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Fatty acid trophic markers in Lake Baikal phytoplankton: A comparison of endemic and cosmopolitan diatom-dominated phytoplankton assemblages

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ABSTRACT

Recent changes in phytoplankton community in Lake Baikal suggest the reduction in bloom of endemic diatoms takes place. How much these changes affect primary production remains unknown. Fatty acids are suitable biomarkers of the phytoplankton food quality. In our work we present the data of the qualitative and quantitative analysis of fatty acids (FA) in net samples of Lake Baikal phytoplankton. According to microscopic analysis of the samples the main phytoplankton groups were diatoms (Bacillarophyceae), golden algae (Chrysophyceae), green algae (Chlorophyceae), and Dinophytes (Dinophyceae). Visual quantification of cells showed that *Synedra acus* subsp. *radians*, *Aulacoseira islandica*, *Synedra ulna*, and *Aulacoseira baicalensis* were dominant diatom species. The first three species are cosmopolitan and the last one is endemic. Gas chromatography–mass spectrometry analysis revealed that the fatty acid biomarker values reflect changes in phytoplankton community structure in Lake Baikal. The trophic markers of the phytoplankton dominated by endemic *A. baicalensis* were higher compared to the phytoplankton dominated by cosmopolitan *S. acus* subsp. *radians*. The long-chain essential FA (LCEFA, %DW) and FA-based food quality index (FQI) for endemic assemblage were 1.85 and 0.75, while for the cosmopolitan assemblage − 0.70 and 0.28, correspondingly. Our data suggest that reduction in bloom of endemic A. baicalensis may negatively affect the Lake Baikal ecosystem due to probable lower LCEFA production and, consequently, lower total productivity of the lake.

1. Introduction

Microalgae are dominant producers of organic matter in aquatic ecosystems (Dalsgaard et al., 2003). Phytoplankton play a major role in primary production and trophic interactions (Winder and Cloern, 2010; Strandberg et al., 2015) in oceans and deep-water lakes (Parsons, 1963). Phytoplankton are sensitive to changes in climatic and anthropogenic factors (Beaugrand, 2014). Thus, it is necessary to consider their role in energy transfer to the higher trophic levels of aquatic ecosystems when making predictions about the dynamics of aquatic ecosystem functioning under global environmental changes.

Lake Baikal is the deepest and largest oligotrophic lake in the world (Kozhov, 1963), where pelagic phytoplankton play a major role in the organic matter cycle, accounting for about 90–95% of the Lake's primary production (Boulion, 2016). The peak of phytoplankton bloom and its seasonal succession depend on hydrodynamic processes in the Lake (Kozhov, 1963; Goldman et al., 1996), such as surface water temperature, penetration of solar radiation through the water mass (transparency), and availability of nutrients. Lake Baikal's primary productivity shows little inter-annual variation in the summer-autumn season (Pomazkina et al., 2010) as opposed to the spring–summer period, when the intensity of these processes show significant inter-annual differences (Popovskaya, 2000). Therefore, the lake's primary productivity depends on the spring–summer diatom bloom. Earlier studies have showed that endemic and cosmopolitan species of diatoms are primary contributors to the Lake's primary production. Mackay et al. (2006) demonstrate that during the 1950s, productive years in the lake were dominated by large cells, endemic species belonging to the genera *Aulacoseria* and *Cyclotella* (Popovskaya, 2000), although *Synedra* tends to bloom following years of peak *Aulacoseira* production.

Global climate changes affect the stability of hydrological parameters in Lake Baikal (Moore et al., 2009; Shimaraev and Domysheva, 2012; Izmest'eva et al., 2016). It is obvious by the example of surface water temperature rising, altered thermal stratification (Hampton et al., 2014), shorter periods of ice cover (Mackay et al., 2006), and changes in silicon concentrations (Shimaraev and Domysheva, 2002). These changes have a negative impact on the development of psychrophilic endemics such as *Aulacoseira baicalencis* and *Cyclotella minuta* (Mackay et al., 2006; Izmest'eva et al., 2011). At the same time, cosmopolitan diatom species such as *Stephanodiscus meyerii* and *Synedra acus* subsp.

