ISSN 1995-0829, Inland Water Biology, 2024, Vol. 17, No. 2, pp. 354–364. © Pleiades Publishing, Ltd., 2024.

METHODS

Early Detection of Contamination with Microplastics by Changing the Phototaxis of Freshwater Mesozooplankton to Paired Photostimulation

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Received December 6, 2022; revised July 28, 2023; accepted August 24, 2023

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. This paper proposes a modification of the photostimulation method: paired photostimulation involving the 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 study demonstrates good reliability and increased sensitivity of this method of detecting changes in environmental toxicity when compared with single photostimulation or traditional bioindication through the survival rate of test organisms.

Keywords: zooplankton, behavioral response, paired photostimulation, water pollution monitoring, submersible holographic camera

DOI: 10.1134/S1995082924020056

INTRODUCTION

Although the use of plastic materials has brought enormous social benefits, the "plastic age" has come with great challenges (Wagner et al., 2014). One emerging issue of increasing concern is the accumulation of plastic in marine and freshwater ecosystems. The huge amount of microplastics (particles from 1 μm to 5 mm) floating on the ocean surface (Jambeck et al., 2015; Wagner and Lambert, 2018), currently estimated at tens to hundreds of thousands of tons (Weiss et al., 2021; Pedrotti et al., 2022), is particularly alarming. Most marine microplastics are thought to come from terrestrial sources, including the surface waters of rivers, lakes, and reservoirs (Ilyina et al., 2021; Ivanova et al., 2021; Frank et al., 2021; Lisina et al., 2021; Weiss et al., 2021; Pedrotti et al., 2022; Nava et al., 2023).

Microplastics are a heterogeneous class of pollutants with a broad spectrum of effects. The diverse characteristics of microplastics (material type, particle size, and particle shape) make them potentially accessible to a wide range of neustonic (floating materials, density <1 g/cm^3), pelagic (materials in suspension), and benthic species (sedimentary materials with a density >1 g/cm^3) (Scherer et al., 2018). This allows microplastics to enter aquatic food webs more easily than larger particles.

It is known that a wide range of organisms, including zooplankton (crustaceans *Daphnia magna* Straus, 1820; *Gammarus pulex* L., 1758; and *Notodromas monacha* O.F. Müller, 1776 and gastropods *Potamopyrgus antipodarum* JE Gray, 1843), fish, and cetaceans, are able to absorb microplastics. Rotifers, cladocerans, and mussels are thought to be particularly prone to ingesting microplastics, as they typically feed on suspended solids. For example, rotifers (*Anuraeopsis fissa* Gosse, 1851) and cladocerans (*Daphnia* sp.) can readily feed on plastic pellets (Cózar et al., 2014; Van Sebille et al., 2015). Filter feeders (e.g., daphnids) use an advanced filtration apparatus to filter suspended particles, and copepods actively capture and process

suspended particles with modified appendages (Wagner et al., 2014).

When ingested, microplastics can have a variety of negative effects on aquatic organisms in the form of physical and/or chemical damage (Wagner et al., 2014; Wagner and Lambert, 2018). To date, research into the potential adverse effects caused by exposure to microplastics compared to marine species in freshwater organisms is sparse and has primarily been conducted on filter feeders *Daphnia magna* (Besseling et al., 2014; Ogonowski et al., 2016; Rehse et al., 2016); amphipods *Hyalella azteca* Saussure, 1858 (Au et al., 2015) and *Gammarus pulex* L., 1758 (Weber et al., 2018); and freshwater snails *Potamopyrgus antipodarum* JE Gray, 1843 (Romero-Blanco et al., 2021), as well as several fishes (Karami et al., 2016; Lu et al., 2016; Rochman et al., 2013). While existing research suggests that a wide range of aquatic taxa are susceptible to the adverse effects of microplastic ingestion, the toxicological consequences for freshwater species are largely unknown.

Studies in marine species have shown nutrient deficiencies caused by the extensive ingestion of microplastics replacing part of the natural diet (Cole et al., 2015; Phuong et al., 2016; Welden and Cowie, 2016). Additional nutrition-related effects have also been found: gastrointestinal obstruction and damage (Wright et al., 2013), inflammatory responses (Von Moos et al., 2012), and xenobiotic desorption (Browne et al., 2013). The significant reduction in algae consumption by marine copepods *Centropages typicus* when exposed to microplastics affects the growth and development of fertility and survival of organisms (Svetlichny et al., 2021).

Long-term exposure to microplastics disrupts reproduction dynamics and population survival (Browne et al., 2015), which can have serious consequences for food chains, as filter feeders are at the base of food chains, and ultimately lead to an imbalance in the ecological balance (Li et al., 2016).

There is evidence that the negative impact on aquatic organisms is exacerbated by the ability of microplastic particles to absorb a wide range of persistent organic pollutants and trace elements from the environment. The review paper (Saprykin and Samoilova, 2021) provides a detailed analysis of numerous laboratory studies on the negative effects of exposure to chemicals associated with microplastics—causing cellular toxicity and negatively affecting fish populations, energy reserves of coastal crabs, and the metabolic rate and survival of Asian green mussels, as well the growth, development, and survival of daphnia.

There is evidence of a positive correlation between the abundance of microplastics in seawater and the total abundance of zooplankton, especially copepods (Vasilopoulou et al., 2021**)**. In addition, filter feeders are more vulnerable to exposure to suspended microplastics (Scherer et al., 2018), and their individual behavior may change in response to environmental contaminants or stressors. Changes in behavior are early warning signals of consequences that can affect the entire ecosystem, because they link physiological changes in organisms and ecological processes in the system (Wong and Candolin, 2015).

