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Features of phototropic response of zooplankton to paired photostimulation under adverse environmental conditions

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Abstract Our previous studies showed that the change in the plankton response to light could be an indicator of environmental pollution. This study experimentally reveals that the response of *Daphnia magna* Straus and *Daphnia pulex* plankton ensembles to photostimulation depends on the intensity of the attracting light. This makes it difficult to identify the occurrence and change of pollutant concentration. The large variability in the magnitude of the behavioral response is caused by the nonlinear response of plankton ensembles to the intensity of the attractor stimulus. As the intensity of the photostimulation increases, the variability of the phototropic response passes through increase, decrease, and relative stabilization phases. The paper proposes a modification of the photostimulation method—paired photostimulation involving successive exposure to two photostimuli of increasing intensity. The first stimulus stabilizes the behavioral response, while the increase in response to the second stimulus makes it possible to more accurately assess the responsiveness of the plankton ensemble. The paper studies the sensitivity of the method of paired stimulation of the behavioral response of different types of freshwater plankton ensembles: *Daphnia magna* Straus, *Daphnia pulex* to the effects of pollutants (potassium bichromate, microplastic). The study demonstrates good reliability and increased sensitivity of this method of detecting changes in environmental toxicity compared to single photostimulation or traditional bioindication through the survival rate of test organisms.

Keywords Zooplankton · Behavioral response · Paired photostimulation · Water pollution monitoring · Submersible holographic camera

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Introduction

Along with the established methods for detecting large-scale pollution of water bodies, the problem of early detection of small but biologically significant concentrations of pollutants is becoming ever more urgent. The occurrence of small concentrations of pollutants in a water body, that do not have a visible toxic effect and do not violate the biological well-being impression, may lead to a shift in the species ratio under biota chronic exposure (Bownik, 2017). This will inevitably lead to a change in the qualitative profile of the ecosystem and a potential disastrous decrease in the number of commercial bio-objects or their quality impairment (Sukharenko et al., 2017).

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Reliable findings on a threshold sublethal harmful effect require the following conditions: the responsiveness of test organisms to alteration effects and their resistance to natural changes in habitat conditions. These requirements best match the response of the autochthonous mesoplankton adapted to the conditions of a particular water body.

Water sample biotesting methods have been actively used in recent years. They are based on recording the survival rate (OECD, 2013, 2019), reproduction, offspring quality, changes in morphological parameters (OECD, 2013), and physiological functions (Lechelt et al., 2000; Morgalev et al., 2015; Nikitin, 2014; Wang et al., 2019) of aquatic organisms. The most promising are the methods of biomonitoring the state of the aquatic environment using bivalves (Sukharenko et al., 2017), Branchiopoda (Carreño-León et al., 2014), and Copepoda (Lechelt et al., 2000; Pan et al., 2015, 2017; Ren et al., 2016). They filter a large amount of water for eating bacteria and algae contained in it; therefore, even small concentrations of harmful substances cause drastic changes in their condition. Among the test responses used for control, the most interesting are such behavioral responses as the swimming speed and trajectory, frequency of swimming movements, etc. (Lechelt et al., 2000; ISO 6341:2012). Behavioral responses link chemical exposure to subsequent molecular, cellular, physiological, and behavioral changes that lead to a disease or injury of individuals (Ankley et al., 2010). The central nervous system perceives, processes, and transmits information on the external and internal environment of a test organism. Therefore, the musculoskeletal behavioral responses it controls serve as highly sensitive indicators of toxic effects (Mora-Zamorano et al., 2018). Besides, the most reasonable scheme for assessing the state of any organism is to register the parameters of its response to a standard stimulus (Baevsky & Berseneva, 2017). One of the most convenient and easily adjustable standard stimuli is photostimulation, which causes a phototropic response of both individuals and their ensembles.

A large number of works were devoted to the study of phototaxis. It is especially clearly seen in the diel vertical migration along the water column (Cousyn et al., 2001)—the largest migration of biomass. On the one hand, the variability of diel vertical migration indicates the involvement of the nervous system in the phototropic response: in environments where the influence of predators and other factors changes over time, a change in normal migration (nocturnal ascent) to reverse migration (nocturnal descent) is possible (Ohman, 1990; Ohman et al., 1983). Changes in phototaxis during the introduction of psychotropic substances are shown in laboratory conditions (Rivetti et al., 2016).

A correlation between daily vertical migration and illumination conditions was established for *Daphnia* crustaceans (Gehring & Rosbash, 2003). Gehring and Rosbash (2003) showed that the daylight ultraviolet radiation suppresses the synthesis of cryptochrome acting as a key protein of the molecular circadian system. Cryptochrome is most likely part of the response related to crustaceans escaping the ultraviolet radiation in daylight and returning to the surface at night (Nesbit & Christie, 2014). This molecular pacemaker sets the rhythm for synchronizing physiological and behavioral processes (Golombek & Rosenstein, 2010). The circadian system of *Daphnia* includes the gene/protein cryptochrome 2 (CRY2), which inhibits the CLK/CYC heterodimer-mediated transcription (Nesbit & Christie, 2014). The endogenous molecular circadian pacemaker is corrected by external stimuli, among which the main one is the alternation of day and night (Golombek & Rosenstein, 2010). The visual organ is important to control the circadian rhythm (Golombek & Rosenstein, 2010). Light is perceived by the retinal neurons, and the signal is transmitted to the suprachiasmatic nucleus of the hypothalamus with the molecular circadian system.

There is data on the influence of various external factors on the circadian rhythms of crustaceans. For example, anthropogenic chloride contamination of the aqueous medium (chloride at the concentrations of 15, 100, 250, 500, 1000 mg/L) changed the circadian rhythm of actin and Na + /K + ATPase content in *Daphnia pulex* (Coldsnow et al., 2017). The authors recorded the reduction of the circadian rhythm exposed to a high level of mineralization.

Moreover, there are conflicting data regarding the phototropic response: for example, with a sudden increase in light intensity, *D. magna* try to escape from light, which in natural conditions allows escaping the attack of predatory fish in the daytime (Simão et al., 2019). On the contrary, other researchers observed that the crustaceans gather in the illuminated zone and that this response is suppressed in the presence of toxicants

(Dyomin et al., 2020a, 2021; Lertvilai, 2020; Michels et al., 2000). At the same time, De Meester (1991) describes both swimming towards (positive phototaxis) and away from light (negative phototaxis). Besides, phototaxis depends on light intensity and may change its polarity depending on it (Bedrossiantz et al., 2020; Simão et al., 2019). This requires the standardization of photostimulation parameters to ensure the comparability of results obtained both by different groups of researchers and under different laboratory and field conditions.

