Original article

Experimental Study of Ultrasound Effect on Microperiphyton of Artificial Substrates for Fouling Protection of Technical Water Supply Circuit of Nuclear Power Plants

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Abstract

During exploitation of nuclear power plants, biofouling forms in the elements of the technical water supply circuit, which results in equipment malfunction, underproduction of electricity, and economic losses. One of the methods to prevent biofouling on immersed surfaces is ultrasound exposure. To study the peculiarities of biofouling development in water pipelines of a nuclear power plant, the impact of an ultrasonic device on the formation of benthic diatom algae (Bacillariophyta) – the primary stage in the succession of the microfouling community – was assessed. Microperiphyton consisting of diatoms, bacteria, and protozoa, forms biofilm on surfaces and promotes active development of macrofouling community leading to further reduction of efficiency of nuclear power plants. Long-term experiments were carried out in the laboratory and nearshore marine area to study the influence of ultrasonic device at different power and duration of exposure on periphyton development on steel and concrete samples. It was found that increasing the intensity of the ultrasonic device has a pronounced effect on microfouling of substrates reducing the abundance and species richness of diatoms. Based on the results, it was recommended to extend the experiments using a full-function ultrasonic device of higher power during exploitation of a nuclear power plant.

Keywords: biofouling, ultrasonic protection methods, nuclear power plant process equipment, benthic diatom, Bacillariophyta

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Экспериментальное изучение воздействия ультразвука на микроперифитон искусственных субстратов с целью защиты от биопомех систем технического водоснабжения атомных электростанций

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Аннотация

При эксплуатации атомных электростанций в элементах системы технического водоснабжения формируются биопомехи, приводящие к нарушению эксплуатации оборудования, недовыработке электроэнергии и экономическим потерям. Одним из методов предотвращения биообрастания на погружных поверхностях является воздействие ультразвука. С целью изучения особенностей развития биообрастания в водоводах атомной электростанции оценено воздействие ультразвукового устройства на формирование таксоцена бентосных диатомовых водорослей (Bacillariophyta) – первичного звена сукцессии сообщества микрообрастания. Микроперифитон, состоящий из диатомовых, бактерий и простейших, образует биопленку на поверхностях и способствует активному развитию сообщества макрообрастания, приводя к дальнейшему снижению эффективности атомных электростанций. В условиях лаборатории и морской акватории проведены длительные эксперименты по исследованию влияния работы ультразвукового устройства при разной мощности и продолжительности излучения на развитие перифитона на образцах субстрата из стали и бетона. Выявлено, что повышение интенсивности работы ультразвукового устройства оказывает выраженное влияние на микрообрастания субстратов, снижая плотность поселения и видовое богатство диатомовых. По итогам исследования рекомендовано расширение экспериментов с использованием полнофункционального ультразвукового устройства более высокой мощности при эксплуатации атомной электростанции.

Ключевые слова: биообрастание, ультразвуковые методы защиты, технологическое оборудование АЭС, бентосные диатомовые, Bacillariophyta

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Introduction

In recent decades, there has been a tendency to rearrange thermal and nuclear power plants (TPP, NPP) to sea coasts to take in large volumes of water required for technical water supply circuit (TWSC). This raises various problems related to the development of fouling organisms in water pipelines. Conventionally, these problems are divided into three types [1]: physical fouling of pipelines and operational biofouling caused by it; mechanical damage and death of hydrobionts (plankton and larvae of benthic species, fish eggs, and juveniles) after their passing through water supply circuits [2]; impact of waste water (including thermal water) on coastal aquatic ecosystems [2,3]. Thus, marine fouling is both an environmental and anthropogenic-technological phenomenon that must be taken into account when developing a concept for biofouling protection [1, 4].

The formation and growth of fouling communities (micro- and macrophytes, mollusks, barnacles, tubular polychaetes, ascidians, etc.) on the TWSC of an NPP is facilitated by a bacterial-algal biofilm, the initial stage of periphyton succession on the submerged equipment surfaces in water. Intensive development of fouling in the NPP heat sinks and replenishment supply reservoirs results in complex disruptions to the TWSC and corrosion intensification. Moreover, the working cross-section of pipelines and heat exchangers decreases, technological systems (including safety ones) fail, which leads to a decrease in the efficiency of NPP power units and economic losses [5–7]. In recent years, the total damage from biofouling at NPPs and TPPs has exceeded 11 billion rubles.

