

## Study of the effect of anthropogenic load on the gene expression levels and elemental composition of endemic sponges of Lake Baikal

*Thursday, 12 November 2020 15:45 (15 minutes)*

Sponges are one of the oldest multicellular animals that have survived almost unchanged to this day. The earliest finds of sponge remains date back 680 Ma. At the moment, the planet is inhabited by both marine and freshwater sponges. By the type of nutrition, all sponges are filter feeders, so they play an important role in aquatic ecosystems. At a certain moment events of mass diseases and mortality of sponges were noted in different aquatic ecosystems.

Lake Baikal is inhabited with 15 species of endemic sponges. Baikal sponges are not just one organism, but a whole community of sponge with symbionts - unicellular algae and bacteria. Due to the symbiosis, the sponges are green colored. In the last decade, events of mass diseases and mortality of sponges have been observed on Lake Baikal, which indicates negative changes in the ecosystem of the lake. Endemic sponges are an important object to study, since they make up a bulk of benthos biomass and play a key role in the ecosystem of Lake Baikal, which contains 20% of the world's fresh water and is a UNESCO World Heritage Site.

To reveal background of these events we perform analyses of elemental composition and gene expression of Baikal endemic sponge *Lubomirskia baikalensis*. Two sampling points were chosen by monitoring data, showed places with the highest and the lowest rates of sponge disease. It was Listvyanka bay, which is considered to be the most anthropogenic impacted point and Bolshie Koty bay, which looks like almost intact. Samples of *L.baikalensis* were collected by SCUBA diving at Listvyanka Bay, n=10, and Bolshie Koty Bay, n=10. Samples were collected at August 2020. Immediately after collection, the samples were frozen at -20°C for Neutron Activation Analysis (NAA) and tissue parts were placed in IntactRNA solution for gene expression analyses.

Changing the level of gene expression is one of the main mechanisms of adaptation of a living organism to changes in the environment. Since the endemic Baikal sponges have lived in very stable conditions for the past several million years, even small changes in the environment should trigger a response at the level of gene expression. To study the mechanisms of sponge cells protection from the effects of heavy metals, an analysis of the gene expression, whose products are involved in the most important metabolic pathways of cell interaction with heavy metals, will be carried out.

To characterize expression patterns in *L.baikalensis* four candidate stress response genes were chosen for later qPCR analyses, based on previous assessments of upregulation in other aquatic invertebrate species [1,2] and studies of gene expression response to heavy metal exposure for different types of organisms. For glutathione-s-transferase mu class gene expression differences have been shown on Mollusca, collected in points with different anthropogenic load. Copper transporting ATPase 1 and copper chaperone for superoxidodismutase were also used in this study.[2] MTF1 gene is shown to be transcriptional regulator involved in cellular adaptation to exposure to heavy metals [3]

Specific primer pairs for these genes were selected based on previously published transcriptome data of *L.baikalensis*. [4] Primer design and further analyzes is carried out in Molecular Genetics Group DLNP, JINR. As a result, a test system will be developed based on the expression levels of heavy metal defense genes to quickly assess the state of the ecosystem of Lake Baikal

Sample preparation for Neutron Activation Analysis was carried out in In the Sector of Neutron Activation Analysis and Applied Research, FLNP, JINR. Sponge samples were dried to dry weight and divided into 6 cm pieces. Each piece will be homogenized and analyzed separately. This approach will allow us to find out the dynamics of accumulation of heavy metals in sponges with age and differences in heavy metal concentrations at points with high and low percentage of diseased sponges. The elemental composition of the Baikal sponges was previously studied in the 1980s and 2000s [5,6]. The ability of sponges to accumulate copper and aluminum has been shown. Taking into account the dynamics of the development of the tourism industry on the shores of Lake Baikal and the annual increase in anthropogenic load, it is necessary to study how much the levels of accumulated heavy metals have changed in sponges during last 20 years.

All these data will help us assess the contribution of anthropogenic load to the development of the ecological crisis on Lake Baikal, which is currently being observed.

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**Session Classification:** Life Science

**Track Classification:** Applied Research