In natural conditions, the registration of autochthonous plankton phototropism is based on the trawling method with the subsequent calculation of the number of crustaceans in water layers with different lighting, which a priori eliminates the possibility of prompt assessment. There are some studies devoted to the use of submersible photographic cameras with different illumination wavelengths (Lertvilai, 2020), which showed different responsiveness of zooplankton in the daytime and at night, as well as depending on the wavelength of light, but the number of such studies is insufficient. Unlike photographic cameras, holographic cameras register information on all particles in a volume per one light exposure, which allows obtaining a focused image from one hologram, determining geometric parameters, and classifying the type of each particle in the registered volume. The studies of Dyomin et al. (2019), Giering et al. (2020), and Nayak et al. (2021) present the features, capabilities, and comparison of such cameras.

The digital holographic camera designed at Tomsk State University (Dyomin et al., 2020b) also makes it possible to measure the parameters of individual particles but differs from known similar devices in the possibility of zooplankton photostimulation by attracting radiation with controlled intensity causing the phototropic response (Dyomin et al., 2020a). The advantages include the significant amount of the controlled volume (0.5 dm^3) and the representativeness of data thus determined. Besides, the design of the cameras makes it possible to conduct the study in field and laboratory conditions, as well as to develop and verify algorithms in order to assess the environmental toxicity. The purpose of this study was to develop a method of paired photostimulation standardization for the unambiguous interpretation of plankton phototropic response for early detection of environmental disorders in freshwater bodies.

Materials and methods

Test organisms

The studies were conducted using two zooplankton freshwater species: Cladocera *Daphnia magna* Straus and *Daphnia pulex*. *D. magna* was received from LLC Europolitest (Russia). *D. pulex* were taken from the natural population in freshwater bodies in the vicinity of Tomsk and then were adapted to laboratory conditions for 8 months. Cultivation and experiments were carried out under the conditions recommended in the procedures (ISO 6341:2012): temperature (t) = 22 ± 2 °C, pH = 7.0–8.5, control culture medium = fresh water, oxygen (O_2) content = 6 mg/dm^3, photoperiod = 12/12 h. The design of a submersible digital holographic camera (DHC) was described in detail in Dyomin et al. (2021).

Pollutants

Potassium bichromate ($K_2Cr_2O_7$, Merck KGaA, Germany)—a standard model toxicant with pronounced toxicity—and microplastics (mPl) made of the biologically inert material in macroform were used as pollutants.

Microplastic particles were prepared from fibers of woven polypropylene bags (LLC Terra, Russia) aged in natural conditions for 12 years according to a procedure developed in our laboratory (Morgalev et al., 2022).

The concentration and size of microparticles were determined from images obtained with a confocal microscope when excited with a 405-nm laser. The concentration of mPl in the resulting primary suspension was ~ 10^7 particles/dm^3 (200 mg/dm^3). The content of particles smaller than 3 × 3 μm was 70.0 ± 6.4% and larger particles—30.0 ± 5.8%, respectively.

Digital holographic camera

The design of a submersible digital holographic camera (DHC) was described in detail in Dyomin et al. (2019). The DHC allows registering a hologram of the entire working volume of the medium (the volume illuminated by the laser beam, Fig. 1a) with further gradual reconstruction of images of the medium layers at a given step and study of the particles in these layers. The given DHC modification used two lasers—one with a wavelength of 532 nm for plankton photostimulation and the second with a wavelength of

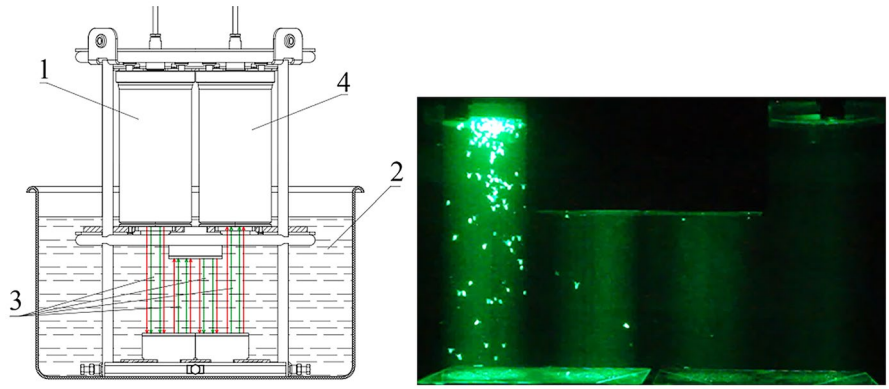

Fig. 1 Arrangement of beams forming the working volume for hologram recording (1, lighting module; 2, water tank; 3, working volume limited by recording (red) and attracting (green) light beams; 4, recording module) (**a**); photo of light columns with hydrobionts (**b**)

650 nm for hologram recording. The wavelength of attracting light used for photostimulation was close to the local maximum of the reflection spectrum of microalgae being the main nutrient source for plankton. The maximum laser emission power at the output of the lighting module window was 4 mW, which was controlled by a change in the control voltage. At the same time, the illumination variation range was 0–4600 lx.

During laboratory experiments, the DHC was placed in a 90 dm^3 water tank filled with 50 dm^3 of the test medium thus providing for the optical part being below the liquid level (Fig. 1a). Since the beam of the attracting light passed through the mirror-prism system, as a result of the Fresnel reflection, partial water absorption, and scattering by microsuspensions, the brightness of water columns of light decreased in the direction from the lighting to the recording module (Fig. 1b).

Paired photostimulation

The use of plankton behavioral response as an indicator of the alternating exposure is based on recording a change in phototaxis compared to the background.

The paired photostimulation described in detail in the article by Morgalev et al. (2022) was used to reduce the effect of behavioral response variability in the background (inter-individual distribution) and in case of insufficient photostimulation intensity that does not activate systems that ensure the behavioral response. The first, smaller exposure activates the functional system (Anokhin, 1974) responsible for the behavioral response, which reduces inter-individual distribution and entropy in the system (Morgalev & Morgaleva, 2007). The second, more intense exposure causes the crustaceans to move at a rate appropriate to their physiological state.

