УДК 594.72:591.613

DOI: 10.30694/1026-5600-2018-1-31-43

М. И. Орлова[1, 2]*, Е. В. Строгова[2, 3], Т. Личи[4], М. Лурье[4]

К стратегиям защиты систем циркуляционного и технического водоснабжения (СТВ) от обрастания колониальными беспозвоночными с покоящейся стадией в жизненном цикле: *Plumatella emarginata* (Tentaculata) и ультрафиолетовое излучение uv — контроль vs. уничтожение.

Часть I. Новые данные о выходе из диапаузы и их применение для экологического мониторинга СТВ

[1] ФБГУН Зоологический институт Российской академии наук (ЗИН РАН)
Россия, 199034 Санкт-Петербург, Университетская наб., д. 1

[2]ООО Научно-технический центр «Техноэкотон»
Россия, 171842, Тверская область, г. Удомля, ул. Космонавтов, д. 5а, пом. 141

[3] ФБГУН Санкт-Петербургский научный центр Российской академии наук (СПБНЦ РАН)
Россия, 199034 Санкт-Петербург, Университетская наб., д. 5

[4] Атлантиум технологии ООО,
Израиль, 99100 Индустриальный парк Хар Тув, ул. Хамелаха, д. 11, п/я 11071

*E-mail: marina.orlova2012@gmail.com

Цель сообщения представить наглядную и другую базовую информацию, необходимую для организации и ведения мониторинга расселительных стадий видов-обрастателей, для создания системы контроля развития биопомех в СТВ объекта энергетики. В сообщении представлены результаты, в том числе фотодокументы, лабораторных наблюдений за феноменологией (последовательность, тайминг, количественные характеристики) выхода статобластов мшанки рода *Plumatella* из диапаузы. В частности, выявлены новые особенности этого процесса, такие как формирование в летнее время агрегаций, состоящих из статобластов, их остатков и развивающихся зооидов-основателей колоний. Эту особенность можно рассматривать как один из вариантов преадаптации данного экологически пластичного рода к колонизации искусственных биотопов.

Ключевые слова: покоящиеся и расселительные стадии обрастателей, выход из диапаузы, системы циркуляционного и технического водоснабжения, *Plumatella*

Toward strategies for protection of cooling water and service water systems (CSWS) from overgrowth by colonial invertebrates with resting stages in their life cycle: *Plumatella emarginata* (Tentaculata) and UV — control vs. combating.

Part i. New data on dormancy release and their value for ecological monitoring of CSWS

M. I. Orlova[1, 2]*, E. V. Strogova[2, 3], T. Lichi[4], M. Lurie[4]

[1]Zoological Institute of the Russian Academy of Sciences (ZIN RAS)
1, Universitetskaya nab., St. Petersburg, 199034, Russia

[2] LTD Scientific and Technical Center "Technoecoton"
off. 141, 5a, ul. Kosmonavtov, Udomlya, Tver' oblast, 171842, Russia

[3] St. Petersburg Research Center of the Russian Academy of Sciences (SPBRC RAS)
5, Universitetskaya nab., St. Petersburg, 199034, Russia

[4] Atlantium Technologies Ltd
11 Hamelacha Street, Har Tuv Industrial Park, POB 11071, 99100, Israel

*E-mail: marina.orlova2012@gmail.com

This paper is aimed to provide visual documentation as well as basic information required for setting up and carrying out monitoring of dispersal stages of undesirable (biofouling) species. Such monitoring is designed to help develop systems for control of biological fouling in Cooling and Service Water System (CSWS) of power plants. This communication

presents results (including photographs) from laboratory observations of various stages of the genus *Plumatella* life cycle. Some previously unknown peculiarities of the life cycle have been observed, such as formation of aggregations consisting of statoblasts and newly hatched zooids-founders of colonies in the summer time. This peculiarity has not been previously documented and can be considered as one of pre-adaptations of this ecologically flexible genus for colonization of artificial habitats.

Keywords: dormant and dispersal stages of biofouling species; dormant release; industrial cooling water system biofouling; *Plumatella*.

Introduction

For the last 15 years there have been numerous reports of biofouling of CSWS in hydroelectric facilities by Bryozoa [1, 2, 6, 7, 8, 15, 16], worldwide. In the last 4 years, two new species of common freshwater bryozoan from genus *Plumatella* have been found in the cooling reservoirs of nuclear power plants (NPPs) in the North-West of Russia [1, 2, 6, 7].

The life cycle of freshwater bryozoans inhabiting heated water bodies and causing biofouling in industrial complexes has been studied less comprehensively than that of fouling species with larval dispersal (e.g. zebra/quagga mussels). Plumatellids — the most common freshwater bryozoans are well studied by taxonomists [1–3] who deal with preserved material and electronic microscope, but they are poorly studied as living organisms [4, 17]. Therefore it is not surprising that, generally, the control of bryozoan fouling is focused on manual or mechanical removal of colonies. Little or no attention is given to presence of microscopic dormant lifestage (statoblasts) in industrial systems, which are the real prerequisite of massive fouling. Statoblast germination which leads to rapid recolonization of cleaned surfaces by new colonies has not been well studied. Until 2014, statoblasts and biological processes related to them have been out of scope of ecological monitoring carried out in cooling water bodies of power plants in Russia [7]. As no attention was given to the dormant stages of bryozoans, there is insufficient information on their distribution, abundance, viability, or the mechanisms of their germination.

This paper presents results and photographs from laboratory studies. It describes the germination of statoblasts (flotoblasts) and hatching of zooids in Phylactolemata bryozoan species *Plumatella emarginata* and *P. geimermassardi*. This study revealed peculiarities of the plumatellids life cycle which may lead to understanding of the limits of common antifouling strategies and provides criteria for estimation of UV illumination effectiveness described in the companion paper [13].

Background

1. Overview of the bryozoan life cycle for developing appropriate monitoring and strategy for control of biofouling

Most taxa of freshwater Bryozoans, including genus *Plumatella*, belong to class Phylactolemata. They are biofouling species with a complex life cycle. The fouling is caused by moss-like colonies of live individuals which develop within the cooling water system, growing attached in place within the piping systems and impeding water flow. From the literature [3], representatives of this class are hermaphrodites and sexually produce free-swimming larvae — ancestrula-like (very rarely found by researchers[1)]). They are capable of asexual reproduction through the formation of vegetative "bags" (statoblasts) produced by colonies through internal gemmation. Statoblasts are the obligatory dormant (resting) stage [4, 16]. These dormant stages and the period of rest (diapause) both serve as strategies for survival of unfavorable conditions (1)[2)] and for passive long distance dispersal (2). The vegetative bags act as seeds and it has been shown that they are able to quickly germinate under favorable conditions [4]. They are also the tool for subsequent rapid colonization of appropriate habitat either natural or artificial (3).

Infestation of any facility by Phylactolemata occurs during the mobile stages of the life cycle, either as planktonic larvae (rare) or more often by

[1)] it is not possible to reliably assess the role of the larvae in biofouling development at this time. In our 5-year study [7] we have observed that dispersal of *P. emarginata* occurs primarily through statoblasts;

[2)] primarily it is a "tool" for survival in unfavorable conditions: in temperate zone during the winter season and in tropical zones during the dry season, when water bodies can completely or partially dry-up;

those resting statoblasts which are able to passively drift with the incoming water and accumulate in sediments and on the surfaces of cooling water systems.