Against the background of an altered physiological state of aquatic organisms when exposed to pollutants, including microplastics (Mattson et al., 2017), the response of euryhaline zooplankton to photostimulation changes (Dyomin et al., 2020, 2021). Although the mechanism of this effect on zooplankton remains poorly understood to date, our studies have shown that paired photostimulation makes it possible to detect the appearance of pollutants in the aquatic environment by behavioral reactions (Morgalev et al., 2022).

The current trend in monitoring the world ocean using submersible instruments that record parameters of behavioral reactions of autochthonous organisms in real time in situ provides a highly representative sample and reliable bioindication (Dyomin et al., 2019a).

The equipment created at Tomsk State University (digital holographic cameras and hydrobiological probes based on them) (Dyomin et al., 2019b) makes it possible to measure the parameters of individual particles, but differs from similar devices in the possibility of photostimulation with attractor radiation, causing a phototropic reaction of zooplankton (Dyomin et al., 2020). Along with the early detection of contamination of freshwater ecosystems with microplastics, the advantages of this method include the operational monitoring of the state of natural water bodies, which makes it possible to register the negative impact of pollutants in low concentrations on the biota, which, as a rule, cannot be detected by similar devices and traditional bioindication methods.

The purpose of the work is to identify the possibility, features, and conditions of using the method of paired photostimulation for the early detection of contamination of water areas with microplastics.

MATERIALS AND METHODS

Organisms under study. The studies were carried out using two species of freshwater zooplankton, cladoceran crustaceans (Cladocera) *Daphnia magna* and *Daphnia pulex*. The *D. magna* culture was received from OOO Evropolitest (Russia). *D. pulex* individuals were isolated from a natural population in the vicinity of Tomsk and adapted to laboratory conditions for 8 months. The introduction of wild species into laboratory culture was described by us previously (Dyomin et al., 2021). The maintenance of cultures and experiments were carried out under conditions according to the recommendations of the method (ISO 6341, 2012):[1] temperature 22 ± 2°C, pH 7.0–8.5, cultivation

[1] ISO 6341:2012 "Water quality—Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)—Acute toxicity test."

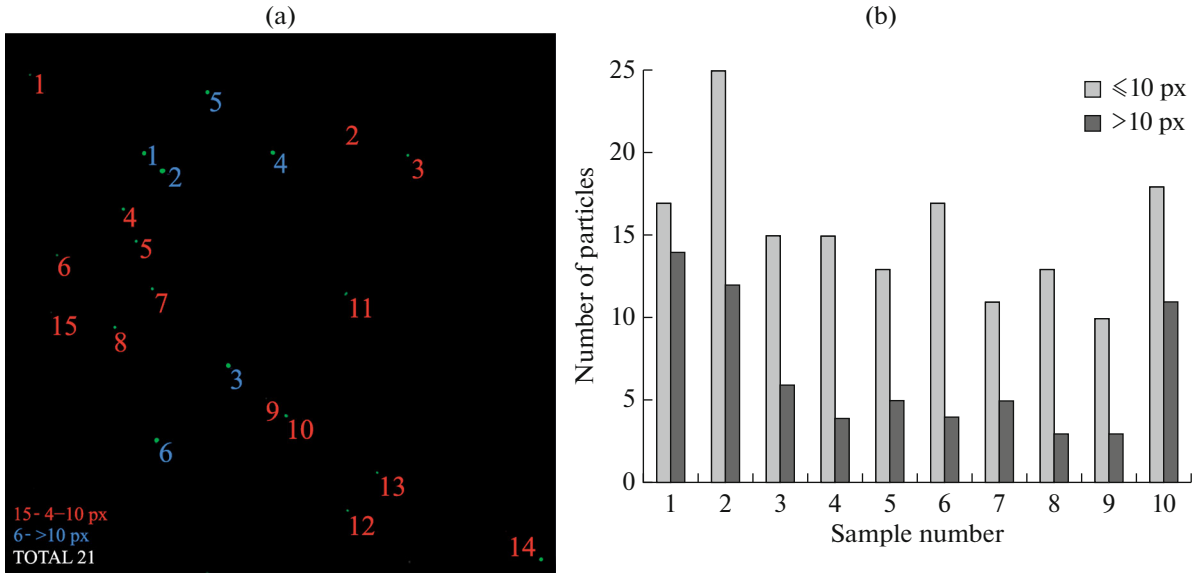

Fig. 1. Microplastic concentration and size in the image under a confocal microscope (size ≤10 px, numbers 1–15 and size >10 px, numbers 1–6) (a) and on the diagram in particle samples (b).

medium drinking water (SanPiN 2.1.3684-21),[2] O_2 content 6 mg/dm^3, photoperiod 12 h light/12 h dark.

Pollutants. A substance with pronounced toxicity, the standard model toxicant $K_2Cr_2O_7$ was used as pollutant (Merck KGaA, Germany), as were microplastics made from materials that are biologically inert in macroform. The microplastics were prepared according to a method developed in the laboratory from fibers of woven polypropylene bags (OOO Terra, Russia), aged under natural conditions for 12 years (Morgalev et al., 2022). The concentration and size of microparticles were determined from images obtained using a confocal microscope (Zeiss, LSM 780 NLO) excited by a laser with a wavelength of 405 nm. The autoluminescence of microplastic particles excludes the inclusion of particles from other materials. In layers measuring 850 × 850 × 2.7 μm (Fig. 1a), microplastic particles were visualized as compact groups with an area of 1–25 pixels (0.7–19 μm^2). The number of particles with sizes <10 pixels (diameter of a circle with an equivalent area of ~4 μm) and >10 pixels was counted (Fig. 1b). The content of particles <10 pixels in size in the layer was 15.4 ± 1.4 pcs and the content of larger sizes was 6.7 ± 1.3 pcs (70 and 30% respectively). The concentration of microplastics in the resulting initial suspension was ~10^7 particles/dm^3 (200 mg/dm^3).