Design of the experiment

On the day of the experiment, 1 h before the start, pre-selected and counted crustaceans of the same age (3 days) were fed by adding a suspension of *Chlorella vulgaris* Beijing algae according to the procedure (ISO 6341:2012). After 1 h, using a sieve, 200 ± 10 crustaceans were placed in a container with the DHC, which created a concentration of 4 ind/dm^3 (4000 ind/m^3) corresponding to the concentration in natural water bodies. The studies were carried out after an hour of adaptation of test organisms to the experimental conditions.

Two holograms separated by a time interval of 40 ms were recorded every 30 s to study the dynamics of the concentration of crustaceans at different intensity of attracting light, which also made it possible to track the direction and speed of movement of certain crustaceans within the controlled volume. The following parameters were determined based on the images reconstructed from digital holograms (Table 1).

The dynamics of the phototropic response during the paired photostimulation in the ecotoxicological experiment was registered for 8 cycles: the first cycle (background) before the introduction of cultivation water (in the control series) or a pollutant (in test series) and 7 consecutive cycles in 10, 30, 60, 90, 120, 150, and 180 min after the introduction of water or a pollutant. Each cycle included a 15-min

Table 1 Phototaxis parameters used for the multiple regression model

N_{25}	Concentration of crustaceans (ind/dm^3) over 5 min of photostimulation with 25% illumination of the maximum
N_{75}	Same with 75% illumination
V gen 25	Average speed of the movement of crustaceans in all directions (cm/s) over 5 min of photostimulation with 25% illumination
V gen 75	Same with 75% illumination
V Z up 25	Average speed of the upward movement (cm/s) of crustaceans over 5 min of photostimulation with 25% illumination
V Z up 75	Same with 75% illumination
V Z down 25	Average speed of the down movement (cm/s) of crustaceans over 5 min of photostimulation with 25% illumination
V Z down 75	Same with 75% illumination
V xy 25	Average speed of the movement of crustaceans (cm/s) in the horizontal plane over 5 min of photostimulation with 25% illumination
V xy 75	Same with 75% illumination
Delta	Difference in the concentration of crustaceans (ind/dm^3) at the first and second intensity of photostimulation
D/N_{25}	Ratio of delta and N_{25}, %
D/N_{75}	Ratio of delta and N_{75}, %

continuous registration of 30 holograms and was divided into 3 equal 5-min intervals, each of which registered 10 holograms: (1) without attracting light (I_0); (2) with attracting light at intensity I_1 (1150 lx); (3) in the subsequent second photostimulation with illumination I_2 (3450 lx). There was a 15-min pause without lighting after each cycle to restore the number of crustaceans.

The processing results of 10 sequentially recorded holograms were used to calculate the average concentration of crustaceans during the first and second photostimulation stages (C_1 and C_2, respectively), as well as the ratio of the increase in crustacean concentration during the transition from the first to the second intensity to the concentration of crustaceans during the second intensity: $\Delta C/C_2 = (C_2 - C_1)/C_2 * 100\%$.

The study of pollutant toxicity by mortality and immobilization of daphnia was fully consistent with ISO 6341:2012.

Solutions or dispersed suspensions of pollutants in the cultivation water were added in a volume of 0.5 dm^3.

Statistical data analysis

Statistical processing was performed using Statistica 10 (StatSoft, Inc., USA). The Shapiro–Wilk *W*-test was utilized to evaluate the distribution of data regarding normality, and Bartlett's test was employed to verify the homogeneity of variance. The Student's *t*-test (two-sided *p* level) was used to assess the differences in normal distribution and the Mann–Whitney *U*-test—for deviations from normal distribution.

The information value of parameters was determined by multiple regressions. The forward stepwise inclusion of predictors in the regression model was used. A ridge regression scheme was also used due to the correlation of some predictors. Ridge regression was used when the independent variables were highly intercorrelated, and stable estimates for the regression coefficients could not be obtained via ordinary least squares methods. The results were presented as mean ± standard error of mean.

Results

Threshold intensity of photostimulation for the detection of the behavioral response of crustaceans

We found that crustaceans began to gather in the illuminated column of the water medium at an attracting radiation power of 200 ± 15 μW, which corresponded to 5% of the maximum laser radiation power and the illumination of ≈50 lx. The threshold of crustacean sensitivity depended on two factors: water transparency and duration of attracting light exposure. The most stable results were obtained under attracting light with a radiation power of at least 20% of the maximum laser radiation power. The following illumination gradations were used in further

studies based on the obtained data: 25%, 50%, 75%, and 100% of the maximum, which corresponded to the illumination of 1150 lx, 2300 lx, 3450 lx, and 4600 lx, respectively. It is important to emphasize that the 650-nm laser used for hologram recording did not make the crustaceans gather in the field of view even at long continuous exposure (15 min). Note that its actuation duration was not more than 50 μs per minute during the experiment.

Dynamics of crustaceans gathering into light columns at different intensity of attracting light

When attracting light was turned on, the crustaceans continued to gather in the illuminated columns of the water medium of the controlled volume for 30 min (Fig. 2a) with gradual stabilization.

In this series of experiments before the attracting light was turned on, the crustaceans were mainly gathered at the bottom of the tank and did not get into the field of view of the camera.

After the attracting light was on, the holograms were recorded at the end of each 5-min interval (in 4, 4.5, and 5 min). The gathering of crustaceans in the light columns of the aquatic environment can be divided into three phases:

1. During the first 2 min after the attracting light was turned on, there was no visible response of crustaceans; even some individuals accidentally getting into the illuminated space swam away.
2. From 2 to 5 min, there was a sharp increase in the concentration of crustaceans.
3. From 6 to 10 min—high, stabilizing concentration of crustaceans.

The rate of crustaceans gathering in the light column depended on the lighting intensity (Fig. 2b).

A significant increase in the concentration of crustaceans in the registered volume was recorded at the end of the third 5-min interval (14–15 min of the observed period): $C = 9.0 \pm 2.5$ ind/dm^3 (1150 lx), $C = 14.0 \pm 2.4$ ind/dm^3 (2300 lx), $C = 12.0 \pm 2.2$ ind/dm^3 (3450 lx), and $C = 14.0 \pm 1.1$ ind/dm^3 (4600 lx). In the next two cycles, the number of crustaceans attracted by light almost did not change.