Statoblasts are lentiform multicellular bodies, covered with a solid shell. Examples of the statoblasts of the two species belonging to common freshwater bryozoan genus *Plumatella* — floating ones called "flotoblast" and sessile ones called "sessoblast"[3)] are shown in Figure 1. These dormant/resting statoblasts are generally resistant to common antifouling strategies, such as chlorination [16][4)], successfully used for dreissenid mussels, e. g. zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) [11, 12] control. It seems [5] that even control strategies effectively used for Hydrozoans (*Cordylophora caspia*), which are considered more resistant than mussels [14], appear ineffective against plumatellids statoblasts. It has been found [10] that statoblasts are highly resistant to both organo-chlorine pesticides and 2,4-D (a dioxin pesticide), which are highly toxic to most life-forms; copper sulfate had no impact on germination success; mercuric chloride had minimal impacts at low levels, but mortality increased with concentration; sodium salts of arsenic and molybdenum ($AsNa_3O_3$; $MoNa_2O_4$), while keeping germination below the level of the control, actually increased germination rates at higher concentrations. As for the colonies (active phase of life cycle) they are relatively sensitive[4)], however in toxicity bioassays it is not uncommon for an apparently dead colony to "revive" when it is returned to clean water [16].

Many common biofouling invertebrates have only the sexually produced larvae (Fig. 2) serving as the dispersal and colonization mechanism. Larvae as a rule are vulnerable to negative environmental factors [8] and antifouling remedies [11], more so than the sessile adults. *Plumatella* in its active phase (colonies) is also known as relatively tolerant organisms even to high ambient temperature. It is able to form monospecific fouling at sites where mussels do not survive [8, our unpublished data] or co-occur with other fouling organisms possessing free-living larvae in cooler habitats [5, 8].

Freshwater bryozoan *Plumatella emarginata* is found in CSWS of power plants (including NPPs of the Russian Federation and adjacent countries [6, 8]). It forms massive colonies (Fig. 2), causing problems with adequate cooling water delivery, particularly during the warm time of the year. The statoblasts (Fig. 1) are present in artificial and quasi-natural habitats throughout the year. Single zooids or germinating statoblasts (active phase) are observed periodically during the warm season in fouling samples collected from surfaces and water inside CSWS. They are found less frequently in zoobenthos and zooplankton samples collected (1–3 times in the

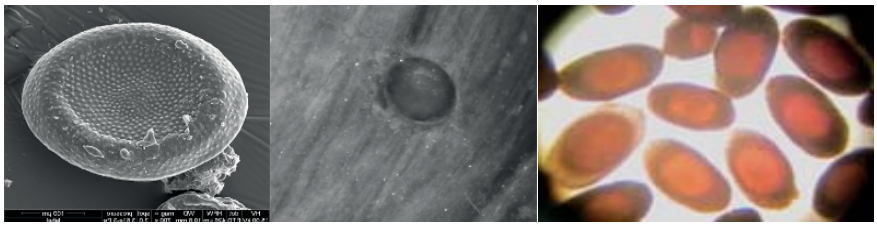

Fig. 1. Resting — statoblasts of *Plumatella* genus. On the left electronic microscopic photos *P. geimermassardi* flotoblast [From Gontar' 2016 [1]); in the center — sessoblast of the same species [the same source], on the right — flotoblasts of *P. emarginata* (central and right photos were taken using binocular light microscope, X40, the same resolution is also used for microscopic photos in figs. 2, 3 and 5, unless otherwise stated.

Рис. 1. Покоящиеся почки обрастателей- статобласты мшанки рода *Plumatella*. Слева — электронная микрофотография флотобласта *P. geimermassardi* [из Gontar' 2016 [1]); в центре — сессобласт того же вида [тот же источник], справа — флотобласты *P. emarginata* (центральная и правая фотография сделаны под бинокулярным микроскопом при увеличении X40, то же самое увеличение использовано при микроскопировании (фотографировании) объектов, имеющихся на рисунках 2, 3 and 5, если не указано иное).

[3)] some sources [17] distinct future flotoblasts and sessoblasts within a mother colony and state that sessoblasts need shorter time for completing their gemmation from mother colony (3.84 days on average), while formation of flotoblasts possessing swimming ring takes longer time — 5.32 days on average; number of future flotoblast in a colony in higher than that of future sessoblasts (from 4:1 to 21:1); released sessoblasts when finding an appropriate substrate attach before dormancy;

[4)] occasional maintenance treatments with sodium hypochlorite of 0.3mg/1 over 24 hours should keep bryozoans colonies under control, but will not kill statoblasts [16].