[2] SanPiN 2.1.3684–21 "Sanitary and epidemiological requirements for the maintenance of territories of urban and rural settlements, for water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, the operation of industrial and public premises, and the organization and implementation of sanitary and antiepidemic (preventive) measures."

Digital holographic camera. The design of the submersible digital holographic camera (DHC) has been described in detail previously (Dyomin et al., 2020). The DHC allows one to register holograms of a controlled volume, which is illuminated by a laser beam (Fig. 2), with further sequential numerical reconstruction of images of layers of the medium with a given step and the study of particles located in these layers. Lasers with a wavelength of 532 nm were used to photostimulate the motor activity of plankton and those with a wavelength of 650 nm to record holograms. The wavelength of attractor lighting for photostimulation is close to the local maximum of the reflection spectrum of microalgae, the main source of nutrition for zooplankton. The maximum laser radiation power at the output of the illumination module illuminator (Fig. 2) was 4 mW and was regulated by changing the control voltage. This created a maximum illumination of 4600 lx.

During laboratory experiments, DHC was placed in a container with a volume of 90 dm^3, filled with 50 dm^3 of the aqueous medium under study, which ensured that the optical part was below the liquid level.

Principles of the paired photostimulation method. Using the phototropic response of plankton as an indicator of an impact that does not cause irreversible changes in the planktonic community involves recording the dynamics of the response to photostimulation compared to the background.

The standard way to measure this indicator is $\Delta R/\Delta I$, i.e., the ratio of the change in reaction (number of crustaceans) per unit increase in lighting intensity. Since the response of biological systems to a stimulus of increasing intensity differs from linear, depending on the intensity of the impact, a wide range

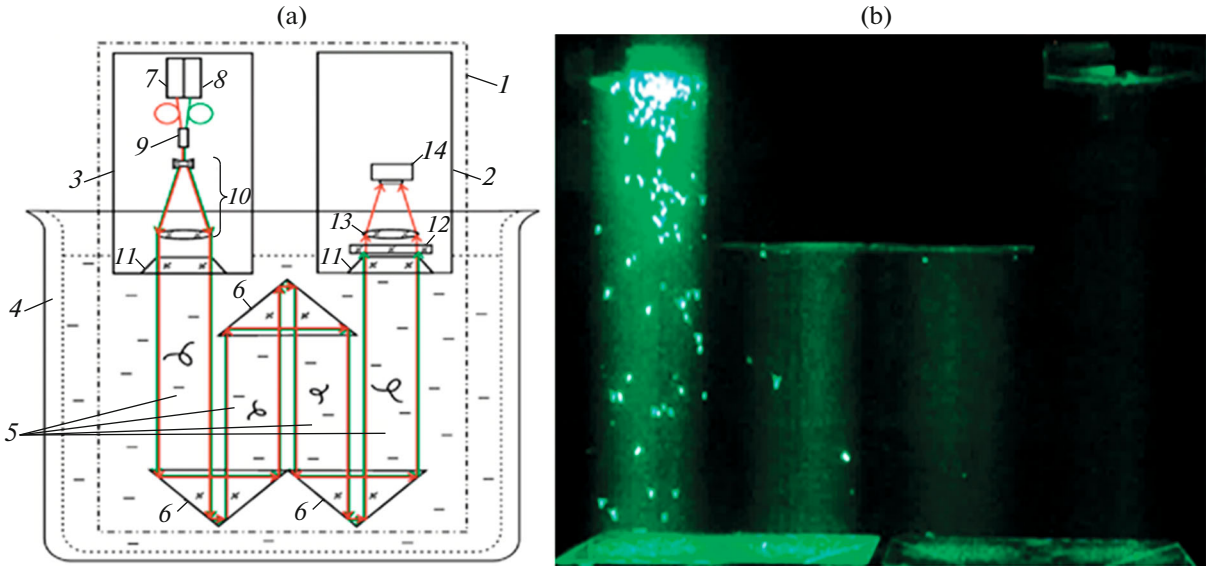

Fig. 2. Scheme of the submersible digital holographic camera (DHC) (a) and pillars of light with aquatic organisms formed laser bunches (b). (*1*) DHC, (*2*) DHC recording module, (*3*) DHC lighting module, (*4*) container with water, (*5*) controlled volume (VR) (limited by beams of recording (red) and attractor (green) light), (*6*) mirror-prismatic system for forming the working volume, (*7, 8*) semiconductor laser diode (λ = 650 and 532 nm, respectively), (*9*) fiber optic multiplexer, (*10*) beam expander, (*11*) portholes, (*12*) selective filter, (*13*) receiving lens, and (*14*) CMOS camera.

of $\Delta R/\Delta I$ values can be obtained. In addition, the accuracy of determining the $\Delta R/\Delta I$ indicator decreases for several reasons: nonzero variability in the behavioral response of plankton in the background (interindividual scatter) and the increasing variability of the behavioral response in the case of the insufficient intensity of photostimulation, which does not lead to the activation of systems that ensure the implementation of the behavioral response. Previously conducted laboratory and field experiments have shown that one of the options for solving this situation is to use paired photostimulation (Morgalev et al., 2022). First, a less intense impact activates the functional system responsible for the behavioral response (Anokhin, 1974), and accordingly, interindividual dispersion and entropy in the system decrease (Morgalev and Morgaleva, 2007). Secondly, a more intense impact causes the movement of crustaceans at a speed appropriate to their physiological state. The use of paired photostimulation significantly reduces the extrapolation error and makes it possible to increase the accuracy of assessing the degree of impact on zooplankton, including the degree of toxic impact.