Another important fact was the reduction of concentration variability of crustaceans (Cv—ratio of the arithmetic mean to the standard error of mean) with increasing intensity of attracting light. So after 10-min illumination, Cv decreased from 80% in the background to 16% at 25% illumination and then to 8% at the maximum illumination.

Thus, the graded dependence of the concentration of crustaceans on the attracting light intensity was maintained within 10 min of photostimulation.

Choice of paired photostimulation parameters

The dynamism of the ability of a pool of crustaceans to change their direction during the second light stimulus was determined to test the use of the paired photostimulation. The choice of sequence and intensity of photostimuli is based on this.

The lability of the phototropic response of test organisms in two modes of photostimulation was studied:

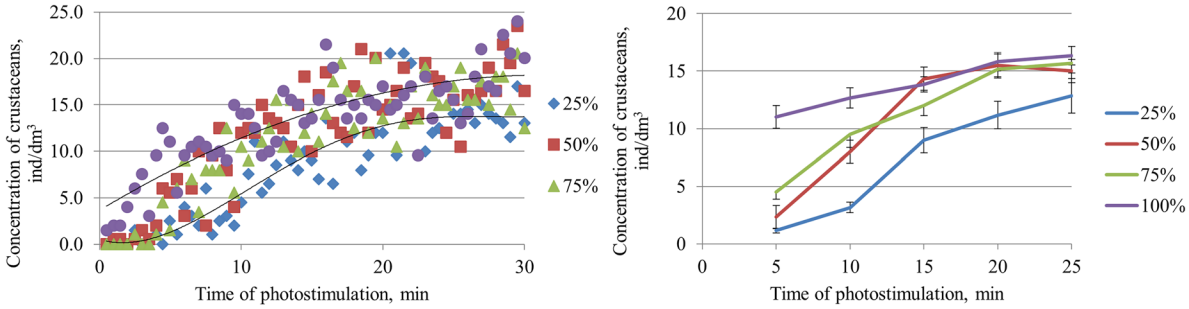

Fig. 2 Dynamics of crustacean concentration in illuminated columns of water medium working volume (**a**) and average crustacean concentration (**b**) depending on the attracting light intensity (in % of the maximum illumination)

with a stepwise continuous increase and a stepwise intermittent increase in the intensity of attracting light.

The stepwise increase in intensity of 5-min photostimulation made it possible to register a stepwise increase in the concentration of crustaceans in light columns (Fig. 3a).

In the absence of attracting light (background), almost all crustaceans were outside the observed columns of the water medium, and their concentration in the working volume (C) averaged 0.1 ± 0.1 ind/dm^3. After the attracting light with the 1150 lx was activated, single individuals ($C = 0.3 \pm 0.2$ ind/dm^3) were recorded in the illuminated columns of the water medium. Starting with 2300 lx, a pronounced positive phototaxis of crustaceans was noted, and their concentration increased significantly ($C = 2.5 \pm 0.6$ ind/dm^3), which at the illumination intensity of 3450 lx and 4600 lx reached a relatively high level: $C = 7.0 \pm 0.7$ ind/dm^3 and $C = 12.3 \pm 0.7$ ind/dm^3, respectively. It should be noted that with an increase in the intensity of attracting light, the variability in the concentration of crustaceans decreased from 100% in the background to 6% at the maximum illumination.

The analysis of obtained data on the degree of recovery of the concentration of crustaceans at a stepwise increase in photostimulation intensity with 5-min interruptions between each 5-min light exposure showed that there was no full recovery of their concentration without attracting light (Fig. 3b). The degree of recovery of the concentration of crustaceans depended on the intensity of attracting light. At the illumination of 1150 lx of the maximum, the concentration of crustaceans during the 5-min interruption decreased to 7.7%. At 2300 lx illumination, C decreased to 25.9%, at 3450 lx, to 31.3%. After 5-min photostimulation with 4600 lx illumination, 50.0% of crustaceans remained in the registration area, i.e., during interruptions some crustaceans were dispersed from the registration area.

When choosing a paired photostimulation scheme, preference should be given to the second most illuminated exposure immediately after the action of the first one and without any interruption between them. In this case, it was necessary to consider the accumulation of crustaceans in the controlled volume, which depended on the intensity of the first exposure.

The first intensity of attracting light in paired photostimulation was chosen as follows. The phototaxis threshold of crustaceans was determined in the range of attracting light intensity of 5–50% of the maximum with 5% increase. The intensity at which there was a local decrease of the crustacean concentration variation coefficient was determined with further increase of the illumination intensity. This value was used as the attracting light intensity of the first stage of paired photostimulation (I_1).

The choice of the intensity of attracting light of the second stage of paired photostimulation (I_2) was related to the degree of increase in the phototropic response of crustaceans. There was a different degree of response increase with different combinations of the attracting light intensity of the first and second stages of paired photostimulation. With a combination of 1150–2300 lx, the transition from the first to the second stage caused an increase in the concentration of crustaceans by 101 ± 14% compared to the increase at the

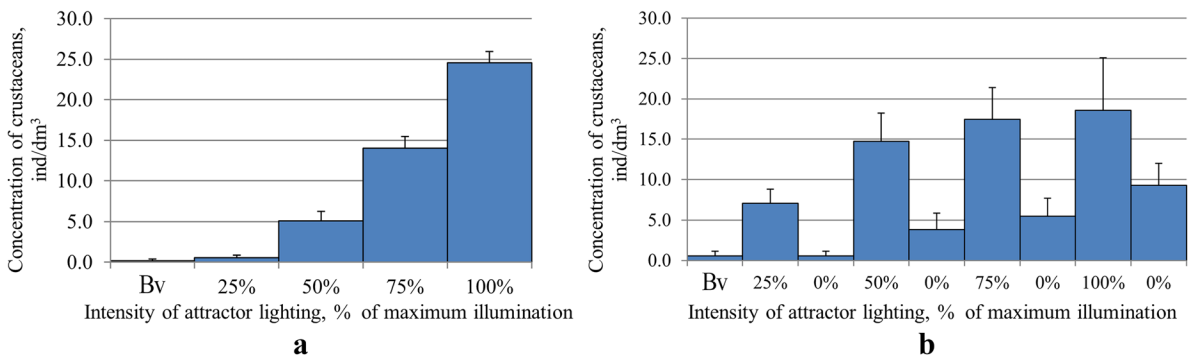

Fig. 3 Increased concentration of crustaceans at stepwise continuous (**a**) and stepwise intermittent (**b**) increase in the intensity of attracting light

first stage, at illumination intensity of 1150–3450 lx, by 211 ± 27%; at 2300–3450 lx intensity, by 86 ± 13%; at 2300–4600 lx intensity, by 122 ± 15%; and at 3450–4600 lx intensity, by 32 ± 8% (Fig. 4).

