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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 — контроль vs. уничтожение.

Часть II. Реакции статобластов и зооидов на облучение ультрафиолетовыми лампами среднего и низкого давления

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В статье изложены основные принципы выбора средств превентивной защиты оборудования СТВ от биопомех, связанных с развитием организмов, имеющих в своем жизненном цикле покоящиеся стадии. Среди таких средств — наиболее экологически-безопасных и «технологичных» - указывается средневолновое ультрафиолетовое излучение (UV). На основе опыта культивирования статобластов мшанки рода *Plumatella*, описанного в предыдущем сообщении и технических возможностей экспериментирования с двумя типами излучателей (ламп) — (полихроматического — с длиной волны 200–400 и монохроматического — 254 нм), дана оценка однократного первичного воздействия промышленных доз обоих типов UV на статобласты (флотобласты), выходящие из состояния диапаузы в лабораторных условиях. Показано наличие биоцидного и биостатического воздействия на статобласты всех использованных режимов облучения за единственным исключением, а также зависимость проявления биоцидного и биостатического эффекта UV от исходного состояния статобластов. В отношении вылупившихся из необлученных статобластов зооидов-основателей колоний все режимы UV, использованные в эксперименте со статобластами, демонстрируют биоциодный и биостатический эффект на зооидах. Важной особенностью воздействия UVявляется множественность «ответов» статобластов, в частности возможны как подавление развития, так и сочетание подавления с временной задержкой развития, сопровождающейся последующим более дружным выходом зооидов из уцелевших статобластов. Полученные результаты экспериментов предоставляют исходный материал для планирования производственных испытаний, направленных как на прямое уничтожение статобластов и подавление их выхода из диапаузы, так и на перевод статобласта в активное состояние — зооид, который более чувствителен к воздействию различных факторов среды, то есть фактически на управление жизненным циклом мшанки. Также этот подход может быть испытан и как метод прямого уничтожения уже вылупившихся зооидов, сохранивших способность к перемещению с током технической воды в составе флотирующих агрегаций.

Ключевые слова: контроль обрастания с использованием ультрафиолета, монохроматическое и полихроматическое средневолновое ультрафиолетовое излучение, выход из диапаузы, биоцидное и биостатическое действие на статобласты и зооиды-основатели колоний, *Plumatella*.

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 exposure by medium and low pressure UV lamps

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This paper presents basic (12) principles for selection of preventative strategies for control of infestation in CSWS by fouling organisms which have a resting stage in their life cycle. Use of medium wavelength UV is suggested as one of the possible, ecologically safe, strategies. Based on our experience of cultivating statoblasts of bryozoans of the genus *Plumatella* (described in previous communication) we were able to experiment with the impact of two types of UV generators on this resting lifestage. We tested the impact of polychromatic lamps with the wavelengths of 200–400 nm and also that of monochromatic lamps with a single wavelength of 254 nm. The objective was to assess what impacts various doses from both types of UV have on statoblasts (flotoblasts) passing through dormancy release under laboratory conditions. We observed both biocidal and biostatic effects from all doses used. The effects were dependent on the initial conditions of the statoblasts. All of the doses tested had biocidal and biostatic effect on zooids which were hatched from statoblasts not exposed to UV lights. There appears to be a number of "responses" of statoblasts to UV exposure such as: suppression of development; combination of suppression and temporary delay of development accompanied by subsequent more simultaneous hatching of zooids from statoblasts which remained viable. These experimental results form the basis for planning field testing aimed at assessing the applicability of UV either for directly combating statoblasts by suppressing their dormancy release or by facilitating the transformation of statoblast into more vulnerable form. The UV should also be tested as a mitigation strategy for zooids that have already hatched but have kept their ability to be moved by the water as floating aggregations.

Keywords: UV based biofouling control; monochromatic and polychromatic medium wavelength ultraviolet radiation (UV); dormancy release; biocidal and biostatic impact on statoblasts and zooids-founders of colonies; *Plumatella*.

Introduction

This paper is a companion publication to the previous paper documenting the basic lifecycle and cultivation of statoblasts of *Plumatella emarginata* [1]. The previous publication was focused mostly on the life cycle of *Plumatella emarginata* and dormancy release as key phase for testing of control strategies aimed to prevent biofouling[1)] caused by organisms possessing dormant bags stages in their life cycle.