Experimental design. On the day of the experiment, 1 h before the start, a synchronized 1-day culture of crustaceans was fed with algae *Chlorella vulgaris* B. according to the methodology (ISO 6341, 2012). After 1 h, the crustaceans were transferred into an aquarium with a DHC chamber in an amount of 200 ± 10 ind., which corresponded to the concentration in natural reservoirs (4000 ind./m^3). Experiments were carried out after the adaptation of the crustaceans (after 1 h) with the registration of the phototropic reaction in a clean (control) and contaminated (experiment) environment for 3 h.

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

The dynamics of the phototropic reaction were recorded over eight cycles: the first cycle (background) before the introduction of cultivation water (in the control series) or pollutant (in the experimental series) and seven consecutive cycles 10, 30, 60, 90, 120, 150, and 180 min after adding water or pollutant. Each cycle consisted of a 15-min continuous registration of 30 holograms and was divided into three equal 5-min intervals with the registration of 10 holograms: without turning on the attractor lighting (I_0), under attractor lighting with intensity I_1 (1150 lx), and with subsequent second photostimulation with illumination I_2 (3450 lx). After each cycle (except for the second), there was a 15-min pause without lighting to restore the number of crustaceans.

Based on the results of processing ten sequentially recorded holograms, the average concentration of crustaceans was calculated during the first and second stages of photostimulation (C_1 and C_2 respectively), as well as the ratio of the increase in the concentration of crustaceans during the transition from the 1st to the 2nd intensity to the concentration of crustaceans during the second intensity: $\Delta C/C_2 = (C_2 - C_1)/(C_2 \times 100$ pp).

Statistical data processing was carried out using the Statistica v.10 program (StatSoft, Inc, United States).

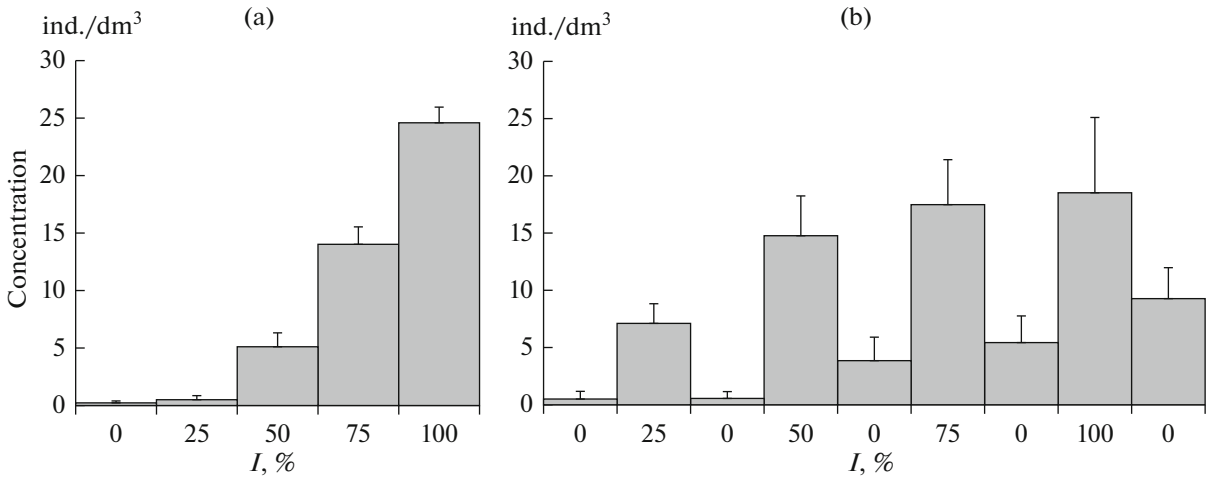

Fig. 3. Concentration of crustaceans (ind./dm^3) with a stepwise continuous (a) and stepwise intermittent (b) increase in the intensity of attractor illumination (*I*, % of maximum).

After testing for normality of distribution using the Shapiro–Wilk test, differences in mean values were determined by Student's *t*-test for independent variables (two-sided *p*-level).

The data is given in the form $M \pm m$, where m is the standard error of the mean M.

RESULTS

Threshold intensity of photostimulation for the appearance of a behavioral response in crustaceans. The crustaceans began to gather in the illuminated column of the aquatic environment at an attractor radiation power of 200 ± 15 μW, which corresponded to 5% of the maximum laser radiation power. Stable results were obtained with attractor illumination with a radiation power of ≥20% of the maximum laser radiation power. Taking into account the data that were obtained, the following gradations of illumination were used in further studies: 25, 50, 75, and 100% maximum, which corresponded to an illumination of 1150, 2300, 3450, and 4600 lx, respectively. It is important to emphasize that the laser with a wavelength of 650 nm, used to register holograms, did not cause the collection of crustaceans even with long-term continuous exposure (15 min), and the duration of its activation under the experimental conditions was ≤50 μs/min.

Selection of paired photostimulation parameters. To use paired photostimulation, it is necessary to determine the ability of an ensemble of crustaceans to rearrange during the second exposure.