The design of experiments during the contamination of the aqueous medium was developed taking into account the obtained data.

Informativeness of hydrobiont responses to pollutants

The relationship of a set of phototaxis parameters with the concentration and time of action of the standard potassium bichromate ($K_2Cr_2O_7$) toxicant was determined in our studies. We studied the toxicant exposure at the concentrations of 0.06 mg/dm^3, 0.12 mg/dm^3, and 0.24 mg/dm^3. The exposure time in a toxicant was 20, 40, and 60 min.

A toxicant response model was built to determine the most reliable predicted values via the multiple regression model. The dependent variable was the toxicity index (Tox), which was calculated as the product of the concentration and duration of exposure to the toxicant.

The regression equation

$$\text{Tox} = 0.54 \cdot \text{"}\mathbf{N_{25}}\text{"} - 0.04 \cdot \text{"}\mathbf{D/N_{75}}\text{"} - 2.11 \cdot \text{"}\mathbf{V\ Z\ down\ 25}\text{"} - 0.97 \cdot \text{"}\mathbf{V\ Z\ down\ 75}\text{"} + 4.63$$

explained the correlation with predicted values by 84% (adjusted $R^2 = 0.841$) with an accuracy of 0.4 ($p < 0.0001$).

Thus, when changing the toxicity of the medium, the most informative were the indicators of the concentration and speed of crustaceans moving down the vertical during paired photostimulation. The subsequent exclusion of all indicators related to the speed of movement of crustaceans from the model led to a slight decrease in the model completeness up to 74% and accuracy up to 0.55 with further significant simplification of computing for the reconstruction of images from holograms. The model efficiency is illustrated in Fig. 5.

This simplifies the equation to the following form:

$$\text{Tox} = 2.51 \cdot \text{"}\mathbf{Delta}\text{"} - 1.60 \cdot \text{"}\mathbf{N_{25}}\text{"} - 0.37 \cdot \text{"}\mathbf{D/N_{75}}\text{"} + 17.32$$

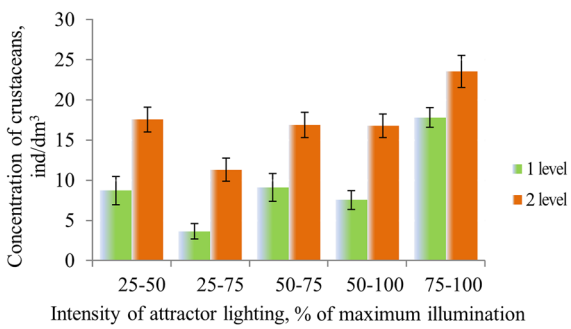

Fig. 4 Dynamics of the concentration of crustaceans at the first and second stages of paired photostimulation with different ratio of attracting light intensity

Response to increasing concentration of $K_2Cr_2O_7$ model toxicant

The introduction of the toxicant showed a decrease in the concentration of *D. magna* crustaceans, both at photostimulation with the intensity of 1150 lx (25%) and with 3450 lx (75%) (Fig. 6). In the medium contaminated with bichromate at the concentration of 0.06 mg/dm^3, there was a decrease in the concentration of crustaceans from 10.6 ± 5.1 to 4.5 ± 3.8 ind/dm^3 ($p = 0.35$, df = 18) at 1150 lx and from 20.4 ± 6.71 to 7.8 ± 7.1 ind/dm^3 ($p = 0.21$, df = 18) at 3450 lx. At the same time, the D/N_{75} decreased from 48.0 ± 5.3 to 42.0 ± 4.7% ($p = 0.41$, df = 18). A further increase in toxicant concentration to 0.12 mg/dm^3 and 0.24 mg/dm^3 significantly reduced this figure to 29 ± 3.2% ($p = 0.007$, df = 18) and 27 ± 3.0% ($p = 0.003$, df = 18), respectively, while the change in the concentration of crustaceans remained unreliable. Thus, the change in D/N_{75} reflected a change in the toxicity of the medium.

Dynamics of phototaxis at zooplankton exposure in the polluted medium

The dynamic response to the pollutant was studied at $K_2Cr_2O_7$ concentration of 0.12 mg/dm^3 and microplastics of 0.5 mg/dm^3 ($5 \cdot 10^5$ particles/dm^3). Due to the fact that in order to create the tested concentrations, the pollutants in the volume of 0.5 dm^3 were added to the tank, and we previously performed a series of tests with the introduction of 0.5 dm^3 of clean cultivation water (control). The average concentration of crustaceans during the first (C1) and second (C2) photostimulation stages was determined based on the reconstructed holograms, alongside with the ratio of the concentration increase during the transition from the first to the second intensity to the concentration during the second stage: $D/N_{75} = (C2 - C1)/C2 * 100\%$.