Anti-biofouling measures are divided into physical, chemical, and biological or complex [1, 5, 8]. Physical methods include cleaning the TWSC with compressed air, increasing the temperature of water in pipelines, cathodic protection, generating an electric field, ultraviolet (UV) or ultrasonic (US) exposure [1, 4, 8]. US anti-fouling methods include sounding of equipment, which causes a cavitation effect in the tissues of aquatic organisms, thus reducing their ability to settle on the substrate and grow subsequently [1, 6]. It is known that almost complete death of the larvae of a bivalve mollusk zebra mussel, is caused by continuous ultrasonic exposure with a power of 100–800 W, a voltage of 438 V, and a frequency of 17–22 kHz for several days, while the percentage of death increases sharply when increasing sound pressure frequency and strength [5, 6, 9, 10]. High energy consumption can be stated as the disadvantage of this method.

Some of the most pressing challenges for *RosEnergoAtom* JSC are to monitor the biofouling development, to take preventive measures, and to minimize its impact on the operation of the NPP TWSC [6]. Based on the implementation of the preliminary project results “Development of Technology for Combating Biofouling of Technological Equipment of Circulating Water Supply Circuits at Nuclear Power Plants (AP-19/246)" by *All-Russian Research Institute for Nuclear Power Plants Operation* JSC, it was revealed that the ultrasonic device (USD) is an effective anti-fouling tool. Thus, its using promotes to perform comprehensive preventive protection of equipment from fouling and avoids shutting down the TWSC, taking it out for repairs and cleaning [1, 6]. Tests in the Laboratory of environmental protection in the Rostov NPP showed that a prototype of the USD destroyed the druses of the zebra mussel (*Dreissena polymorpha L.*) that were strongly adhered to the water-immersed elements of the TWSC [6].

In 2021–2022, together with the Benthos Ecology Department of the Federal Research Center of IBSS, the advanced USD exposure on the formation of fouling on artificial substrates was tested. The laboratory stage of the experiment was performed under conditions that simulating marine environment; the experiment in the water area was performed in the marine water areas near the building of Radiobiological Department (RBD) of the IBSS.

The choice of benthic diatoms (Bacillariophyta) as test objects is based on their importance in aquatic ecosystems as a primary trophic link, mass distribution and predominance in microphytobenthos in terms of numbers, high biomass, and huge species richness [11]. Due to their sensitivity to environmental factors, diatoms can be indicators for the water quality assessing, as well as in testing the effectiveness of anti-fouling devices and biocidal paints and varnishes [11–13]. Microfouling, consisting of bacteria, diatoms and protozoa, forms a primary biofilm on underwater surfaces, including the TWSC [7, 12], which provides to the active development of the macrofouling community and leads to a further decrease in the NPP operating efficiency [7, 14].

This work is aimed to evaluate the USD efficacy with different operation modes for protecting hydrotechnical facilities from microfouling based on studying of benthic diatoms taxocene structure during long-term laboratory and field experiments. The tasks are as follows: 1) to identify differences in the abundance and species richness of benthic diatoms during the formation of microphytoperiphyton on concrete and steel plates; 2) to assess the dynamics of diatom fouling intensity of various substrates in the control and under different operating modes of the USD.

Material and methods

During the first stage, in laboratory conditions simulating marine environment, the following samples of the substrate were used: metal plates 5.5 × 6.5 cm from A-3 stainless steel and M-500 concrete plates 10 × 18 cm. The samples were fixed on holders and placed in 40-liter containers – control (CC) and test (TC) ones, 13 pieces of steel and concrete plates in each (Fig. 1, *a, b*). The containers were filled with natural seawater taken from Sevastopol Bay. The water in the containers was changed every two days, and each series of experiments was run for four weeks. During the experiment, periphyton components were successively deposited on steel and concrete samples: bacteria → diatoms → zoobenthos larvae. The TC was treated with ultrasound generated by a cassette of five high-frequency emitters with their constant power of 500 W, frequency of 27.1–27.3 kHz and current strength of 3 A.

Fig. 1. Long-term laboratory experiment: *a* – control container (CC) with concrete and stainless steel plates; *b* – test container (TC) with the ultrasonic device (USD) and similar plates; *c* – devices for setting the time and power modes of USD operation (*top*) and HAILEA device for water cooling and temperature control (*bottom*)

The frequency of USD exposure was three times a week for 4 hours (Fig. 1, *c*). The distance from the USD to the water surface in the TC was 10 cm; the CC was isolated from the ultrasonic unit with sheets of foam plastic, plexiglass, and dense rubber 1.5 cm thick. To avoid heating of sea water in the TC during the USD operation, a HAILEA device was used, which thermostated the water temperature in both containers to 19.0 ± 0.5 °C (Fig. 1, *c*). The laboratory experiment included constant lighting conditions (8/16 hours) and ventilation necessary for the alive organisms. The experiment continued for four months (September to December 2021).