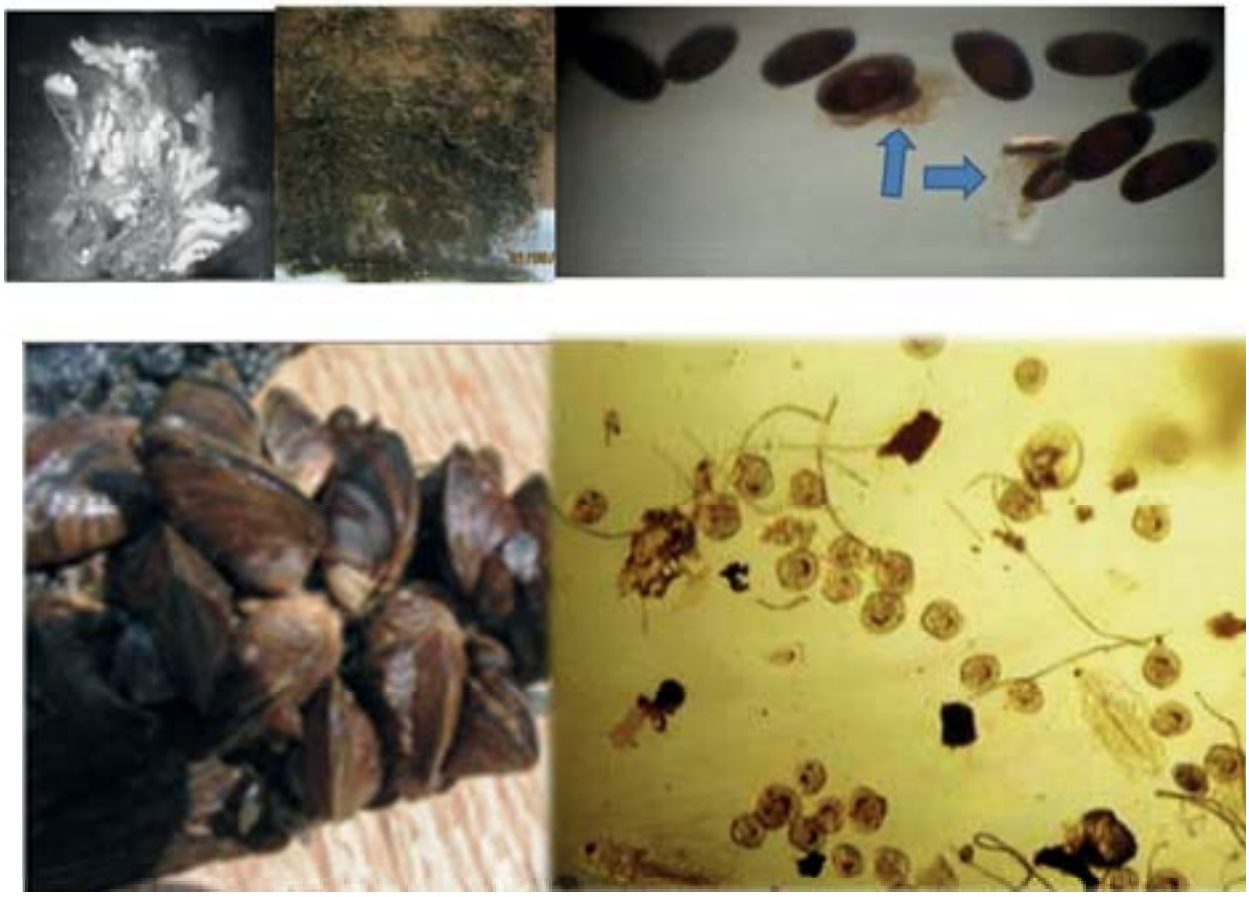

Fig. 2. Active phases of fouling organisms inhabiting cooling and service water systems and outer areas of hydroelectric (canals, dams) power plants from preserved samples. Upper block of photos from left to right: fragment of colony of *P. geimermassadi* [1] (light microscopic photo, X10), colony of *P. emarginata* (naked eye) on a fouling coupon, germinating statoblasts of *P. emarginata* — open shells and releasing content (pointed with arrow, light microscopic photo). Lower block from left to right: adults of *Dreissena polymorpha*, planktonic larvae of Dreissenidae (light microscopic photo).

Рис. 2. Активные фазы жизненного цикла обрастателей, обитающих системы охлаждения и технического водоснабжения электростанций, так как они выглядят в фиксированных гидробиологических пробах. Верхний блок фотографий слева направо: фрагмент колонии *P. geimermassardi* [из 1] (под микроскопом, десятикратное увеличение), колония P. emarginata (невооруженным глазом) на пластине для исследования динамики формирования обрастания, пробуждающийся статобласт *P. emarginata* — открыты створки, выходит наружу содержимое (указано стрелкой, фото сделано под световым микроскопом). Нижний блок слева направо: взрослые особи *Dreissena polymorpha*, планктонные личинки Dreissenidae (фото сделано под световым микроскопом).

warm season) during annual monitoring of cooling water reservoirs and preserved with formaldehyde (Fig. 2). The resting life stages are generally not reported by observers carrying out routine ecological monitoring aimed at environmental protection rather than prevention of biofouling in industrial settings.

Little is known about the role of each statoblasts generation in fouling of cooling water systems. The fouling process appears to be more complicated than that under natural conditions [7]. Partial control of bryozoans colonies in industrial systems through chemical control or dewatering [16] may trigger the formation of resting stages at almost any time.

Resting statoblasts can accumulate in natural water bodies: flotoblasts (Fig. 1, left and right photos) – may be present in the water column and also in the surface film and in the bottom sediments (pers. observation by Orlova and Strogova); sessoblasts (Fig. 1, central photo) — can be found on hard substrata. The statoblasts can also accumulate in the CSWS, both as flotoblasts transported there by water flow from upstream colonies or released from existing colonies growing in place and as sessoblasts[4)] attached within the piping prior to dormancy (mechanisms of such protracted dormancy are yet to be understood).

It is not clear which factors regulate the dormancy release of *Plumatella* statoblasts from generation (1) [3], while activation of statoblasts collected from late summer/autumn colonies (generation (2)?) is well-studied under laboratory conditions [4]. It would appear that under laboratory activation (germination) of statoblasts collected from colonies is facilitated by vernalization (exposure to freezing temperatures) for approximately 1 month or more [4].

When activated under favorable conditions, the statoblasts are able to produce zooids-founders of colonies (Fig. 3). If vernalization and subsequent cultivation are properly done, hatching success under laboratory conditions can reach 90 % for *P. fungosa* [4].

Under natural temperate climate conditions plumatellids produce colonies during the spring and summer [4, 7, personal observations]. However, in the cooling ponds and within the inner part of CSWS, where seasonality is erased with the addition of heat, resting periods and vegetative growth peaks may not be predictable [7]. Therefore, biofouling problems are difficult to anticipate under these artificial conditions. Further, biofouling forecasts are also complicated by annual climate fluctuations. During years with long-lasting cool springs, no massive colony formations are observed in the summer time. [7].

Material and methods

The laboratory research has been done twice with *Plumatella emarginata* statoblasts; once in the winter time (normal period of rest in temperate climates) and once in the early summer (at the peak of vegetative growth). During the summer experiment the influence of pre-germination period on dormancy release was also tested. The description of *P. emarginata* biological material and cultivation methods (Fig. 4) is common with the companion paper [13].

Additionally we tested dried statoblasts of *P. geimermassardi.*

Biological material

Plumatella emarginata. Biological material from the same locality, collected on the same date and stored at the same place, has been used for the two sets of observations described in this paper. Statoblasts (flotoblasts) were collected from a mature colony growing in the basin used for cultivation of fish larvae in Kurchatov town (Kursk region) on September 30, 2016, at an ambient water temperature of 20 ºC. We suggest this material belongs to generation 2 (see Background, section 2).

Immediately after the collected colony was brought to the laboratory, the statoblasts were manually washed out from it, using raw water from the Kursk cooling reservoir. Subsequently, the washed out statoblasts were examined under a microscope. All statoblasts found were flotoblasts which looked intact and mature with well-developed morphological structures (see Fig 1, right photo). They were associated with the surface film of water. The statoblasts were collected from the water

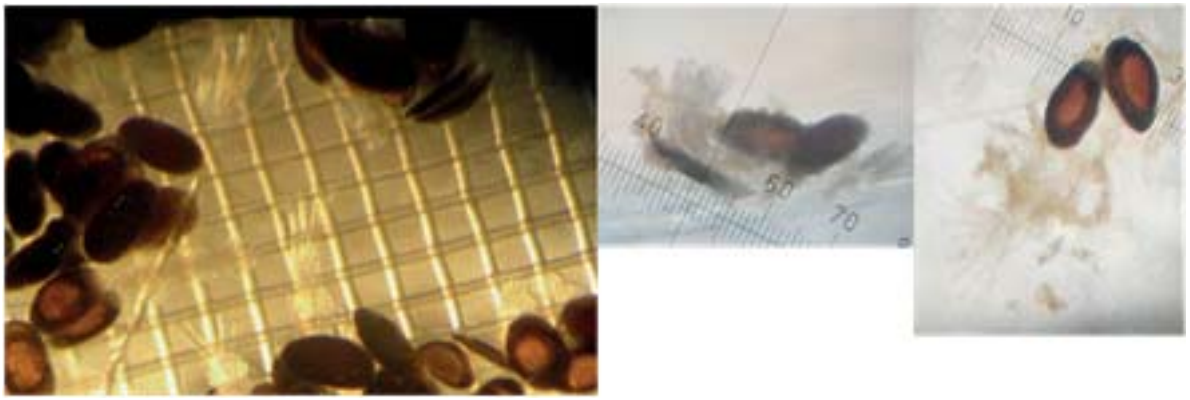

Fig. 3. Examples of zooids-founders of colonies (transparent object with coronas of tentacles) hatched from germinated statoblasts. To the left — example from *Plumatella emarginata* (Kurchatov, collection on September 30, 2016, activation after vernalization June 6, 2017, zooids have hatched June 11–12), to the right *P. geimermassardi* (Kopora Bay, collected in content of dried colonies from fish farm pontoons on July 28, 2017, activated by placement in water on August 1, 2017, ***without vernalization by freezing***).