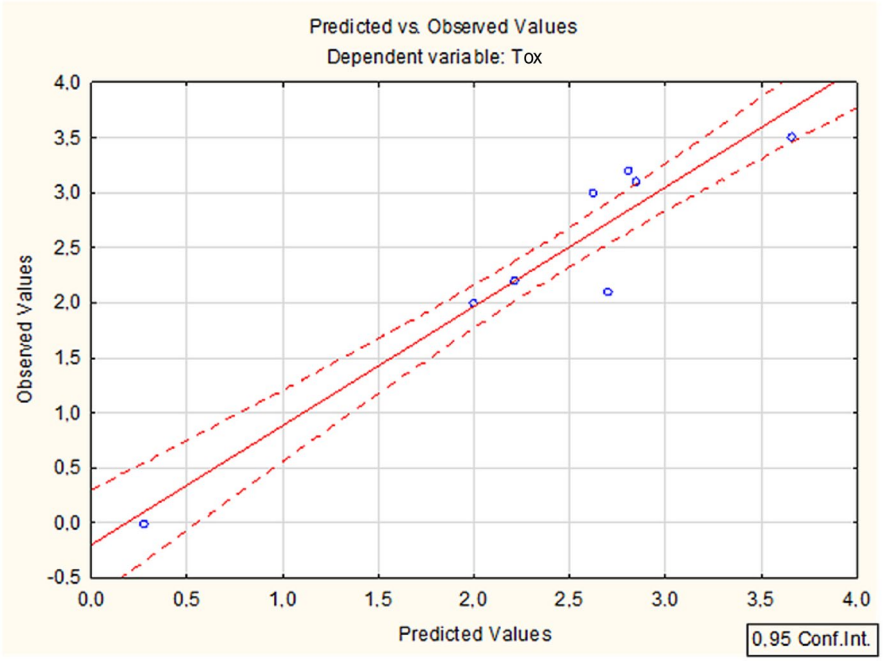

Fig. 5 The correlation of observed and predicted simplified model values

The study showed that the introduction of 0.5 dm^3 of cultivation water without a pollutant did not register significant changes in phototaxis parameters: during the observed period (min. 180), the C_1 of *D. magna* changed from 1.8±0.8 to 0.8±0.6 ind/dm^3 ($p>0.1$) and *D. pulex* from 2.1±0.7 to 1.9±0.5 ind/dm^3 ($p>0.1$). In this case, the C_2 of *D. magna* changed from 6.8±0.7 to 5.8±0.6 ind/dm^3 ($p>0.1$) and *D. pulex* from 6.1±0.7 to 5.2±0.4 ind/dm^3 ($p>0.05$). The change in the D/N_{75} index did not differ significantly at the beginning and end of the experiment: 74±11% and 86±12% ($p=0.22$) for *D. magna* and 66±11% and 63±8% ($p=0.41$) for *D. pulex*.

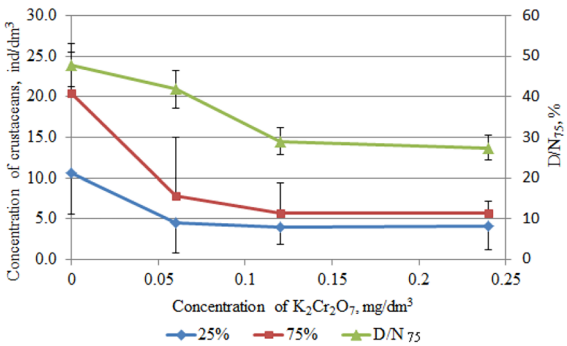

Fig. 6 Dependence of the concentration of crustaceans and their concentration increase at paired photostimulation on toxicant concentration in the medium

Contamination with potassium bichromate

The contamination of the aqueous medium with potassium bichromate at the concentration of 0.12 mg/dm^3 led to a phase change in the D/N_{75} of *Daphnia magna* Straus (Fig. 7a). Prior to the application of a pollutant, the D/N_{75} made 86±12%. After the pollutant was introduced, the indicator variability increased, and only by 120 min, there was a significant decrease in the indicator to 56±8% ($p=0.02$); by 150 min, to 48±5% ($p=0.003$); and by 180 min, to 53±6% ($p=0.01$). Thus, this phototaxis indicator reflected the cumulative action of the toxicant.

The D/N_{75} dynamics of *D. pulex* was somewhat different. When the background value equaled 51±4%, the indicator decreased to 42±7% ($p=0.04$) starting at 30 min. It further decreased to 36±8% ($p=0.02$) by 60 min, to 41±8% ($p=0.03$) by 90 min, and to 38±10% ($p=0.02$) by 120 min. Then the value was increased to the initial value (Fig. 7b).

Contamination with microplastics

The concentration of microplastic particles in the amount of $5{\cdot}10^5$ U/dm^3 (0.5 mg/dm^3) led to a phase change in D/N_{75} of *D. magna* (Fig. 8a). Prior to the application of a pollutant, the D/N_{75} made 67±4% (Bv). After the pollutant was introduced, there was

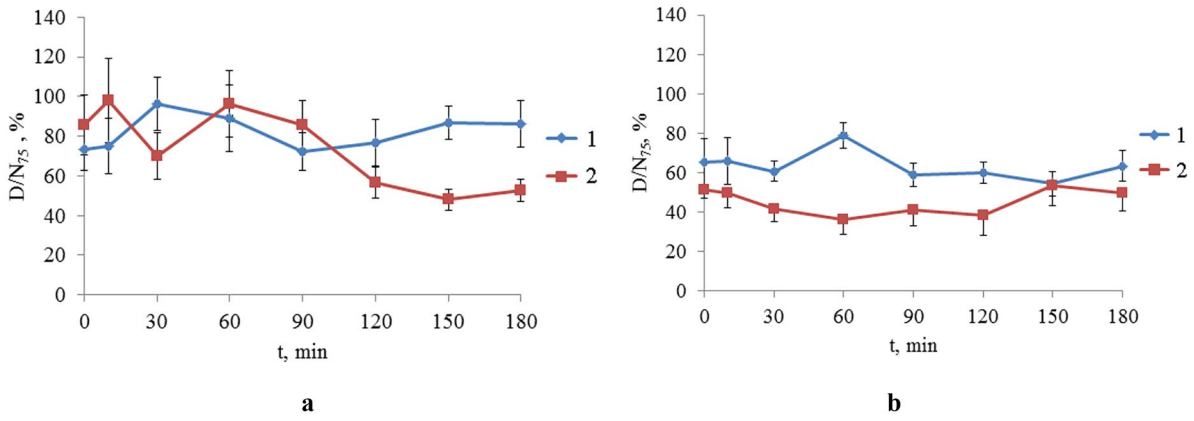

Fig. 7 D/N_{75} dynamics of *Daphnia magna* Straus (**a**) and *Daphnia pulex* (**b**) under introduction of $K_2Cr_2O_7$ (1, cultivation water; 2, potassium bichromate)

a short-term increase of up to 74 ± 5% ($p = 0.05$). Then, there was a decrease in the indicator to 57 ± 4% ($p = 0.006$) by 30 min, to 51 ± 3% ($p < 0.001$) by 60 min, and to 48 ± 4% ($p < 0.001$) by 90 min. After 120 min, we observed an increase in D/N_{75} to 64 ± 6% ($p = 0.26$) and a further decrease of 59 ± 5% ($p = 0.03$) at 150 min and 48 ± 6% (p = 0.003) at 180 min. Thus, similar to potassium bichromate exposure, the phototaxis index reflected the cumulative action of the pollutant.