With a 5-min photostimulation stepwise increasing in intensity, a stepwise increase in the concentration of crustaceans in a controlled volume was recorded (Fig. 3a).

In the absence of attractor lighting (background), almost all crustaceans were outside the observed columns of the aquatic environment, and their concentration (*C*) in the working volume on average over 5 min was 0.1 ± 0.1 ind./dm^3. After turning on the attractor lighting with an intensity of 25% of the maximum laser radiation power in the illuminated columns of the aquatic environment, single individuals were recorded ($C = 0.6 \pm 0.4$ ind./dm^3). Starting from 50% illumination, pronounced positive phototaxis of crustaceans was noted; their concentration increased significantly ($C = 5.0 \pm 1.2$ ind./dm^3 ($R = 0.001$, $df = 18$)), reaching $C = 14.0 \pm 1.4$ ind./dm^3 ($R < 0.0001$, $df = 18$) at 75% illumination intensity and $C = 24.6 \pm 1.4$ ind./dm^3 ($R < 0.0001$, $df = 18$) at 100%. It should be noted that, with increasing intensity of attractor lighting, the variability of the concentration of crustaceans decreases from 100% in the background to 6% at maximum illumination.

With a stepwise increase in the intensity of photostimulation with 5-min breaks between each 5-min illumination with an attractor light, there was no complete restoration of the concentration of crustaceans during periods without attractor illumination (Fig. 3b).

The degree of restoration of the concentration of crustaceans depended on the intensity of attractor lighting. At an attractor light intensity of 25% of the maximum possible for a given laser, the concentration of crustaceans during a 5-min break decreased by 92.3% of the average value during the period of photostimulation; at an intensity of 50% it decreased by 74.1%; and at 75% it decreased by 68.7%. After photostimulation with an intensity of 100%, 50% of the crustaceans remained in the recording zone; i.e., some crustaceans disperse from the recording zone during breaks. Therefore, it is advisable to present the second intensity photostimulation immediately after the first one, without a break between them.

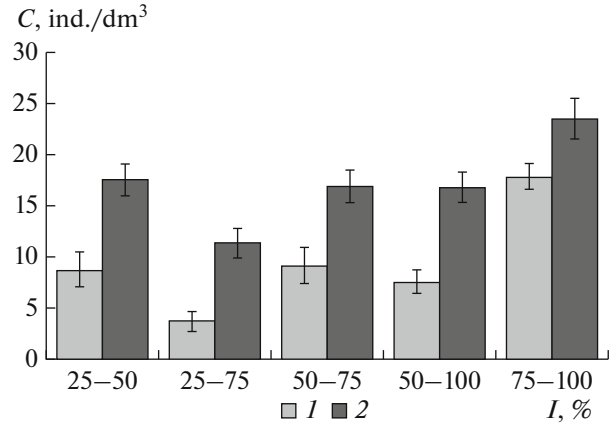

Fig. 4. Concentration of crustaceans (C, ind./dm^3) depending on the ratio of the intensity of attractor illumination (I, % maximum) at the first (*1*) and second (*2*) stages of paired photostimulation.

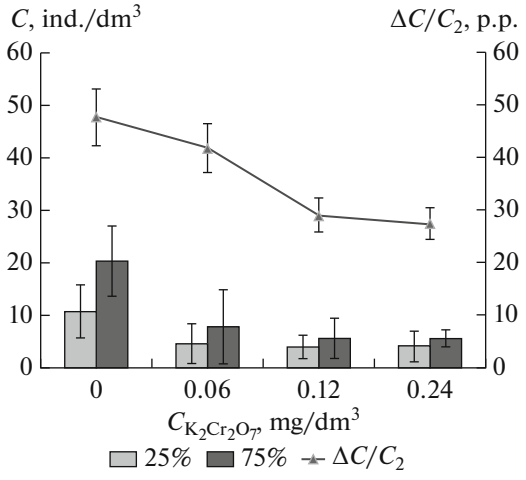

Fig. 5. Dependence of the concentration of crustaceans (C, ind./dm^3) and the increase in their concentration ($\Delta C/C_2$, p.p.) with paired photostimulation depending on the concentration of the toxicant.

To select the intensity of the first stage of photostimulation (I_1), the phototaxis threshold of crustaceans in the range of attractor illumination of 5–50% of maximum power with an increase step of 5% was determined. In the range above the threshold, we determined the intensity at which a local decrease in the coefficient of variation in the concentration of crustaceans occurs (I_1).

With different combinations of the intensity of attractor lighting of the first and second stages of paired photostimulation, the level of increase in the reaction is different. The greatest relative increase in the concentration of crustaceans (211 ± 27%) was observed at a combination of light levels of 25–75% (Fig. 4).

The following features in the behavioral reactions of crustaceans were revealed: the maximum increase in the concentration of crustaceans in response to the second photostimulation was when the intensity of attractor lighting of the first and second stages of paired photostimulation was combined at 25–75% of the maximum intensity; restoration of the initial state of the ensemble of crustaceans occurred in >10 min, so the minimum period of time before the next test is required to be >15 min.

Taking into account the data, a scheme for subsequent series of experiments was developed.

Response to increasing concentrations of model toxicant $K_2Cr_2O_7$. After recording phototaxis parameters in the background, the toxicant concentration was increased step by step every 30 min to 0.06, 0.12, and 0.24 mg/dm^3. The exposure time for each concentration is 30 min.