Peculiarities of possible strategy related to prevention of colonization of CSWS by invertebrates possessing dormant bags on the example of *Plumatella.*

As suggested in the previous paper [1], control of biofouling species with resting phase(s) in their life cycle is more complex than control of biofouling organism with free-living stage and no dormancy life stage. One of the possible ways to facilitate prevention of *Plumatella* fouling could lay in the management of key phase of its life cycle. For example, impacting the dormancy release by suppressing or enhancing hatching of zooid-founders of colonies. If hatching is enhanced, other control strategies can then be applied to this active, and, more vulnerable life stage. Equally, as

[1)] Fresh water and marine sessile organisms form living assemblages that may be called "fouling" or "periphyton" (see more about terminology in [6–8 etc.]). These assemblages form multicomponent systems consisting of unicellular (including pathogens) organisms causing damage to the materials of construction (biofilm [9]) as well as metazoans — obligatory sessile — and their consorts. It is thought [8] that the development of fouling occurs as a succession where the colonization by unicellular organisms is followed by the settlement of metazoans — invertebrates and macrophytes. As a rule, it is at this final and irreversible phase of biofouling that mechanical cleaning and chemicals are applied to combat the fouling, Theoretically, interrupting the fouling process at an earlier stage of development may suppress or prevent the fouling potentially making control of biofilm so far easier and more cost effective.

we know now from our observation (Fig. 5 in [1]), there is one more target for control strategies, such as the floating aggregates consisting of statoblasts and developing zooids. There are no records in the open literature which would describe such "*anti-plumatella*" strategy. Lack of this information is why we carried out this experiment to establish whether it is possible to accelerate or decelerate dormancy release and enhance growth in population number of newly hatched zooids of *P. emarginata* as the final target for control.

UV as an ecologically safe, universal disinfectant for testing suggested strategy of management of dormancy release for prevention *Plumatella* fouling: prerequisites and criteria for selection

As mentioned previously [1], in conditions of CSWS we observed irregular alternating in phases of activity and rest and spontaneous rapid development of plumatellid fouling usually preceded by dormancy release of statoblasts. As predicting of seasonal and biological cycles is difficult in CSWS, they require continuous means of fouling control. Further, *Plumatella* commonly co-occurs with other fouling invertebrates[1)] as well as microbial biofilms [2] which also have to be controlled. Therefore, a control strategy which addresses all of the components of biofouling is desirable instead of those that target only one component such as *Plumattella*. Of the universal strategies, those which are also able to affect dormancy release are the more preferable. There are other basic criteria for selection of control methods as shown in Table 1. When UV is measured up against these criteria it meets most of the requirements: absence of known by-products or pollutants so far and applicability of continuous UV treatment; availability of industrial grade technology(ies); proven effectiveness against co-occurring fouling species and associations [3–5].

This paper aims to answer point № 9 in Table 1 by the laboratory test of efficacy. Below we provide the results of laboratory experiments with UV lights tested as universal disinfectant with suggested biostatic or lethal (biocidal) effect on both germinating statoblasts and newly hatched zooids and probably stimulating effect on statoblasts. Thus tested was the effect of the UV lights as a possible driving factor for dormancy release. We used medium UV spectrum (UVB, UVC) both as polychromatic illumination (generated by medium pressure mercury lamps — 200–400 nm) and as monochromatic (low pressure lamps — 254 nm) one.

Material and methods

1. Material. The same material on *Plumatella emarginata* we used for observation of dormancy release [1] was used in the two experiments described below. The trials (samples) for dormancy release observation described in Part I [1] have been used as control trials in Experiment 1.

2. General description of experiments. Two experiments were performed. Experiment 1 used statoblasts, and in Experiment 2 newly hatched zooids were exposed to eight different UV doses (Table 2). The experiments were carried out on 7–14th June 2017, in the lab. of Atlantium Technologies Ltd. Each dose (L — low pressure lamp's illumination, M — medium pressure) was applied once to statoblasts (Groups A,U,T, see below for their description), once or twice with short interval (20–25 min) to zooids (only Group U).

All individuals treated with UV in Experiment 1 were kept after exposure in cultivation vessels (see Fig. 4 in [1]) under the same conditions as the control individuals in order to observe any post-exposure difference in dormancy release and the state of the statoblasts in treated and untreated samples. This post-treatment cultivation lasted from 5 for Group T to 7–8 days for Groups A, U. Data on abiotic conditions are summarized in Fig. 1.