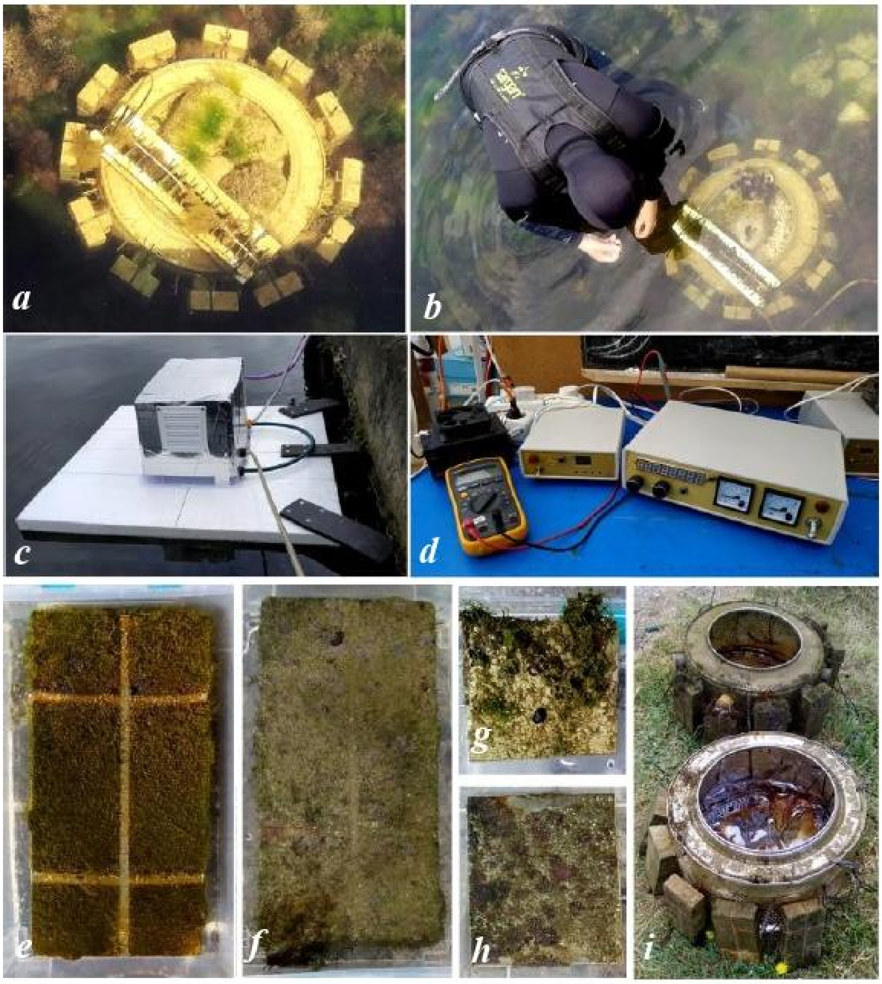
Every month, two samples of concrete and steel plates were removed from each container, microphytoperiphyton from their surface was scraped off from area of 5.5 × 6.5 cm and rinsed with filtered sea water. The total volume of each lavage was adjusted to 100 ml, the cells were counted with Carl Zeiss Axiostar+ light microscope × 400 (LM). Taxonomic identification of diatoms was carried out using permanent slides and micrographs obtained with Hitachi SU3500 scanning electron microscope (SEM).

To assess the microperiphyton abundance, diatom cells were counted in a Goryaev chamber (hemocytometer) in duplicate, then the results were recalculated per 1 m^2 of substrate surface [11]:

$$N = \frac{(aV)}{(S \cdot 10^{-4} \quad 7 \cdot 10^{-3})},$$

where a – number of cells in 0.007 mm^3; V – specified sample volume, 100 mm^3; S – sample surface area, in our experiment – 35.75 cm^2.

For the second stage of experiment (in the seawater area), control (CS) and test (TS) stands were made with steel and concrete plates attached to them. The stands had been exposed for five months (from April to September 2022) at a depth of 0.5–0.7 m at the water area bottom (Fig. 2). The USD was installed above the TS .



F i g . 2 . Long-term experiment in the water area: *a* – general view of the control stand (CS) with concrete and steel plates; *b* – sampling by a diver; *c*– USD installed above the test stand (TS); *d* – devices for setting and maintaining the time and power modes of USD operation; concrete plates after 5-month exposure: *e* – control; *f* – after exposure to USD; steel plates after 5-month exposure: *g* – control; *h* – after exposure to USD; *i* – control (*top*) and test (*bottom*) stands after 5-month exposure to sea water on a floating platform and was protected by a special cover from the wave, precipitation, and solar irradiation impact (Fig. 2, *c*). The distance from the emitters to the water surface was 20 cm. The control stand (CS) was installed in the water area at a distance of 30 m from the TS behind a concrete block, which shielded the CS from the USD exposure. During the experiment, the water temperature in the water area increased seasonally from 10 to 25.0 ± 0.5 °C. Every month, two plates of each type of substrate were removed from both stands and the abundance and species richness of diatoms were determined in the laboratory using the aforementioned method [11].