The reduced concentration of crustaceans *Daphnia magna* in a controlled volume occurred with the photostimulation of both low and high intensity (Fig. 5). However, these changes in the concentrations of crustaceans were unreliable due to the large variability of values. The resulting lighting multiple regression model I_1 = 1150 lx (25% max) and I_2 = 3450 lx (75% max)

$$T_{ok} = 2.51\Delta C - 1.60C_1 - 0.37\Delta C/C_2 + 17.32,$$

where T_{ok} is the product of concentration and time of action of the toxicant, $\Delta C = C_2 - C_1$ is the difference in the concentration of crustaceans (ind./dm^3) for 5 min of photostimulation at the first and second photostimulation intensity, $\Delta C/C_2$ is attitude ΔC to concentration at the second intensity, explains the relationship with predictors by 74% (adjusted $R^2 = 0.74$) with an accuracy of 0.55 ($p < 0.0001$). This made it possible to abandon the speed characteristics of the movement of crustaceans.

Indicator $\Delta C/C_2$ decreased gradually as the concentration of the toxicant increased; i.e., the suppression of the phototropic reaction was observed. A significant decrease in this indicator (from 48.0 ± 5.3 pp in the background to 29.0 ± 3.2 pp, $p < 0.0001$, $df = 18$) occurred at a toxicant concentration of 0.12 mg/dm^3.

Dynamics of phototaxis during plankton exposure to contaminated environments. The modeling of different toxicity of the environment can be carried out in two ways: increasing the concentration of the toxicant or increasing the time the crustaceans spend in an environment with its constant concentration.

Control series (application of cultivation water). A preliminary study showed that adding 0.5 dm^3 of cultivation water without a pollutant did not lead to a significant change in indicators, including the indicator $\Delta C/C_2$.

Contamination with potassium dichromate. Adding potassium dichromate in an amount creating a con-

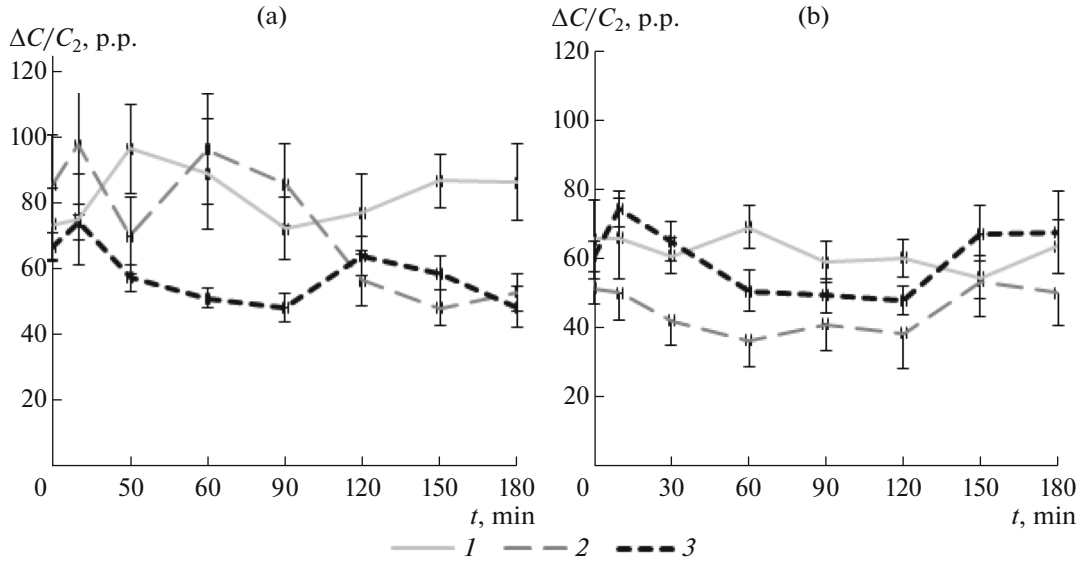

Fig. 6. Dynamics of the indicator $\Delta C/C_2$ for crustaceans *Daphnia magna* Straus (a) and *Daphnia pulex* (b) when adding cultivation water (*1*) and $K_2Cr_2O_7$ (*2*) and microplastics (*3*).

centration of 0.12 mg/dm^3 in the aquarium led to a change in the indicator $\Delta C/C_2$ in crustaceans *D. magna* (Fig. 6a): before adding the pollutant, the value of $\Delta C/C_2$ was 86 ± 12 pp; afterward, an increase in the variability of the indicator was observed, and only after 120 min did it significantly decrease to 56 ± 7 percentage points (R = 0.04, df = 18). A further decrease reached 48 ± 5 pp (R = 0.009, df = 18) after 150 min and 53 ± 6 pp (R = 0.02, df = 18) up to 180 min. Thus, this phototaxis indicator reflected an increase in the cumulative dose of the toxicant.

For *D. pulex,* the dynamics of the indicator $\Delta C/C_2$ was more complex. With a background value of 51 ± 4 pp, the indicator decreased to 36 ± 5 pp (R = 0.03, df = 18) after 60 min and remained almost unchanged until 120 min. Next, the value of $\Delta C/C_2$ increased to the initial level (Fig. 6b).

Microplastic contamination. Microplastic particles in a concentration of 5 × 10^5 units/dm^3 (0.5 mg/dm^3) led to multidirectional changes in the $\Delta C/C_2$ indicator of crustaceans *D. magna* (Fig. 6a). Before adding the pollutant, the $\Delta C/C_2$ value reached 67 ± 4 pp. After the addition of the pollutant, this indicator decreased to 51 ± 3 pp (R = 0.004, df = 18) after 60 min and to 48 ± 4 pp (R = 0.003, df = 18) after 90 min. After 1.5 h, the indicator increased after 120 min to 64 ± 6 pp (R = 0.68, df = 18) and further to 59 ± 5 pp (R = 0.2, df = 18) after 150 min and 48 ± 6 pp (R = 0.017, df = 18) after 180 min. Thus, as with the action of potassium dichromate, the phototaxis index reflected an increase in the cumulative dose of the pollutant.

