others]. In most reservoirs, the maximal cell number is normally 10^5–10^7 cells mL^{-1}. The growth of PSB is usually limited by the insufficient amount of light reaching the chemocline [2, 26]. In Lake Shunet, the number of PSB is extremely high, an order of 10^8 cells mL^{-1}; this lake may be therefore considered a unique object. A similar phenomenon has been recorded only in one other lake, the Canadian Lake Mahoney, which, in the opinion of its investigators, is the world's record holder in the PSB biomass in the chemocline [3]. Importantly, the PSB species, which is phenotypically and phylogenetically close to the dominant species in Lake Shunet, dominates in Lake Mahoney as well. Thus, it seems to be expedient to compare the two lakes from the point of view of the conditions of habitation of PSB.

In both lakes, the chemocline is located at a relatively low depth: 6.7 and 5 m for Lake Mahoney and Lake Shunet, respectively. The intensity of PAR at the upper chemocline boundary in both reservoirs is therefore relatively high, about 50 μE m^{-2} s^{-1} [3, 12], and at the uppermost boundary of the purple layer the cells do not experience limitation by light throughout the period of open water. However, as was shown above, the cells forming the purple layer in both lakes are under conditions of severe self-shading so that about 10% of the cells in Lake Mahoney (according to Overmann's estimates [3]) and not more than 20% of the cells in Lake Shunet (according to our estimates) were able to carry out generative growth. Obviously, the specific rates of photosynthesis assessed by the vial method are the averaged values and do not reflect the real distribution of physiological activity in a dense population.

The level of sulfide, the second factor necessary for photosynthesis, was approximately the same in both reservoirs. In Lake Shunet, the flow of S^{2-} from the bottom horizons into the zone of PSB development was about 90 mmol S m^{-2} day^{-1} in all the seasons, even not considering sulfate reduction in the chemocline itself. In July 2002, the sulfate reduction rate in the chemocline was approximately 1.6 mmol S m^{-2} day^{-1} (estimated using the data of Kallistova et al.) [10]. The rate of photosynthesis within the chemocline zone calculated on the basis of the data for August 2003 was about 24 mmol C m^{-2} day^{-1}. Based on the generalized equation of anoxygenic photosynthesis:

$$H_2S + 2HCO_3^- = 2[CH_2O] + SO_4^{2-}$$

the sulfide flow exceeded the demand for anoxygenic photosynthesis more than 7-fold (according to the estimates made for August). In Lake Mahoney, the PSB cells also experienced no sulfide deficiency in all the seasons, except the beginning of summer when the photosynthesis was the most intense [3]. Thus, it can be asserted that the flows of light and sulfide into the zone of active PSB development were sufficient to provide for the maximal rate of cell growth in spring and in summer if it were not for the density-dependent effects leading to the greater part of the population being deeply limited in light and, possibly, in sulfide at increasing cell concentrations.

In turn, the low elimination rate of the cells from the purple layer contributes to the increase in their abundance. As it was shown for the related species *Amoebobacter purpureus* inhabiting Lake Mahoney, the sedimentation rate of the PSB cells is very low due to the fact that the cells have the capacity for light-dependent regulation of density (buoyancy) by varying the volume of gas vesicles and the intracellular carbohydrate content [3]. Research on pure cultures showed that the cells were capable of a 9-fold increase in the volume of gas vesicles when they appeared under the dark conditions [27]. In Lake Mahoney, the density of cells with high content of gas vesicles was about 1.002 g/mL, which was significantly less than the water density in the chemocline (1.015 g/mL). In Lake Shunet, the water density at the upper chemocline boundary, where the purple layer is local-