Рис. 3. Примеры зооидов-основателей колоний (прозрачные объекты с венчиком щупалец), вылупившиеся из взошедших статобластов. Слева пример *Plumatella emarginata* (г. Курчатов, сбор 30 сентября 2016 г., активированы после яровизации 6 июня, 2017 г., зооиды вылупились hatched 11–12 июня), направо — *P. geimermassardi* (Копорский залив, собраны 28 июля в составе высохших колоний с понтонов садкового хозяйства, активированы путем помещения в воду 1 августа 2017 ***без яровизации путем промораживания***).

surface, subdivided into small groups and placed into separate plastic bags for storage and future transportation. All bags were immediately placed in a freezer where they were maintained at –10 to –15 ºC until required for further experiments.

Statoblasts were activated twice; first on December 25th 2016 and for a second time on June 4–6, 2017. The first experiment was carried out in the "Gidrotechproject" Ltd lab. in St-Petersburg, Russia. The second experiment was done in Atlantium Technologies Ltd lab. in Bet-Shemesh, Israel. Facilities used for cultivation are shown in Fig. 4. In December the raw water used was taken from the Udomlya Lake (cooling reservoir of the Kalininsk power plant) and had salinity of 0.24 ppt and pH above 8 [7]. In June, water from the Kinnereth Lake (Israel) diluted by deionized water to keep salinity within 0.2–0.4 ppt and pH within 8–8.6 was used [13]. The temperature for both experiments was the ambient room temperature (16–20 ºC in the winter experiment and 24–29 ºC in the summer experiment). Photoperiod corresponding to the time of the year and latitude of locality (St-Petersburg or Bet-Shemesh) was maintained.

Activation — melting and acclimation to room temperature of *P. emarginata* statoblasts occurred differently in each experiment in order to imitate different environmental conditions (see Table 1 and section 1.3.1. in [13] for more details).

P. geimermassardi. Statoblasts from dried colonies collected during the monitoring of periphyton in the discharge canal of Leningrad power plant on July 28, 2017, belonged to generation (1). They were activated to check their ability to germinate by placing them in water on August 1, 2017, without vernalization by freezing.

Germination is defined as the growth of cellular mass within a statoblast resulting in visual changes — opening valves, visibility of live whitish content between valves, capacity to tightly close in response to touch or shaking with subsequent reopening of valves. Not all statoblasts hatch into zooids. Hatching is defined as the final part of the process of dormancy release. Dormancy release describes the whole sequence from awakening of the statoblasts up to the appearance of fully developed zooids (Fig. 3).

Results and discussion

1. Photoimages as basic visual information for monitoring of dormant and dispersal stages of plumatellids

Mature colonies of two plumatellid species *Plumatella emarginata* and *P. geimermassardi* are shown in Fig. 2. Both species pose risk of biofouling in power plants. The same figure shows the image of dormancy release of *P. emarginata* statoblasts from preserved sample. Figure 3 shows hatched

Fig. 4. Laboratory conditions for cultivation of *P. emarginata*. On the left — cultivation vessels prepared for experiment, on the right — cultivation vessels with treated [12]) and control (used here (below) for observations of germination of statoblasts and hatching of zooids — founders of colonies) samples of statoblasts (each sample had 400–600 individuals in the individual cages, which allowed for isolation of individuals from those in other samples but provided unhampered access of water to each individual).

Рис. 4. Лабораторные условия для культивирования *P. emarginata*. Слева — емкости для культивирования с аэрируемой водой, готовые для использования в экспериментах, справа — те же емкости, куда помещены обработанные [12] и контрольные (использованные для наблюдений за всхожестью статобластов и вылуплением зооидов-основателей колоний) пробы статобластов (каждая проба, состоящая из 400–600 статобластов находится в индивидуальном садке, который позволяет изолировать каждую пробу, но не затрудняет водообмен между садком и основной массой воды в культивационной емкости и доступ кислорода к статобластам).

zooids-founders of colonies. This stage can only be observed in live samples. Additional materials on the third plumatellid species — *Plumatella similirepens* can be found in reference [2].

2. Observation of dormancy release in *Plumatella emarginata*

2.1. General sequence. Figure 5 shows the sequence of transformations from intact, frozen, closed statoblasts into zooids. This sequence is reconstructed primarily from the observations carried out during June 6–14, 2017, and partly (2b) from observations done in December in St. Petersburg. Additional data collected by the authors in the field were also used.

The sequence shows (1) *variation in statoblasts undergoing the processes of germination and hatching* (see variations numbered 2, 3, 5 and indicated with Cyrillic letters а, б, в). Another observation not previously recorded in open literature is (2) *the process of clumping of viable statoblasts to each other and with remnants of dead statoblasts and empty shells using an excreted jelly-like matrix* (code (way) 4 in Fig. 5). We suggest that these aggregates may serve as nurseries for groups of newly hatched zooids and thereby increase the probability of successful colonization when an appropriate substrate is encountered. If the appropriate substrate is absent, the aggregate/colony can remain floating or drifting. The process of consolidation into mobile aggregates is likely to have an adaptive role, including that of withstanding damage from an external impact.

The presence of the floating colonies calls into question the regulatory role of biofilm in adhesion, attachment and settlement of dispersal stages of invertebrates and the development of macrofouling [6, 13]. We can compare the development of travelling[5)] *Plumatella* aggregates/colonies to translocation by adult dreissenid mussels [9]. However, unlike the translocators, the floating aggregates with hatching zooids can serve as the initial macrofouling colonies of plumatellid in the proximal and distant portions of CSWS. Unlike the individual translocators, these colonies will grow and multiply in-situ by asexual division.

2.2. Timing. Results from the activation experiments carried out in December were significantly different from those carried out in June.

December 2016. Process of swelling (1 in Fig. 5) of corrugated (dehydratated by freezing) statoblasts took 2–3 days; excretion of jelly-like "adhesive" by statoblasts was found on day 4–5, coinciding with the opening of the valves (3 in Fig. 5); the first zooids, very few in number, were found on day 7. Flattening of statoblasts (2b) or consolidation into aggregates (5) were not observed at all.

June 8–14, 2017. Results from observation done on days 1–4 are summarized in Table 1 (9 characteristics, including the beginning of hatching). Almost no corrugated statoblasts were found in the cultures. The first zooids appeared 3–4 days sooner than in December (columns 6, 7 in Table 1). Hatching started after, or simultaneously with the formation of aggregates.