The introduction of microplastics led to multidirectional changes in $\Delta C/C_2$ in crustaceans *D. pulex* (Fig. 6b). After adding the pollutant, after an unreliable increase to 74 ± 5 pp (R = 0.01, df =18) at the tenth minute, the indicator decreased to 51 ± 6 pp (R = 0.001, df = 18) after 60 min, to 49 ± 5 pp (R = 0.002, df = 18) after 90 min, and to 48 ± 4 pp (R = 0.001, df = 18) after 120 min. After this, there was an increase in the indicator to 67 ± 8 pp (R = 0.46, df = 18) after 150 min and to 68 ± 12 pp (R < 0.65, df = 18) after 180 min. The change in indicator C/C_2 occurred similarly to its change during contamination with potassium bichromate.

It should be noted that, according to the results of data obtained using DHC, after the introduction of microplastics in response to photostimulation, different dynamics of the number of crustaceans *D. magna* and *D. pulex* were observed (Fig. 7). If, during a stay in a contaminated environment in a DHC-controlled volume, the concentration of *D. magna* at the first and second stages of photostimulation remained approximately constant (5.1 ± 0.5 ind./dm^3 and 11.7 ± 0.5 ind./dm^3 respectively), then the concentration of *D. pulex* decreased at the first stage from 7.7 ± 2.9 ind./dm^3 (30 min) to 1.3 ± 0.6 ind./dm^3 (180 min) and, and at the second stage, from 21.9 ± 2.0 ind./dm^3 (30 min) to 4.0±1.2 ind./dm^3 (180 min).

DISCUSSION

In the literature available to us, there is no data on the use of paired photostimulation in the bioindication of pollution of aquatic ecosystems. However, there is extensive evidence of light-dependent migration of zooplankton (Overholt et al., 2016; Kim et al., 2018; Colangeli et al., 2019; Moeller et al., 2019; Simão et al., 2019; Sha et al., 2021). Moreover, this

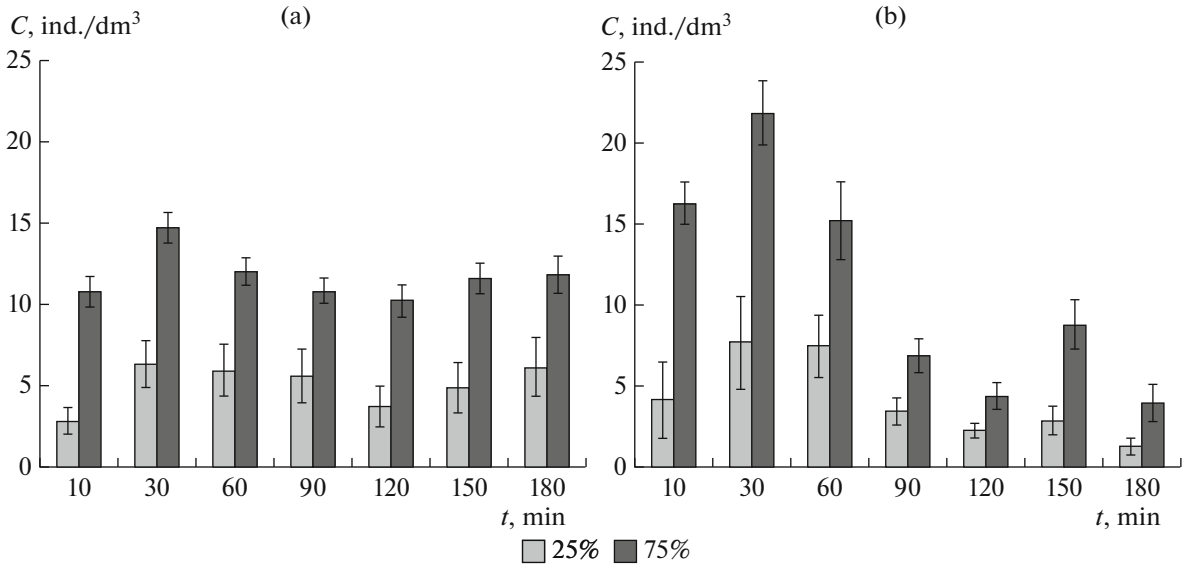

Fig. 7. Dynamics of the concentration of *D. magna* (a) and *D. pulex* (b) in an environment contaminated with microplastics.

migration changes up to the inversion of the sign of phototaxis during the contamination of the aquatic environment (Maher et al., 2014; Colangeli et al., 2019; Simão et al., 2019; Bedrossiantz al., 2020; Sha et al., 2021), including photoreactive particles such as nanozinc oxide and microplastics (Bhuvaneshwari et al., 2017; Lehutso et al., 2021).

There is evidence that the phototropic response correlates with the wavelength, intensity, and duration of the presented light stimuli (Mimouni et al., 1993; Storz et al., 1998; Maher et al., 2014; Overholt et al., 2016). In addition, differences in the behavior of copepods have been identified both at the interspecific and intraspecific levels (Overholt et al., 2016).

Potassium bichromate is considered a model toxicant in most standard bioassays (OECD 202, 2004[3]; ISO 6341:2012; OECD 236, 2013[4]; and OECD 203, 2019[5]) to determine the sensitivity of aquatic organisms; therefore, it is used by numerous authors as a testing influence when conducting laboratory studies. The response to toxic exposure to $K_2Cr_2O_7$ in increasing concentrations is unambiguous and gradual, which makes it possible to compare the sensitivity of test organisms and test reactions during biotesting.