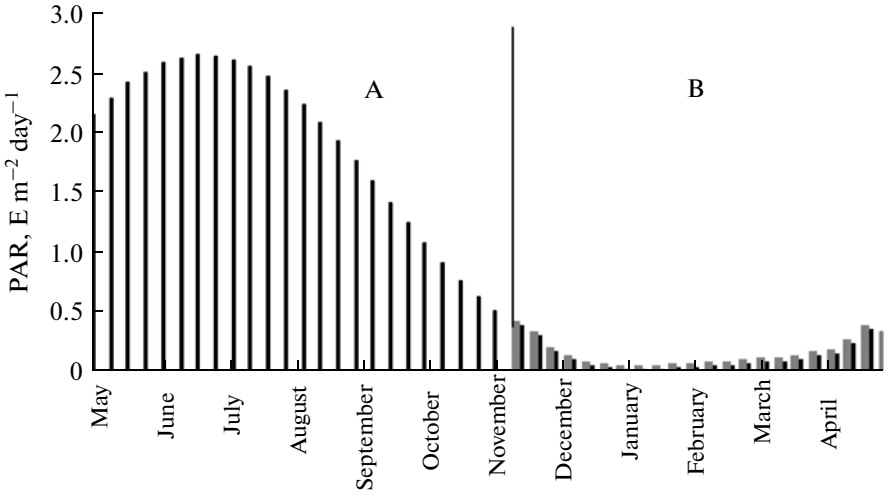

Fig. 4. Seasonal dynamics of the calculated flow of photosynthetically active radiation (PAR) reaching the upper boundary of the Lake Shunet chemocline in fine weather and in the absence of snow on ice in the open water period (A) and in the under-the-ice period (B).

ized, is about 1.04 g/mL and sharply increases with depth reaching the values about 1.08 g/mL near the bottom. Evidently, the high water density slows down the process of cell sedimentation. For example, sedimentation rate of the PSB cells in Shunet (about 0.1 cm day^{-1}) was an order of magnitude lower than in the neighboring Lake Shira, where the water density in the chemocline zone was about 1.02 g/mL and increased at the bottom to 1.03 g/mL [23].

The hydrophysical stability of the water masses near the chemocline is another factor of accumulation. Since the uniform vertical distribution of salinity and temperature is an indicator of the mixing of the water column, it becomes evident that the water layers deeper than 4.5 m were hardly subjected to the processes of mixing during the open water period (Fig. 1). Consequently, the processes of passive transfer of PSB cells caused by the turbulence of the water masses were insignificant in the period of the most vigorous development of PSB.

An additional factor contributing to the accumulation of PSB in the chemocline is their inaccessibility to grazing by zooplankton due to high ambient sulfide concentrations. It was shown that the crustaceans *Arctodiaptomus salinus* dominating in the zooplankton of Lake Shunet lost their activity and died when they arrived to the purple layer zone (Tolomeev, unpublished data). However, under laboratory conditions, these crustaceans could consume the cells of a pure PSB culture washed off sulfide (Tolomeev and Rogozin, unpublished data).

Considering the low sedimentation rate, we may suggest that the major part of the PBS biomass should be utilized above the chemocline zone. Mass upwelling (floating up) of PSB to the aerobic zone and development of cell aggregates as flakes on the surface was shown to occur in Lake Mahoney in autumn when homothermy of the mixolimnion results if its decreased hydrophysical stability. Their biomass is then utilized in the process of aerobic degradation resulting in increased numbers of heterotrophic bacteria [3]. It is possible that a similar phenomenon also takes place in Lake Shunet; however, we did not succeed in recording it due to its short-term character. Nevertheless, on October 9, 2011, aggregated flocculent masses of PSB were observed in the water samples from the chemocline of Lake Shunet (Zadereev, personal communication), probably of the same nature as in Lake Mahoney. The presence of upwelling of PSB may be suggested by analogy with Lake Mahoney, because appreciable decreases in the number of PSB were observed in Lake Shunet in autumn 2003 and in winter 2004 compared to the preceding summer periods (Fig. 2). Thus, utilization of the biomass of PSB from the chemocline is likely to occur in the aerobic zone of the lake as it was shown for Lake Mahoney [3]. Unraveling the ways of utilization of the PSB biomass and their contribution to the trophic chain of this lake is an urgent task for further studies.

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