Thus, timing of two phases of dormancy release (germination and hatching) was different under different temperature regimes of the different seasons despite the fact that the statoblasts had the same origin and cultivation. Further, there was a lack of aggregation during the winter experiment.

2.3. Quantitative and semiquantitative characteristics of hatching from observations done June 8–14, 2017. Hatching started on the 4th day of cultivation (table 1) and continued up to the termination of the experiment (5th day for Group T and 7th day for Groups A and U (Table 2)). Hatching was strongly dependent on the regime of activation (see data for 5th Day in Table 2 for Group T and Groups A, U), like germination was (Table 1). Result of hatching (contribution of zooids to total number zooids+intact statoblasts) in groups with extended period of acclimation to room temperature was impressively *high* — up to 45 % in Group A, 71 % in Group U (Table 2) by the 7th day of observations. Probably these results should be attributed to the time of a year — the best for the processes of dormancy release given the temperature conditions.

2.4. Practical consideration of results for Groups A, U and T

Very high destruction of statoblasts (Table 1) and low number of hatched zooids (Table 2) in Group T activated under conditions of fast melting and rapid oscillation of temperature in the range above zero (Group T transportation from laboratory in St-Petersburg (Russia) to laboratory in Bet-Shemesh (Israel) in June 2017 lasted for almost 3 days) has practical application. In 2017 after a late springs and cool summer *Plumatella* appeared to have lower capacity for colonization of CWSS (personal communication from Kursk NPP staff). Thus, rapid activation of statoblasts especially with subsequent decrease and oscillations of temperature could be considered as a prototype for a preventive anti-fouling method.

3. Additional observation of dormancy release in *Plumatella geimermassardi*

We had no *P. emarginata* statoblasts of generation (1) from the Kurchatov location and used available statoblasts of *P. geimermassardi* likely belonging to

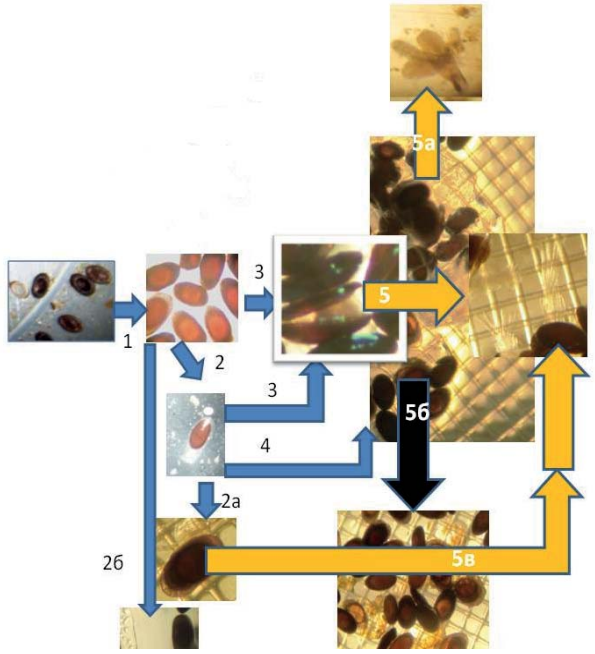

Fig. 5. Major phases of statoblast activation (germination and hatching) during dormancy release by *Plumatella emarginata*. *General legend:* black arrows — stop further germination and development; blue arrows — steps/events of germination processes; yellow arrows — hatching and its results;

Numbers and letters: 1. Swelling and restoration of normal shape by frozen corrugated statoblast (result: closed intact statoblast); 2 Excretion of gel by statoblast; 2a — attachment of single statoblast with gel secretion to substrate (is this secretion adhesive?) or to surface water film; 2б — change of shape and getting appearance characteristic of sessoblast (result: some flattening and stacking to substrate); 3 — reversible opening of shell valves by intact statoblasts (result: beginning of hatching, excretion of additional quantity of secrete); 4 — very slow movement (how?) and consolidation due to excreted material e (result: formation of aggregates= statoblasts partly immersed into transparent jelly-like matrix (consolidated); 5 — hatching of tentaculate zooids by consolidated groups of statoblasts (not all of them are hatching, but many); 5a — finding larvae-like zooids in matrix (single event in experiment (AL100, see in [13]) and mass event in September 2016 in cooling reservoir); 5б — aborted zooids; 5в — hatching zooids from single statoblasts.

Рис. 5. Основные фазы (пробуждение и вылупление зооидов) выхода из диапаузы у *Plumatella emarginata*. *Условные обозначения:* черные стрелки — прекращение дальнейшего прорастания и развитие статобластов и зооидов; голубые стрелки — стадии/наиболее важные события процесса прорастания; желтые стрелки — отрождение (появления) зооидов и основные результаты процесса выхода из диапаузы;

Цифровые и буквенные обозначения: 1 — набухание и восстановление нормальной формы сморщенными после пребывания в морозильной камере статобластами (результат: закрытый интактный статобласт); 2 — выделение геля (желеобразного секрета — «адгезива»?) статобластом; 2а — прикрепление отдельного статобласта с помощью выделенного секрета (является ли секреция частью процесса адгезии?) к субстрату или ассоциация с поверхностной пленкой воды; 2б — изменение формы и приобретение статобластом черт сессобласта (результат: некоторое уплощение и прилипание к субстрату); 3 — обратимое открывание створок интактными статобластами (результат: периодическое появление зооида между створками, выделение добавочного количества желеобразного секрета); 4 — очень медленное разнонаправленное движение формирующихся зооидов относительно створок «материнского» статобласта и консолидация статобластов, содержащих зооиды с другими материалом в выделенном желеобразном секрете (матриксе) (результат: формирование агрегаций, состоящих из прозрачного матрикса и частично погруженных в него прорастающих статобластов с зооидами и мертвого материала); 5 — присутствие (вылупление) сформированных щупальценосных зооидов консолилированными группами статобластов (не изо всех, но из многих выходят зооиды); 5а — обнаружение анцеструлоподобных зооидов в матриксе (единственный раз в эксперименте с кодировкой (AL100, см. [13]) и массово в сентябре 2016 г. в Курчатовском водохранилище; 5б — абортированные зооиды; 5в — выход зооидов из одиночных статобластов.

Table 1

Germination and beginning of hatching (columns 6, 7) of statoblasts from Groups A, U, T* by observations carried out June 8–11, 2017

Group (day of observation)	% in total number of observed statoblasts				Semi-quantitative data (scores)				
	Open intact	Open damaged	Closed intact	Closed damaged	Presence of tentaculate zooids 1 or 0	Presence of larvae-like zooids 1 or 0	Degree of consolidation (aggregation) 0, 1, 2, 3	Presence of ciliates 0, 1, 2, 3	Aborted material 1, 2, 3, 4
1	2	3	4	5	6	7	8	9	10
A(1)	2.13±1.4	0.9	90.9±1.6	5.9±1.6	0.0	0.0	0.0	0.0	0.0
U(2)	2.7±1.7	1.8±1	92.4±2.5	3.1±2.7	0.0	0.0	0.0	0.0	0.0
T(2)	5.4	11.7	70.3	9.1	0.0	0.0	0.0	0.0	3.5
A (4)	25.7±15.5	7.1±5.2	62.8±12.1	4.5±1.0	0.7±0.5	0.3±0	2±1	1.2±0.4	0.0
U (4)	31.4±16.5	0.3	67.0±16.2	1.3±0.8	1±0	0.0	1.1±0.4	0.6±0.2	0.0
T(4)	20.7±2.2	21.0±9.1	53.2±11.2	5.1±3.3	1±0	0.0	1.1±0.4	0.0	1±0

* — Group T, activation occurred in conditions with fast melting and rapid oscillation of temperature in range above zero; Groups A and U were melted gradually as well as acclimation to room temperature conditions occurred for 1.5 (Group A) and 2.5 (Group U) days.