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radians grow intensively (Mackay et al., 2006; Popovskaya et al., 2015). Increasing surface water temperature results in vertical redistribution of Baikal phytoplankton over the photic layer depths (Hampton et al., 2014). Relatively heavy diatoms shift downward in the water column, while the depths of other phytoplankton groups do not change significantly. How much these changes affect primary production remains unknown, especially because many of the lake's diatoms are endemic and their biochemistry is poorly defined in comparison with cosmopolitan taxa.

Microscopic analysis, which is a traditional method of studying phytoplankton communities in Lake Baikal (Popovskaya, 2000; Popovskaya et al., 2015), does not reveal physiological and biochemical changes in the phytoplankton community in response to changing environmental factors. Therefore, fatty acids (FAs) are used as chemotaxonomic markers to study phytoplankton communities in marine and freshwater ecosystems (Dijkman and Kromkamp, 2006). FAs make it possible to estimate the phytoplankton trophic index (TI) and predict trophic interactions in aquatic systems (Hixson and Arts, 2016). Although there are many studies of the Baikal fauna FA composition (Morris, 1984; Kozlova and Khotimchenko, 2000; Grahl-Nielsen et al., 2011), no such studies of phytoplankton have been carried out.

Phytoplankton TI analysis is based on the essential fatty acid (EFA) content. EFAs are normally produced by photosynthetic organisms. Physiological processes in consumers are tightly linked to the dietary uptake of EFAs (Sargent et al., 1999, Müller-Navarra et al., 2004). Among EFAs, there is polyunsaturated fatty acid (PUFA) with long-chain omega-3 and omega-6 essential FA (LCEFA, ═C20) (Galloway and Winder, 2015). High quality of phytoplankton as a food for zooplankton is considered to have a high level of LCEFA.

The FA composition of microalgae depends on both phylogeny (Viso and Marty, 1993; Lowe et al., 2014; Strandberg et al., 2015) and environmental factors (Budge et al., 2001; Gladyshev et al., 2012; Galloway and Winder, 2015; Cañavate et al., 2017). Among marine phytoplankton and freshwater species, diatoms and dinoflagellates have relatively high levels of LCEFA (Mansour et al., 1999; Dalsgaard et al., 2003; Sushchik et al., 2004). Diatoms contain eicosapentaenoic acid (EPA C20:5), while dinoflagellates and freshwater cryptophytes contain docosahexaenoic acid (DHA C22:6) (Brett et al., 2009). Green algae produce omega-3 linolenic acid (18:3) and omega-3 linoleic acid (18:2) (Taipale et al., 2013). Green algae and cyanobacteria have low levels of LCEFA. Therefore they are less valuable for zooplankton than diatoms from the point of view of trophic value (Brett and Müller-Navarra, 1997; Galloway et al., 2014).

Organisms of upper trophic levels need LCEFAs for synthesis of membrane structures and regulation of various physiological processes. LCEFAs are precursors of compounds that support immune protection, reproductive function, etc. (Brett and Müller-Navarra, 1997). When compared with other freshwater ecosystems, upper trophic levels of Lake Baikal have higher PUFA and LCEFA levels, similar to those found in marine organisms (Morris, 1984; Kozlova and Khotimchenko, 2000). Thus, recent changes in Baikal phytoplankton (Mackay et al., 2006; Hampton et al., 2014) may affect LCEFA transfer in food webs and, consequently, lead to changes in bioproductivity in the lake.

In this study, we elucidated the Baikal phytoplankton trophic index and compared the trophic markers in endemic and cosmopolitan diatom-dominated phytoplankton assemblages.