During the first 3 months, the TS was exposed to USD with the following working parameters: power – 500 W, frequency – 27.5 ± 5 % kHz, current strength – 3 A, period of operation – three times a week, 4 hours a day. Since July 2022, duration of TS treatment with the USD was extended to 8 hours a day, five times a week (Fig. 2, *d*). The experiment was terminated on 21.09.2022, due to beginning of storm season.

Results and discussion

Laboratory experiment. After the first stage of experiment, different conditions of sea water in containers were observed. In the TC, the concrete and steel plates remained visually clean, and the water was clear throughout the whole observation period. The water in the CC became muddy after two weeks (despite the regular water replacement every two days), probably due to metabolites released by fouling organisms and diatom polysaccharides. During the experiment, the parameters of abundance and species richness of diatoms in the CC on both concrete and steel plates significantly exceeded the level in the TC (Fig. 3). After four months, the average abundance of diatoms on concrete in the CC was $90.327 \cdot 10^6$ ind./m^2, the number of species was 14 (Fig. 3, *a*), and in the TC – only $0.893 \cdot 10^6$ ind./m^2, with 3 species (Fig. 3, *b*), respectively. It should be noted that in the first month of US exposure, diatom cells were not observed on the steel plates in the CC and TC. This fact can be caused by the smoothness of the steel surface, which does not facilitate the settling and primary adhesion of diatoms and larvae of microzooperiphyton organisms. After four months, the average abundance of diatoms on steel plates in the CC was $124.28 \cdot 10^6$ ind./m^2, the number of species was 11 (Fig. 3, *c*), and in the TC the abundance was $7.14 \cdot 10^6$ ind./m^2 and 3 species only (Fig. 3, *d*).

Based on the laboratory experiment result, it was concluded that even with a relatively weak power of ultrasonic sounding, the USD exposure provided preventive protection of concrete and metal substrates from colonization by the main components of phytoperiphyton. In addition, the effect of US exposure resulted in the mechanical cleaning the plates' surface from contaminants and hydrobiont metabolites.

Field experiment. During the field experiment in the water area, which included the fouling formation, the following phyto- and zoocomponents of periphyton sequentially settled on metal and concrete substrate: bacteria, diatoms, macrophytes seedlings (Chlorophyta, Ochrophyta, Rhodophyta), larvae of mollusks,