Таблица 1

Пробуждение статобластов и начало вылупления зооидов (столбцы 6, 7) в группах A, U, T* по наблюдениям, выполненным 8–11 июня 2017 г.

Группа (день наблюдения)	% в общем числе наблюдаемых статобластов				Полуколичественные данные (баллы)				
	Открытых интактных	Открытых поврежденных	Закрытых инстактных	Закрытых поврежденных	Наличие щупальценосных зооидов 1 или 0	Наличие анцеструлоподобных зооидов 1 или 0	Степень консолидации (агрегированность) 0, 1, 2, 3	Наличие инфузорий 0, 1, 2, 3	Абортированные зооиды и др. материал 1, 2, 3, 4
1	2	3	4	5	6	7	8	9	10
A(1)	2.13±1.4	0.9	90.9±1.6	5.9±1.6	0.0	0.0	0.0	0.0	0.0
U(2)	2.7±1.7	1.8±1	92.4±2.5	3.1±2.7	0.0	0.0	0.0	0.0	0.0
T(2)	5.4	11.7	70.3	9.1	0.0	0.0	0.0	0.0	3.5
A (4)	25.7±15.5	7.1±5.2	62.8±12.1	4.5±1.0	0.7±0.5	0.3±0	2±1	1.2±0.4	0.0
U (4)	31.4±16.5	0.3	67.0±16.2	1.3±0.8	1±0	0.0	1.1±0.4	0.6±0.2	0.0
T(4)	20.7±2.2	21.0±9.1	53.2±11.2	5.1±3.3	1±0	0.0	1.1±0.4	0.0	1±0

* — Т-группа, активация протекала в условиях очень быстрого оттаивания и резких колебаний положительных значений температуры среды; группы А и U подвергались постепенному размораживанию, их акклимация к комнатной температуре также протекала постепенно, в течение полутора суток у группы А и 2, 5 суток у группы U.

Table 2

Portion (%) of intact well-developed tentaculate zooids in total number of observed zooids and statoblasts

Day 5 (12.06.2017)			Day 7 (14.06.2017)		
A	U	T	A	U	T
32.1±11.6	65.8±9.7	6.15±1.1	45.2±10.8	71±12.6	Experiment is terminated

Таблица 2

Вклад (%) целостных зооидов с развитым венчиком щупалец в общую численность всех наблюдаемых в поле зрения зооидов и статобластов

День 5 (12.06.2017)			День 7 (14.06.2017)		
A	U	T	A	U	T
32.1±11.6	65.8±9.7	6.15±1.1	45.2±10.8	71±12.6	Эксперимент прекращен

generation (1) (Kopora Bay, collected in the contents of dried colonies from fish farm on July 28, 2017). Approximately 5 % of these statoblasts when placed into water on August 1, 2017, without any freezing, had hatched into well-developed tentaculate zooids (Fig. 3). Germination of these statoblasts was not as good as that of vernalized statoblasts of *P. emarginata* collected in Kurchatov town in autumn 2016 which belonged to generation (2). The germination was even lower than that of Group T (see Table 2).

4. What is the role of different generations of statoblasts, vernalization and other environmental factors in life cycle and activity of *Plumatella*?

Following the laboratory results we can conclude that the hypothesis regarding the presence of several (probably 2) viable generations of statoblasts in plumatellid biofouling in temperate climate water bodies is valid. Additionally, it would appear that both of these generations have the ability to hatch zooid-founders of colonies. Statoblasts of *P. geimermassardi* (generation (1)), released in spring-early summer 2017 demonstrate limited hatching without vernalization, however we cannot exclude drying as trigger factor for their activation. Statoblasts of *P. emarginata* released in autumn 2016 (conditionally generation (2)), have "overwintered" in freezer (vernalized) and demonstrated massive hatching early next summer. Thus the *Plumatella's* statoblasts of any generation are able to hatch.

All of the relevant information collected [4] deals with statoblasts collected directly from mature colonies growing in semi-natural conditions. There may be other activation factors for sessoblasts and flotoblasts which accumulate in the benthos of natural or artificially heated water bodies or inside CWS. Nevertheless we conclude that vernalization while helpful, may not be absolutely necessary for the activation of plumatellids statoblasts. Therefore, statoblasts in industrial complexes may be activated by other environmental factors such as rehydration after freezing or drying, sub-lethal temperature or sub-lethal antifouling treatments. This ability could help explain multiple peaks of *Plumatella* biofouling of cooling water systems during relatively short periods of time as observed during selected years in Kursk [7].

Summary:

— Monitoring of periphyton has to include monitoring of dormant stages of plumatellids; this paper provides primary photographic materials for identification of both active and dormant stages of two plumatellid bryozoan species. Preparation of an atlas and an identification key for dormant and dispersal stages of fouling species posing a threat for industry could be an important update to current monitoring of periphyton and meroplankton [6].

— *Plumatella* demonstrates variability in statoblast development during the process of germination and hatching;

— The consolidation of germinating statoblasts and hatching zooids into aggregates suggests that the development of *Plumatella* colonies is not as strongly dependent on biofilm precursors as free-living larvae of other biofoulers. Therefore, control of biofilm on industrial substrates may not prevent colonization by plumatellids. Different preventive strategies may be required for these two groups of biofoulers.

— Results of germination and hatching are dependent on conditions of activations (gradual acclimation (Groups A,U) or rapid changes (Group T)). Rapid activation and temperature oscillations could be considered as a possibility for prevention of biofouling;

— Under favorable environmental conditions (temperature, oxygen, salinity etc.) at favorable time of a year, release of zooids-founders of new colonies is higher than that during periods of normal rest; it occurs in different generations of statoblasts; it is facilitated by vernalization. Questions about other environmental and men-mediated factors facilitating dormancy release remain. Thus fouling prevention planning should focus on the known period of high colony formation. Additional experimental studies on activity and dormancy of plumatellids in industrial systems with erased or altered seasonality and photoperiod as well as impacts of biofouling controls are required.

Acknowledgement:

The work is supported by state governmental theme № AAAA-A17-117021310121-0 "Investigations of dynamics of trophic ralationships, biological diversity and patterns of nutrients' cycling in ecosystems of continental water bodies which is caused by anthropogenic impacts and climatic fluctuations" (for M. Orlova) and 0240-2018-0001:

"Development of the theory of transformation of scientific-innovative space of St-Petersburg in the context of innovative development of Russian economics with account of theoretical and methodical background of sustainable technological development of the region on the base of innovative activities and investment, reproduction and formation of scientific and educational potential of St-Petersburg (for E. Strogova).