Our work has established that, when the environment is contaminated with a model toxicant and microplastic particles, the general pattern is the suppression of the phototropic reaction of crustaceans *D. magna* and *D. pulex* for paired photostimulation.

Similar to Haber's formula $E = Ct$, where E is the effect and C and t are the concentration and duration of action (quoted from Erzhanova, 2023), the cumulative exposure dose can be calculated as $D = Ct_{ex}$, where C is the toxicant concentration and t_{ex} is the time of exposure of crustaceans to a contaminated environment. For *D. magna* and *D. pulex* when testing sensitivity to a model toxicant in accordance with (OECD 202, 2004), 50% mortality occurs within 24 h in the concentration range of 0.9÷2.0 mg/dm^3; i.e., the semilethal dose (D_{L50}) is in the range of 21–48 mg/dm^3 h. Then the maximum permissible impact that does not cause a reliable reaction, (<10%) D_{L10}, reaches ~3 mg/dm^3 h.

In our experiment, the reaction to the introduction of a toxicant was manifested in *D. magna* after 2 h of exposure and in *D. pulex* after 1 h. This corresponds to a cumulative exposure dose of 0.24 mg/dm^3 h and 0.12 mg/dm^3 h, which is significantly less than D_{L10} ~ 3 mg/dm^3 h, as determined by the death of crustaceans. Thus, the sensitivity of individuals of *D. pulex* is higher, and they have an earlier but transient reaction, which must be taken into account when creating early response systems.

Differences in the phototropic response of *D. magna* and *D. pulex* communities when the environment is contaminated with microplastics are not as great. A significant response to the introduction of microplastics was observed in *D. magna* after 30 min of

[3] OECD, 2004. Test No. 202: Daphnia sp. Acute Immobilization Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069947-en.

[4] OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines for the Testing of Chemicals, Section 2, 1-22. https://doi.org/10.1787/20745761.

[5] OECD, 2019. Test no. 203: Fish, Acute Toxicity Test, OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris. https://doi.org/10.1787/9789264069961-en.

exposure and in *D. pulex* after 60 min. This corresponds to cumulative exposure doses of 2.5×10^5 particles/dm^3 h and 5×10^5 particles/dm^3 h. As in the case of potassium bichromate contamination, for *D. pulex* a transient reaction was recorded and for *D. magna* the inhibition of phototaxis upon paired photostimulation was increasing. At the same time, the rate of decline in $\Delta C/C_2$ (tangent of the angle of inclination of the line approximating the dynamics of this indicator (Morgalev et al., 2022)) of *D. pulex* in the first 2 h after the introduction of the pollutant is significantly higher (14.4 ± 0.5 pp/h) than in *D. magna* (5.6 ± 0.7 pp/h, $p > 0.0001$), which indicates that they have a higher sensitivity.

Findings about the hypersensitivity of *D. pulex* to the molecular toxicant $K_2Cr_2O_7$ obtained as a result of applying the above method correspond to those obtained using standard biotesting methods. However, the method of paired photostimulation is more sensitive in the initial stages after the introduction of the toxicant.

With an increase in the size of microplastic particles and a decrease in their bioavailability, it is likely that they will no longer pose a danger to this link in the food chain, at least as an antinutrient or ballast substance that adsorbs and concentrates harmful substances.

The connection between the sensitivity of mesoplankton (plastic toxicity) and the species of crustaceans, their size, and even food preferences has been shown previously (Bai et al., 2021). Thus, there is a need to take into account the integral reaction of the entire mesoplankton community, which is only possible when recording the reactions of autochthonous plankton in situ.

A comparison with the literature data is very difficult due to different experimental designs, and mainly with a limited amount of data on the phototropic response of crustaceans. Due to the variety of microplastic particles used by researchers, it is impossible to determine whether our proposed method is more sensitive to microplastic contamination. However, the very fact that there is a dependence of the response to paired photostimulation on the cumulative dose of microplastic particles, and in a fairly short time, shows the promise of using this method for the rapid detection of water pollution.

CONCLUSIONS

Laboratory experiments have shown that the contamination of the habitat with microplastics leads to changes in the parameters of the behavioral phototropic response of mesozooplankton. To increase the sensitivity of this method for detecting small sublethal concentrations of microplastics, a method of paired photostimulation of the behavioral response of mesozooplankton has been developed that consists of the sequential presentation of light stimuli of increasing intensity. The optimal parameters and modes of attractor lighting have been determined. The use of paired photostimulation significantly increases the sensitivity of the method for detecting pollution based on the behavioral reactions of autochthonous mesoplankton. The sensitivity of the phototropic reaction during paired stimulation to the presence of pollutants exceeds the sensitivity of the method for determining the toxicity of the environment by the death of test organisms. Minimum variability and maximum response when exposed to pollutants are characteristic of the indicator $\Delta C/C_2$, the ratio of the difference in the concentration of crustaceans at the first and second intensity of photostimulation to the concentration of crustaceans at the second intensity. The studies showed the promise of using the phototropic reaction of zooplankton to monitor the quality of its habitat for the early diagnosis of microplastic pollution of water areas.

FUNDING

The expedition part of the work was carried out with financial support from the development program of Tomsk State University (Priority-2030); experimental research was carried out as part of the State Task of the Ministry of Science and Higher Education of the Russian Federation (project no. FSWM-2020-0019).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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