The introduction of microplastics led to a phase change in D/N_{75} of *D. pulex* (Fig. 8b). After the pollutant was introduced, the indicator decreased over 120 min from 74 ± 5% ($p = 0.01$) by 10 min, to 65 ± 6% ($p = 0.4$) by 30 min, to 51 ± 8% ($p = 0.01$) by 60 min, to 49 ± 10% ($p = 0.02$) by 90 min, and to 48 ± 12% ($p = 0.03$) by 120 min. After 2 h, the indicator increased to 67 ± 11% (p = 0.3) by minute 150 and to 68 ± 19% (p < 0.001) by minute 180. The change in D/N_{75} was similar to its change during contamination with potassium bichromate and could be explained by the same reasons.

Discussion

The available literature does not reflect data on the use of the paired photostimulation in the bioindication of pollution of aquatic ecosystems. However, there are numerous data on light-dependent zooplankton migration (Colangeli et al., 2019; Kim et al., 2018; Moeller

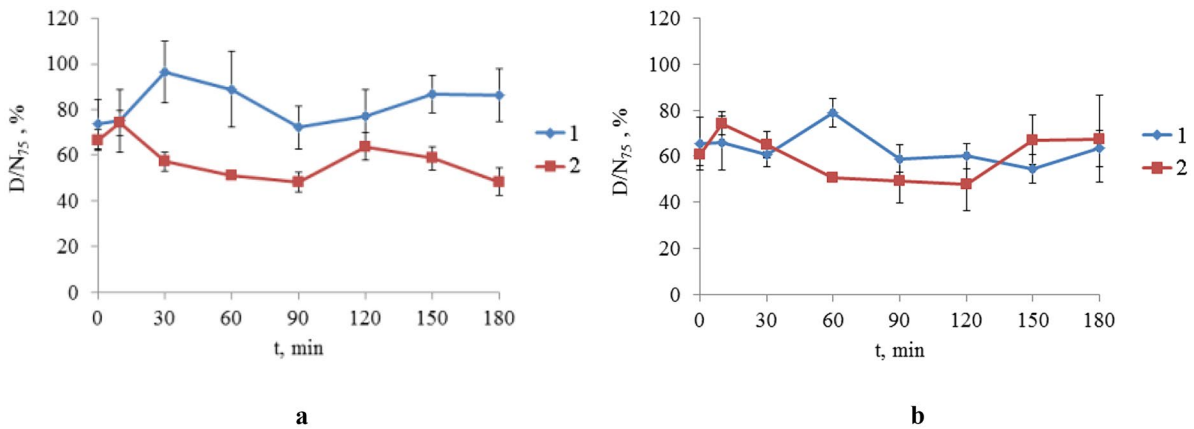

Fig. 8 D/N_{75} of *Daphnia magna* Straus (**a**) and *Daphnia pulex* (**b**) under introduction of microplastics (1, cultivation water; 2, microplastics)

et al., 2019; Overholt et al., 2016; Sha et al., 2021; Simão et al., 2019). The insignificant increase in the phototropic response of daphnia that we observed with an increase in the intensity of attracting light from 3450 to 4600 lx may be associated with the change in the positive phototaxis of some individuals to negative at high lighting intensity. The mechanism of this behavioral response in crustaceans has not yet been fully studied (Simão et al., 2019, Bedrossiantz et al., 2020). Similar changes, up to the inversion of the phototaxis sign during water contamination, were also noted by some authors (Simão et al., 2019; Maher et al., 2014; Bedrossiantz et al., 2020; Sha et al., 2021; Colangeli et al., 2019), including the contamination with photoreactive particles, such as zinc nanoxide and microplastics (Bhuvaneshwari et al., 2017; Lehutso et al., 2021). We found that the introduction of 0.5 dm^3 of cultivation water without a pollutant causes a slight increase in the motor activity of crustaceans and does not significantly change their response to the contamination of the medium. The response to $K_2Cr_2O_7$ exposure at an increasing concentration (of a model toxicant of most standard biotests for determining the sensitivity of hydrobionts in assessing the quality of the aqueous medium in laboratory conditions) is quite unambiguous and gradual, which makes it possible to compare the sensitivity of test organisms and test responses.

The study revealed that despite the phase directionality of changes, the general pattern during the contamination of the medium with potassium bichromate and microplastic particles is the inhibition of the phototropic response of *D. magna* and *D. pulex* crustaceans to paired photostimulation. It was noted that the cumulative dose of exposure depends on the concentration and time of exposure to the toxicant: $D = C \cdot t_{ex}$, where C is the concentration of the toxicant and t_{ex} is the time of exposure to the toxicant. For *D. magna* and *D. pulex*, the LC_{50} by the death rate occurs within 24 h in the concentration range of $0.9 \div 2.0$ mg/dm^3, which is consistent with the requirements of test culture sensitivity techniques (ISO 6341:2012). Therefore, the half-lethal dose (LD_{50}) is in the range of $21 \div 48$ mg·dm^{-3}·h, and the maximum permissible dose that does not cause a reliable response (LD_{10}; $LD \leq 10\%$) is ≈3 mg·dm^{-3}·h.

The analysis of the phototactic behavior of 7–8-day-old *D. magna* individuals in the presence of 11 chemicals commonly found in aqueous media showed that phototaxis is quite useful to detect a wide range of potentially toxic substances (Martins et al., 2007). The lowest concentrations found by the authors using this method were from 2 to 43 times lower than the LC_{50} for *D. magna* (Martins et al., 2007).