We are thankful to Dr. Renata Claudi for helpful comments and ideas. This helped improve our manuscript and gave us further ideas for research into freshwater bryozoans and the impact they have on industrial facilities.

We appreciated "Hydrotechproject" LTD for travel support and experimental equipment and aquarial room in St-Petersburg.

Литература

1. *Гонтарь В. И.* Описание первой находки пресноводной мшанки *Plumatella geimermassardi* Wood & Okamura (Phylactolemata) в Копорской Губе восточной части Финского залива (краткое сообщение) // Региональная экология. 2016. № 49(1). С. 57–62.

2. *Гонтарь В. И.* Описание первой находки пресноводной мшанки *Plumatella similirepens* Wood, 2001 (Bryozoa, Phylactolemata) из озера Удомля, Тверской области // Региональная экология. 2018. С. 59–65.

3. *Гонтарь В. И.* Мшанки (Bryozoa, Polyzoa, Ectoprocta) пресных водоемов России // Алтайский зоологический журнал. 2010. № 1 С. 52–62.

4. *Михаевич Т. В.* Методика культивирования пресноводных мшанок (Bryozoa, Phylactolemata) // Зоологический журнал. 1996. Т. 75. № 10. С. 1586–1589.

5. *Орлова М. И.* Долгосрочная программа предупреждения развития биопомех на АЭС, связанных с обрастанием: особенности организмов-источников биопомех, общие принципы формирования программы и значение мониторинга // Труды IV научно-практической конференции с международным участием «Экологическая и радиационная безопасность объектов атомной энергетики». Калининград, АО ИО РАН, 2017. С. 10–17.

6. *Орлова М. И., Строгова Е. В., Досмедов И. Х., Кузьмин В. В*. Предупреждение и борьба с биообрастанием в системах технического водоснабжения, как один из аспектов безопасности эксплуатации АЭС: ШАГ 1 — обновление системы биолого-химического мониторинга (БХМ) // МНТК Десятая международная научно-техническая конференция «Безопасность, эффективность и экономика атомной энергетики», 25–27 мая, 2016, тезисы докладов. М., Росэнергоатом. 2016. С. 172–174.

7. Отчет по договору № 127/12, Этап 5, Итоговый, "Биолого-химический мониторинг водоема-охладителя Курской АЭС, разработка долгосрочных мероприятий по улучшению качества технической воды, расчет оценки воздействия предлагаемых методов на окружающую среду", ООО НПО «Гидротехпроект», Санкт-Петербург. 2016. 204 с.

8. *Протасов А. А.* Пресноводный перифитон. Киев, Наукова думка. 1994.

9. *Хлебович В. В*. Критическая соленость биологических процессов. Ленинград. Наука. 1974.

10. *Bushnell J. H., Rao K. S.* Dormant or quiescent stages and structures among the Ectoprocta: physical and chemical factors affecting viability and germination of statoblasts // Transactions of the American Microscopy Society. 1974. Vol. 93. pp. 524–543.

11. *Claudi R., Mackie G. L.* Practical Manual for Zebra Mussel Monitoring and Control. Boca Raton, Ann Arbor, London, Tokyo: Lewis Publishers. 1993.

12. *Claudi R., Prescott T. H*. Evaluation of UV Technology at Hoover Dam as Means of Eliminating Downstream Settlement of Dreissenid Mussel Veliger // US Bureau of Reclamation Order R12PD80180, IDIQ Contract R10PC80264. 2013. 45 p.

13. *Orlova M. I., Strogova E. V., Lichi T., Lurie M*. Toward strategies for protecting cooling and service water systems (CSWS) from overgrowth by colonial invertebrates with resting stages in their life cycle: Plumatella emarginata (Tentaculata) and UV — control vs. combating. Part II. Statoblasts' and zooids responses to illumination by medium and low pressure UV lamps // Региональная экология. 2018. С. 30–42.

14. *Pucherelli S., Claudi R*. Assessment of UV Irradiation Effect on Downstream Settlement of Colonial Hydroid Cordylophora caspia // ICAIS 2017, Abstracts. p. 126.

15. *Railkin A. I.* Marine Biofouling Colonization Processes and Defenses. Boca Raton, London, New York Washington D.C.: CRC Press. 2004.

16. *Wood T. S*. The Pipeline Menace of Freshwater Bryozoans// Denesia 16, zugleich Kataloge der OÖ. Landesmuseen Neue Serie. 2005. no 8. pp. 203–208

17. http://docplayer.ru/69641371-Ciao-plumatella-ili-mshanochnyy-rif-korallovyy-rif.html. (дата обращения 1.02.2018).

References

1. Gontar V. I. *Opisanie pervoi nakhodki presnovodnoi mshanki Plumatella geimermassardi Wood&Okamura, 2004 (Phylactolaemata) v Koporskoi gube vostochnoi chasti Finskogo zaliva* [Description of first finding of freshwater Bryozoa Plumatella geimermassardi Wood&Okamura, 2004 (Phylactolaemata) in the Kopora Bay of the eastern part of the Gulf of Finland]. *Regional'naya ekologiya* [Regional Ecology], 2016, no. 1(43), pp. 57–62. (In Russian).

2. Gontar' V. I. R*egional'naya ekologiya* [Regional Ecology], 2018. pp. 59–65. (In Russian)

3. Gontar' V. I. *Mshanki (Bryozoa, Polyzoa, Yetstoprotsta) presnykh vodoyemov Rossii* [Bryozoans (Bryozoa, Polysoa, Yetproprots) of freshwater reservoirs in Russia]. Altaiskii zoologicheskii zhurnal [Altai Zoological Journal], 2010, no. 1, pp. 52–62. (In Russian).

4. Mikhaevich T. V. *Metodika kul'tivirovaniya presnovodnykh mshanok (Bryozoa, Pkhylatstolemata)* [Method of cultivation of freshwater bryozoans (Bryozoa, Pkhylaclotomata)]. Zoologicheskii Zhurnal [Zoological Journal], 1996, vol. 74, no. 10, pp. 1586–1589. (In Russian).

5. Orlova M. I. *Dolgosrochnaya programma preduprezhdeniya razvitiya biopomekh na AES, svyazannykh s obrastaniem: osobennosti organizmov-istochnikov biopomekh, obshchie printsipy formirovaniya programmy i znachenie monitoringa* [Long-term program to prevent the development of bio-disturbances at nuclear power plants associated with fouling: the features of organisms-sources of bio-disturbances, general principles of program formation and the importance of monitoring]. Trudy IV nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem "Ekologicheskaya i radiatsionnaya besopasnost' ob"ektov yadernoi energetiki" [Proceedings of the IV Scientific-Practical Conference with International Participation "Ecological and Radiation Safety of Nuclear Power Facilities"]. Kaliningrad, Atlantic Department of Institute of Oceanology, RAS Publ., 2017, pp. 10–17. (In Russian).