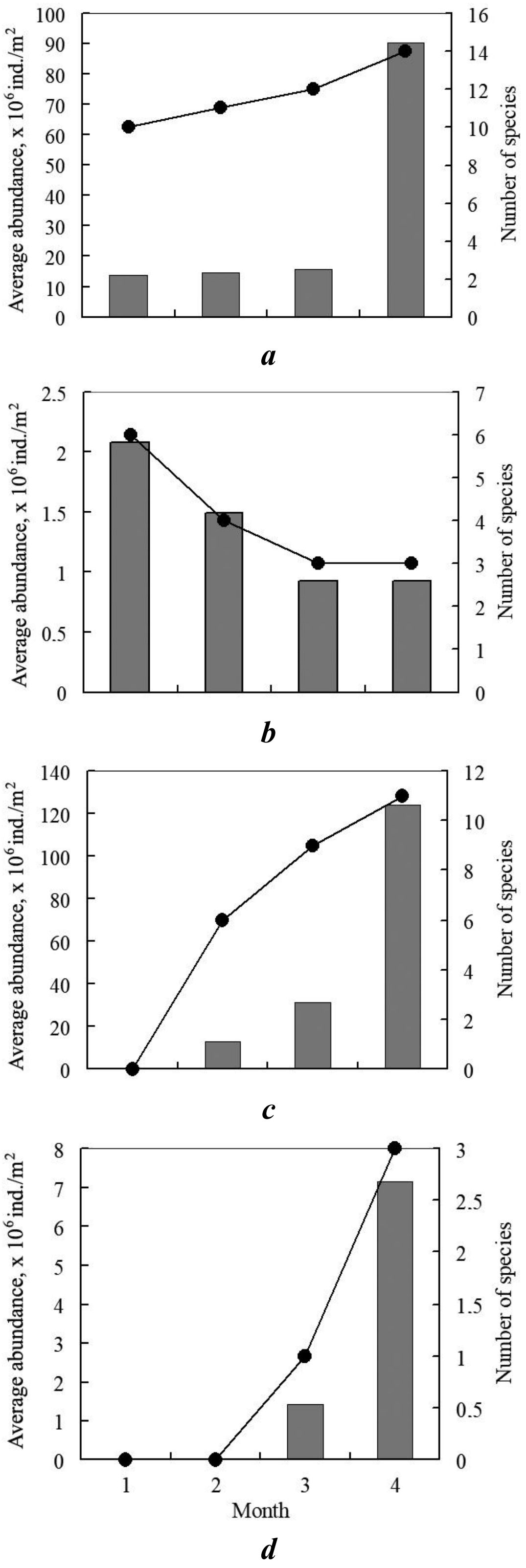


Fig. 3. Laboratory experiment: values of benthic diatoms in the control and exposed to the USD on concrete (*a* – CC; *b* – TC) and steel plates (*c* – CC; *d* – TC). The bars are abundance and the circles are species richness of diatoms

polychaetes, and barnacles. Variability in parameters of micro-phytoperiphyton on both types of substrates was revealed. It might be caused by a number of reasons, including changes in the hydrological regime and activation of diatoms reproduction as a stress response to the USD impact in the initial period of experiment.

The results of the first month of field experiment showed that USD action with its power of 500 W, sounding frequency of 27.5 ± 5% kHz, and current strength of 3 A with the exposure duration four hours a day, three times a week, significantly stimulated micro-phytoperiphyton development on concrete and steel samples at the experimental stand (TS) (Fig. 4).

At the first month of exposure, the lowest abundance of diatoms was observed on the CS concrete and steel plates ($45.5 \cdot 10^6$ and $14 \cdot 10^6$ ind./m^2, respectively). On the contrary, the abundance of diatoms at the TS were tens of times higher than at the CS: $1302 \cdot 10^6$ ind./m^2 on the concrete samples and $472 \cdot 10^6$ ind./m^2 on the steel ones.

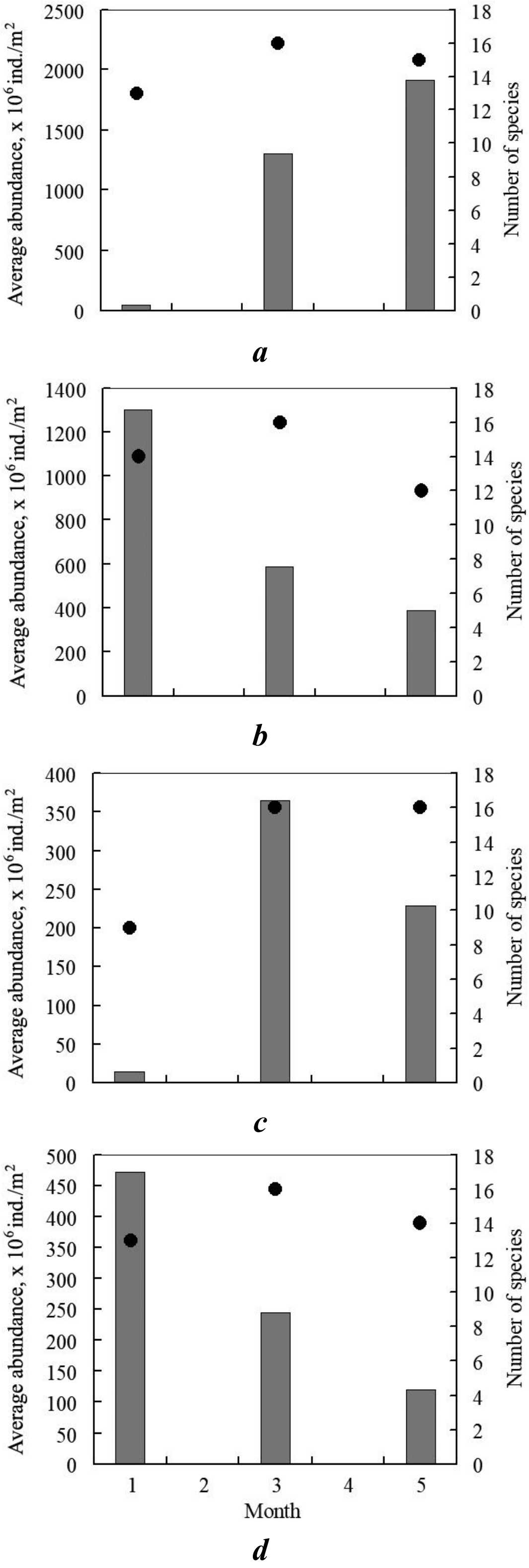


Fig. 4. Field experiment: values of benthic diatoms in the control and exposed to the USD on concrete (*a* – CS; *b* – TS) and steel (*c* – CS; *d* – TS). The bars are abundance and the circles are species richness of diatoms