We recorded a different latent period in the phototropic response of two Crustacea species in the contaminated medium: 2 h for *D. magna* and 1 h for *D. pulex*. It corresponds to the cumulative doses of exposure 0.24 mg·dm^{-3}·h and 0.12 mg·dm^{-3}·h that is significantly less than LD_{10} ≈3 mg·dm^{-3}·h determined by the death rate of crustaceans. Thus, the sensitivity of *D. pulex* is higher; they have an earlier but transient response, which must be taken into account for alarm response systems. This fact may be interpreted as the expansion of the compensatory response either by the avoidance type or by the type of adaptation syndrome, which is less pronounced compared to *Daphnia magna* Straus due to the smaller size of the body and the amount of the absorbed pollutant (Bogolyubov & Kravchenko, 2018).

The differences in the phototropic response of *D. magna* and *D. pulex* during the contamination of the medium with microplastics are not so large. *D. magna* demonstrated a reliable response to the introduction of microplastic in 30 min of exposure and *D. pulex* in 60 min. This corresponds to the cumulative doses of exposure $2.5 \cdot 10^5$ particles·dm^{-3}·h and $5 \cdot 10^5$ particles·dm^{-3}·h. Similar to the contamination with potassium bichromate, *D. pulex* showed a transient response, while the inhibition of phototaxis to paired photostimulation was increasing for *D. magna*. At the same time, the rate of decline of the $\Delta C/C_2$ ratio—the slope ratio of the line approximating the dynamics of this indicator (Morgalev et al., 2022)—in the first 2 h after the application of the pollutant is significantly greater (14.4 ± 0.5 p.p./h) for *D. pulex* than for *D. magna* (5.6 ± 0.7 p.p./h, $p > 0.0001$), which indicates their higher sensitivity.

Similar results were obtained by the authors establishing the differences between the responses of *D. magna* and *D. pulex* under the action of nanoscale and molecular pollutants (Noskov, 2011; Völker et al., 2013. One possible explanation is the difference in the size of crustaceans: the size of *D. pulex* is 1.5–2 times smaller than *D. magna* (Bogolyubov & Kravchenko, 2018). The smaller size of crustaceans, including the smaller size of their digestive system, may lead to faster and more complete filling with mPl particles, as well as to a higher specific concentration of microparticles adsorbed

on the surface of the body (Bergami et al., 2017; Varó et al., 2019). It can be assumed that by increasing the mPl particle size and reducing its bioavailability, it will cease to pose a threat to this food link, at least as an anti-nutrient or ballast substance adsorbing and concentrating harmful substances.

Since mesoplankters as filtrators form the core of food chains, (i) they filter a large amount of water by eating the bacteria and algae contained therein, as a result of which the presence of harmful substances even in small concentrations causes significant changes in their condition, (ii) even slight changes in their behavior (for example, changes in the severity of their phototropic responses, or even the inversion of phototaxis) may result in their increased death due to the inhibition of the predator avoidance during the daytime (Simão et al., 2019). Poor correlation between daily vertical migration and light conditions (Gehring & Rosbash, 2003) may change the likelihood of crustaceans meeting predators of a higher level of the food chain and hamper the food balance in this contaminated biotope.

Thus, the toxicity of plastics to mesoplankton depends on the species of crustaceans. The correlation between the sensitivity and species membership, size, and even nutritional preferences of crustaceans is described in the work of Bai et al. (2021). This fact implies the need to take into account the collective response of the mesoplankton community of the controlled water body, which is only possible using the method that allows measuring the responses of autochthonous plankton in situ.

It is quite difficult to compare the obtained results with literature data due to different experimental designs and mainly due to limited amount of data on the phototropic response of crustaceans. It is impossible to determine the sensitivity of the proposed method to mPl contamination due to the variety of mPl particles used by researchers (we did not find acute toxicity within a 48-h test). However, the very fact that the response to paired photostimulation is dependent on the cumulative dose of mPl particles in a fairly short time is quite promising for the prompt detection of pollution of the water area.

Conclusion

The alarm signal should be formed at the early stages of the appearance of small concentrations of pollutants in the water body in order to monitor and manage the condition of water bodies based on the responses of test organisms sensitive to alternating effects but resistant to natural changes in habitat conditions. These requirements are most applicable to the response of an autochthonous mesoplankton filtrator adapted to the conditions of a particular reservoir or water area.

We studied the variability of the behavioral phototropic response of freshwater mesozooplankton during habitat contamination in laboratory conditions. The study revealed the inhibition of phototaxis after the application of pollutants (standard toxicant—potassium dichromate and microplastics). However, the large variability in the concentration of crustaceans in the background and in response to photostimulation does not provide for reliable determination of the dose dependence of this change and makes it impossible to determine its critical level.

In order to unambiguously interpret the phototropic responses of plankton for the early detection of violations in environmental well-being, we proposed to modify the method of initiating the behavioral response—paired photostimulation. It includes sequential presentation of two light stimuli of increasing intensity. The first, lower intensity exposure, activates the functional system responsible for phototaxis, which reduces the inter-individual dispersion. The second, more intense exposure, makes the crustaceans gather in the illuminated area at a rate corresponding to their physiological state.

The study determined the optimal modes of attracting light. When the toxicity of the medium changed, the most informative was the change in the concentration of crustaceans in the illuminated volume, but not the speed or direction of their movement. The minimum variability and maximum response when exposed to pollutants were typical for $\Delta C/C2$—the ratio of the difference in the concentration of crustaceans during the first and second intensity of photostimulation to the concentration of crustaceans during the second intensity.

It was found that the sensitivity of such modification of the method to the introduction of pollutants exceeds the sensitivity of the method for determining the toxicity of the medium in terms of the reduction of mobility and death rate of test organisms. This indicates the potential of using the phototropic response of zooplankton to monitor the quality of its habitat for the early diagnostics of the water area pollution.

In the near future, we plan to compare the results obtained using this method in the laboratory with field data obtained in a freshwater reservoir—Lake Baikal.

Author contribution Victor Dyomin: conceptualization, funding acquisition, methodology, supervision, writing—review and editing. Yuri Morgalev: data curation, investigation, methodology, validation, writing—original draft. Sergey Morgalev: data curation, formal analysis, investigation, validation, visualization. Tamara Morgaleva: investigation. Alexandra Davydova: data curation, software. Igor Polovtsev: conceptualization, data curation. Nikolay Kirillov: data curation, software. Alexey Olshukov: funding acquisition, project administration, resources. Oksana Kondratova: investigation.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The study does not involve human participants, their data, and biological material. All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

Conflict of interest The authors declare no competing interests.

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