2. Materials and methods

2.1. Phytoplankton collection

Phytoplankton samples (PSi) were collected from across the length of Lake Baikal during an expedition that took place from May 28 to June 5, 2014 (all PSi except PS_8) and in March 2016 (PS_8) (Fig. 1). Sampling occurred at five transects that included 16 stations located in the three basins of the lake and two stations in Maloye More Strait (or

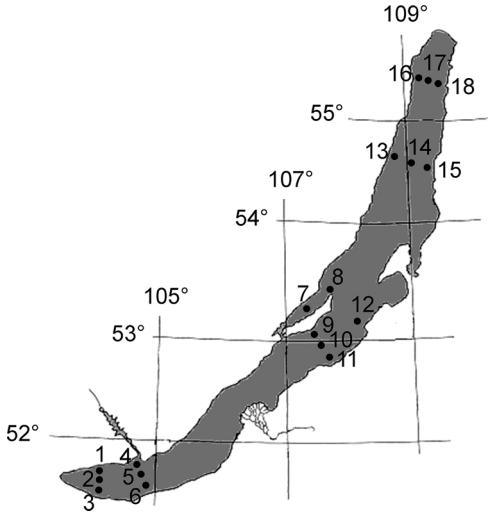

Fig. 1. Locations of the sampling stations in Lake Baikal.

"Little Sea") (PS_7 and PS_8). Integrated vertical samples over the top 50-m stratum of each sampling site were collected using a Jedy net with a mesh size of 40–60 μm (all samples except PS_8). An under ice sample at Hoboy cape (PS_8) was collected by a diver using a syringe sampler in March 2016. For chemical analysis, phytoplankton samples were filtered onto 47-mm glass fiber filters, wrapped in aluminum foil, and immediately frozen at −20 °C. Phytoplankton samples were fixed in acidified Lugol's iodine solution. Phytoplankton cells were identified and counted using Carl Zeiss Axiovert 200 microscope with magnification of ~ 400. Microscopic analysis showed that contamination by zooplankton was negligible at all stations (< 0.5%). Cell counts were converted to phytoplankton biomass taking account of measurements of individual cells according to Makarova and Pichkily (1970). Diatom biovolumes were estimated from mean cell dimensions for the main species of phytoplankton.

2.2. Chemical analysis

2.2.1. Methylation of fatty acids

Fatty acid methyl esters (FAMEs) were obtained by esterification of phytoplankton lipid fraction. To extract lipids, 1.2 ml of Folch solution (trichloromethane:methanol 2:1 by volume) was added to the sample. The mass of the wet samples (humidity is ~ 90%) was 0.15–0.20 g (the precision weight up to fourth character). This quantity of the previously homogenized microalgae biomass provides sufficient precision and sensitivity to determine FAMEs by GC–MS as well as GC-FID at the level up to 0.03 wt percent (in the samples under the study). Further biomass increasing results in the GC-column overload and distortion of the chromatographic peaks. The sample was then extracted three times over 5 min using an ultrasonication bath (50 Hz). The humidity of the samples was calculated gravimetrically. Then, 1.2 ml of distilled water was added to the extract. The obtained mixture was strongly shaken for 0.5 min and centrifuged at 3000 rpm. The chloroform layer was separated, dried over anhydrous Na_2SO_4 for one hour, poured into 10-ml glass bottles, and evaporated under an argon stream down to 1 ml. Then, 4.5 ml of H_2SO_4 solution (2%) in MeOH was added to the obtained fraction. The solution was then covered with aluminum foil and kept for two hours at 65 °C Then, 0.8 ml of *n*-hexane was added to the FAME solution; the solution was shaken and cooled to room temperature. FAMEs were extracted twice with 3 ml of *n*-hexane. An internal standard (didecyl ether solution in *n*-hexane, C = 2.6 mg/mL) was used to quantify FAMEs. We added 0.25 ml of this solution to each sample before the analytics.