6. Orlova M. I., Strogova E. B., Dosmedov I. Kh., Kuzmin V. V. *Preduprezhdenie i bor'ba s bioobrastaniem v sistemakh tekhnicheskogo vodosnabzheniya kak odin iz aspektov bezopasnosti ekspluatatsii AES: SHAG 1 – obnovlenie sistemy biologo-khimicheskogo monitoringa (BKkM)* [Prevention and control of biofouling in technical water supply systems, as one of the safety aspects of NPP operation: STEP 1 – updating the biological and chemical monitoring system (BCM)]. *MNTK 10th mezhdunarodnaya nauchno-tekhnicheskaya konferentsiya "Bezopasnost', effectivnost' i ekonomika atomnoi energetiki": tezisy dokladov* [MNTK Tenth International Scientific and Technical Conference "Safety, Efficiency, and Economy of Nuclear Power Industry", May 25–27, 2016, Abstracts of Papers]. Moscow, Rosenergoatom Publ., 2016, pp. 172–174. (In Russian).

7. *Otchet po dogovoru № 127/12, Etap 5, Itogovyi, "Biologo-khimicheskii monitoring vodoema-okhladitelya Kurskoi AES, razrabotka dolgosrochnykh meropriyatii po uluchsheniyu kachestva tekhnicheskoi vody, raschet otsenki vozdeistviya predlagayemykh metodov na okruzhayushchuyu sredu"* [Final Report, Contract No. 127/12, Stage 5: "Biological and Chemical Monitoring of the Cooling Pond of the Kursk NPP, Development of Long-Term Measures to Improve the Quality of Technical Water, Environmental Impact Assessment of Proposed Methods"], St. Petersburg, "Hydrotechproject" LTD Publ., 2016, 204 p. (In Russian).

8. Protasov A. A. *Presnovodnyi perifiton* [Freshwater Peryphyton]. Kiev, Naukova Dumka Publ., 1994.

9. Khlebovich V. V. *Kriticheskaya solenost' biologicheskikh protsessov* [Critical Salinity of Biological Processes], Leningrad, Nauka Publ., 1974.

10. Bushnell J. H., Rao K. S. *Dormant or quiescent stages and structures among the Ectoprocta: physical and chemical factors affecting viability and germination of statoblasts.* Transactions of the American Microscopy Society, 1974, vol. 93, pp. 524-543.

11. Claudi R., Mackie G. L. *Practical Manual for Zebra Mussel Monitoring and Control.* Boca Raton, Ann Arbor, London, Tokyo, Lewis Publishers, 1993.

12. Claudi R., Prescott T. H. Evaluation of UV Technology at Hoover Dam as Means of Eliminating Downstream Settlement of Dreissenid Mussel Veliger, US Bureau of Reclamation Order R12PD80180, IDIQ Contract R10PC80264, 2013, 45 p.

13. Orlova M. I., Strogova E. V., Lichi T., Lurie M. *Regional'naya ekologia* [Regional Ecology], 2018. pp. 30–42.

14. Pucherelli S., Claudi R. Assessment of UV Irradiation Effect on Downstream Settlement of Colonial Hydroid Cordylophora caspia, ICAIS, 2017, Abstracts, p. 126

15. Railkin A. I. Marine Biofouling Colonization Processes and Defenses, Boca Raton, London, New York Washington D.C., CRC Press, 2004.

16. Wood T. S. The Pipeline Menace of Freshwater Bryozoans, Denesia 16, zugleich Kataloge der OÖ. Landesmuseen Neue Serie. 2005, no. 28, pp. 203–208.

17. http://docplayer.ru/69641371-Ciao-plumatella-ili-mshanochnyy-rif-korallovyy-rif.html. (accessed 1.02.2018).

Краткие сведения об авторах:

Орлова Марина Ивановна, д. б. н.

[1]Ведущий научный сотрудник,

лаборатория пресноводной и экспериментальной гидробиологии,

[2]Главный научный сотрудник.

Научные интересы: водные и прибрежные экосистемы, включая техногенные; сообщества обрастателей: структурно-функциональные особенности и предупреждения развития биопомех, чужеродные виды: систематика, эволюция, расселение; научные основы морского пространственного планирования.

E-mail: marina.orlova2012@gmail.com

Marina I. Orlova, DSc, PhD (Biol.).

Leading Researcher, Chief Researcher.

Areas of interests: aquatic and coastal ecosystems, including technogenic; fouling communities: structure and functioning, prevention of biofouling; non-indigenous species: taxonomy, evolution, dispersal; scientific background for marine spatial planning.

E-mail: marina.orlova2012@gmail.com

Строгова Елена Владимировна.

[2] Заведующая отделом экологии техногенных и искусственных систем

[3]Исполнитель гостемы, эксперт.

Научные интересы: техногенные и техногенно-трансформированные экосистемы; сообщества обрастателей, динамика расселительных стадий организмов-источников биопомех; зоопланктон; зообентос.

E-mail: elenastrogova@bk.ru

Elena V. Strogova

[2]Head of Department of Ecology of Techogenic and Artificial Systems.

[3] Executor of state theme, expert.

Areas of interests: technogenic and technogenically transformed ecosystems; fouling communities, dynamics of dispersal stages of nuisance fouling organisms; zooplankton, zoobenthos.

E-mail: elenastrogova@ bk.ru

Личи Товит

Заведующая лабораторией.

Научные интересы: микробиальные сообщества, микроперифитон, воздействие универсальных дезинфектантов на микробиальные сообщества в техногенных экосистемах.

E-mail: tovit@atlantium.il

Lichi Tovit

Head of laboratory.

Areas of interests: microbial communities, microperiphyton, impact of universal desinfectans onto microbial communities in technogenic ecosystems.

E-mail: tovit@atlantium.il

Лурье Максим Абрамович

Заведующий отделом продаж

Научные интересы: автоматизация блоков дозирования и контроля в системах дезинфекции водных потоков с использованием ультрафиолетового излучения.

E-mail: maxlurie@atlantium.il

Maxim A. Lurie

Head of Sales Department.

Areas of interests: automatization of dosage and control in UV based disinfection systems for treatment of water.

E-mail: maxlurie@atlantium.il

Для цитирования: *Орлова М. И., Строгова Е. В., Личи Т., Лурье М.* К стратегиям защиты систем циркуляционного и технического водоснабжения (СТВ) от обрастания колониальными беспозвоночными с покоящейся стадией в жизненном цикле: *Plumatella emarginata* (Tentaculata) и ультрафиолетовое излучение uv — контроль vs. уничтожение. Часть I. Новые данные о выходе из диапаузы и их применение для экологического мониторинга СТВ // Региональная экология. 2018. № 1/ С. 31-43. DOI: 10.30694/1026-5600-2018-1-31-43

For citation: Orlova M. I., Strogova E. V., Lichi T., Lurie M. [Toward strategies for protection of cooling and service water systems (CSWS) from overgrowth by colonial invertebrates with resting stages in their life cycle: *Plumatella emarginata* (Tentaculata) and UV–control vs. combating. Part I. New data on dormancy release and their value for ecological monitoring of CWS]. Regional'naya ekologiya [Regional Ecology], 2018, no. 1. pp. 31–43 (In English). DOI: 10.30694/1026-5600-2018-1-31-43