Original Article

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.

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 and research in laboratories and undernatural 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/rbdata/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. dybowski* 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.

Acknowledgments

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