At the TS, the highest species richness of Bacillariophyta was registered at the first month: 14 species on the concrete (Fig. 4, *b*) and 13 species – on the steel (Fig. 4, *d*). Apparently, during this period of the experiment, the USD effect with the specified operation parameters does not suppress the microperiphyton development and also causes stimulating effect, which was previously registered in similar studies by other authors [1, 10]. Taking into account the results of the first month, the USD intensity was increased: from July, the duration of sounding was 8 hours a day, 5 times a week. The parameters of sonic power, frequency and current strength did not change. After a three-month exposure at the CS, the abundance of diatoms on concrete was $1305 \cdot 10^6$ ind./m^2, while it decreased by more than half at the TS – to $585 \cdot 10^6$ ind./m^2 (Fig. 4, *a*, *b*). On steel at the CS, $365.5 \cdot 10^6$ ind./m^2 were counted, and at the TS – $244.5 \cdot 10^6$ ind./m^2 (Fig. 4, *c*, *d*). The species richness of diatoms at the CS and TS was the same: 16 species each on both types of substrates.

After five months of the TS exposure, the abundance of diatoms decreased significantly compared to the CS (see Fig. 2, *i*). The same pattern was observed upon final examination: the phytoperiphyton density on concrete and steel plates at the CS differed significantly (see Fig. 2, *e*, *f*) from such samples at the TS (see Fig. 2, *g*, *h*). The lowest abundance of diatoms at the final stage of experiment was observed in the TS steel plates fouling (121·10^6 ind./m^2). Twice as many cells were recorded on the steel CS samples – 228·10^6 ind./m^2 (Fig. 4, *c*, *d*). On the TS concrete substrate, the abundance of Bacillariophyta was four times lower (385·10^6 ind./m^2) than at the CS (1553.5·10^6 ind./m^2) (Fig. 4, *a*, *b*). Obviously, the intensity of diatom colonization of a smooth surface of steel plates is lower than that on concrete substrate with a rough surface providing better cell adhesion. Importantly, the abundance of diatoms on both types of plates at the TS after five months of exposure to the increased USD operation mode reduced significantly in compare to the initial stage of the experiment.

Within microphytoperiphyton 30 mass benthic species belonging to 21 genera, 17 families, 13 orders and 3 classes of Bacillariophyta were identified (Fig. 5). Representatives of class Bacillariophyceae predominated with 22 species against 5 species from class Fragilariophyceae and 3 species from class Coscinodiscophyceae. Regardless of the experimental conditions and type of substrate, small-celled species from genera *Navicula* Bory 1822 and *Nitzschia* Hassall 1845, characterized by the highest division rate and resistance to stress factors, sharply prevailed.

At the fifth month of experiment on concrete plates at the CS, the maximum abundance of *Nitzschia* sp.1 and *Navicula perminuta* Grunow (1178.5·106 ind./m^2 and 831·10^6 ind./m^2, respectively) was marked. The list of subdominants included *Thalassiosira excentrica* (Ehrenb.) Cleve (203.5·10^6 ind./m^2), *Nitzschia longissima* (Bréb. ex Kütz.) Grunow (87·10^6 ind./m^2), *Caloneis liber* (W. Sm.) Cleve (31.5·10^6 ind./m^2), *Nitzschia* sp. 2 (19·10^6 ind./m^2), *Amphora marina* (W. Sm.) Chase (11·10^6 ind./m^2), *Licmophora gracilis* (Ehrenb.) Grunow(9·10^{6}ind./m^2), *Pleurosigma elongatum* W. Sm. (4.5·10^{6}ind./m^2), *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin(4·10^6 ind./m^2), *Entomoneis paludosa* (W. Sm.) Reimer (3·10^6 ind./m^2). The abundance of other species varied from 0.5 to 2.5·106 ind./m^2.

Thus, an increase in the duration and frequency of the USD action after five months of exposure in the marine water area had a pronounced inhibitory effect on the diatom taxocene formation (the main component of microperiphyton), contributing to reducing in its abundance and species richness on both types of substrate. In general, the results from our experiments are consistent with the previously obtained results of other researchers on assessing the ultrasound effect on the resistance and survival of different groups of fouling. In particular, it was shown [10] that even with a short-term (up to 1–2 min.) of USD exposure with a frequency about 17 kHz and sound pressure from 1700 to 5000 bar, loss of ability to settle and subsequent almost 100% elimination of zooperiphyton larvae (cyprises of barnacles, etc.) were registered. Combined application of USD (power 0.12 kW and frequency 25 kHz) and 30 W UV-lamp revealed that UV-US effect

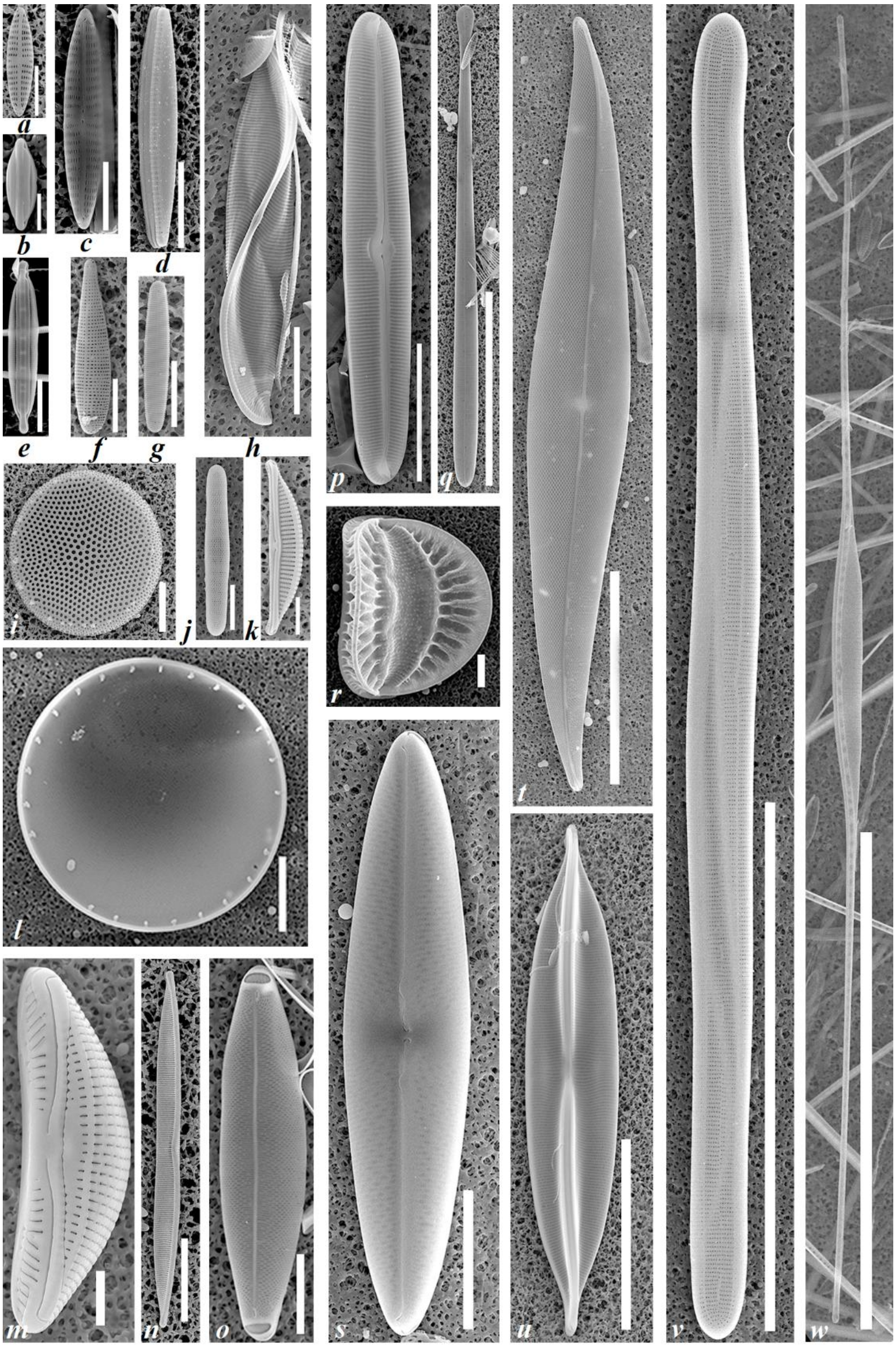

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Fig. 5. Dominant species of benthic diatoms on concrete and steel substrates (SEM): *a – Navicula perminuta; b – Nitzschia* sp.1*; c – Navicula parapontica;d – Tabularia affinis; e – Nitzschia sp.2; f – Licmophora gracilis; g – Microtabella delicatula; h – Entomoneis paludosa; i – Thalassiosira excentrica; j – Grammatophora marina; k – Amphora sp.; l – Actinocyclus subtilis; m – Amphora marina; n – Nitzschia hybrida; o – Striatella unipunctata; p – Caloneis liber; q – Licmophora flabellata; r – Campylodiscus thuretii; s – Trachyneis aspera; t – Pleurosigma elongatum; u – Plagiotropis lepidoptera; v – Ardissonea crystallina; w – Nitzschia longissima. Scale bar: a, c,e, f, g, k, m –* 5 µm; *b –* 3 µm; *d, h, i, j, o, s, r –* 10 µm; *l, p, s –* 20 µm; *q, t –* 50 µm; *u –* 30 µm; *v, w –* 100 µm

on fouling of water supply device lead to significant decrease in the occurrence of *Ciliata* and *Oligochaeta* after 72 hours of exposure, but at the same time it had not noticeable effect on the number of amoebae, rotifers, and other forms of parasitic ciliates [9].

We have to point out that effect of microalgae development stimulation observed at the beginning of the *in situ* experiment was noted before 1) as the first stage (activation) of a test object response when exposed to various toxicants, electromagnetic fields, and other stressors [13, 14]. Apparently, this phenomenon is stipulated by the short-term reaction of microalgae to the USD impact, which is expressed by mobilization of their adaptive capabilities and cell division intensification [15]. At the third month of the USD exposure with increased parameters, a reduction of abundance of diatoms was noted, that reflects a falling resilience at the second stage of stress (inhibition). It is known that the USD sounding to a biological object and their direct impact can destroy the membranes and organelles of unicellular organisms, as well as inactivate its enzymes [15–17].

And finally, at the fifth month of the experiment, the third stage of stress begins (depletion of adaptive capabilities and elimination of diatoms), which was expressed in the diatom cells number reduction by several times on both types of substrates at the TS. The ultrasound spreads well over a large area in water and can prevent the attachment of free-living forms of organisms, especially microperiphyton, destroy microplankton and bacteria even at a distance from the operating USD, thereby worsening the food resource of predators, and also suppress

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the viability of some groups of fouling. The effectiveness of ultraviolet and ultrasound combined treatment to increase water quality during its purification and disinfection from pathogenic organisms when used in aquaculture and in closed water supply circuits has been approved [17, 18].

It should be noted that US sounding, unlike hard radiation, do not have an accumulating effect, therefore long-term US action with low intensity does not cause any noticeable changes in periphyton organisms. In contrast, short-term high-power pulse US treatment can have a more pronounced efficacy on fouling than weak, but continuous US exposure. However, even with prolonged US exposure by a weak and obviously non-lethal doses, it is still impossible to establish the degree of periphyton inhibition only by the absence of a response from organisms. Thus, there can be no signs of suppressive effect to different groups of biota when the ultrasound action is below a threshold level, but the opposite effect can occur – referring to the aforementioned stimulation of the development and growth of fouling organisms, as was observed at the TS in the initial stage of the field experiment.

Based on the experimental results in the water area, we can conclude that since the sensitivity of different groups of periphyton to ultrasound treatment is different, we have to find universal threshold parameters to achieve the greatest USD effect (excluding hard doses dangerous for biota). For example, larvae of barnacles can die and fall off experimental plates even at low levels of the US action, while adhered juvenile barnacles or small mollusks can remain viable even at higher sounding levels. To improve the USD efficacy, the seasonal aspects of periphyton succession on various substrates should also be taken into account. Evidently that during the spring-summer's peak fouling development and the highest intensity of formation of the primary biofilm on the TWSC surfaces, the USD action should be higher than at the autumn-winter period.

In general, the results of laboratory and field studies revealed that the problem to provide the US protection of TWSC equipment against biofouling should be solved not only by increasing the USD power and intensity, which apparently causes the rapid and complete death of all ecological and taxonomic groups of periphyton on treated surfaces. Also, one should define such parameters of sounding that can preventively supress the fouling: settling and adhesion of microphytes, macrophytes, and zoobenthos, their subsequent growth and development. It seems important to expand laboratory and field experiments in order to study the US action of various durations and frequencies on the survival and development of different groups of periphyton to achieve the maximum effect in TPP and NPP TWSC protection. Minimisation the negative impact of ultrasound on the state of other components of aquatic ecosystems of water-cooling reservoirs should also be taken into consideration.

Conclusion

The stimulation of microperiphyton development on both concrete and steel substrates at the initial stage of the experiment studying the ultrasound effect was revealed.

Increasing the duration and frequency of the USD exposure had a pronounced effect on the diatoms – main component of the microperiphyton on concrete and steel substrates. After five months of the experiment with the increased USD intensity, a significant reducing of abundance and species richness of diatoms on steel and concrete was registered, compared to previous months. Small-celled species from genera *Navicula* and *Nitzschia* dominated on both types of substrate, regardless of the experimental conditions. A total of 30 species of benthic diatoms belonging to 21 genera, 17 families, 13 orders and 3 classes of Bacillariophyta were marked.

The results of laboratory and field tests make it possible to recommend to extend the experiments using a full-function USD of higher power directly during the exploitation of an NPP.

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