Treated and untreated zooids (Experiment 2) have also been checked during post-treatment cultivation for their behavior in order to estimate presence of suppression (damage) and to observe repair of damage caused by exposure to UV. Damage may be reversible or irreversible. The post-treatment cultivation lasted 60 hours.

3. Experimental factors, Groups A, U and T of statoblasts

3.1. Experiment 1

Factor 1 (integrated factor, expected to imitate (through transportation and acclimation to the laboratory maintenance) ***influence of weather and environmental conditions in piping on the germination of statoblasts***. In total there were 3 levels of this factor [(1) — (3)], reflected in the three groups of statoblasts, used in experiments. *(1) Group T ("travel")* — was transported from St-Petersburg to Tel-Aviv in summer time, in packed luggage (total of 2 days) followed by exposure to room temperature without air-conditioning for 24 hours. This group experienced stressful conditions with rapid defrost and subsequent oscillations of temperature, limited access for air etc., like those experienced during unstable spring weather or in harsh conditions of CSWS. It was expected that *such stressful activation would facilitate biocidal and biostatic effect (or even destruction) of statoblasts during UV exposure. (2) Groups A ("active") and (3) U ("inactive")* — were transported from St-Petersburg to Tel-Aviv in the winter time, in a cooler, and immediately placed in a freezer upon arrival.

Table 1

Example of selection of UV-based technologies for laboratory and further industrial testing for preventing *Plumatella* fouling development.

Таблица 1

Пример выбора технологий, основанных на воздействии УФ излучения для лабораторного тестирования и последующих производственных испытаний для предупреждения развития обрастания мшанкой р. Плюмателла

Requirements and criteria	UV (medium, low pressure lamps)
Basic criteria and requirements	
(1) Accessibility, including industrial equipment, availability. Information on efficacy of testing in energy sector published in open sources;	+ (e. g. "Atlantium HOD technology" — sold in a variety of modifications, resident companies able to provide engineering and technical services including Russian, results of testing are published [3, 5])
(2) Availability of regulatory permissions to apply in inland waters or positive experience of the use against fouling;	+ (UV is natural factor, required experience is available [3, 5])
(3) Applicable to large cooling water systems of power plants;	+(Equipment for flows of 900 m^3/hour are available and tested in industrial conditions)
(4) Availability of automatic equipment (including correct dosage) for use under different regimes and doses;	+ (On-line automatic control on UV dose and environmental parameters while sending results to consumer)
(5) Efficiency is independent of important water chemical and physical characteristics and under unpredictable conditions	+ (Is reached due to 4 and by automatic adjustment of dosage following changes in environmental characteristics)
(6) There are no by-products and therefore no bio accumulation in water-bodies. No toxicity to receiving water body;	+ (see (2))
(7) Provides multiple effects: destroys biofilm and prevents settlement of fouling organisms;	–/+ (Not appropriate: does not destroy already existing biofilm; Appropriate: prevents/moderates formation of biofilm by eliminating spores and active microorganisms drifting in raw water)
(8) Controls not only dispersal stages of fouling metazoans but also settled juveniles and mature biofouling;	– (Not appropriate: appropriate to prevention of settlement controlling dispersal states (larvae and dormant bags) drifting in raw water)
(9) Ideally: efficient against resting stages of invertebrates;	+/? (Laboratory (step 1, this paper) and then industrial (step 2) tests are required)
(10) Economically attractive;	?
(11) Does not damage materials of construction (e.g. by corrosion);	+
(12) Can be applied as monomethod and in combination with other methods;	+
Additional criteria and requirements	
(13) Applicable as a continuous treatment regime.	+ see 2–6 and 11

Требования и критерии	УФ (излучатели (лампы) среднего и низкого давления)
Базовые критерии и требования	
(1) Доступность, включая промышленное оборудование, его наличии на рынке. Наличие информации об эффективности применения (тестирования) оборудования в секторе энергетики, опубликованной в открытых источниках;	+ (Один из примеров соответствия критерию "Atlantium гидрооптическая технология (HOD)" (продается ряд модификаций, в России имеются местные инжиниринговые компании, осуществляющие установку, наладку и обслуживание оборудования, опубликованы результаты тестирования контроля обрастания, в том числе производственного [3, 5]))
(2) Наличие разрешений и допустимость применения на внутренних водоемах, наличие опыта использования против обрастания ;	+ (УФ является естественным фактором, имеется опыт применения против обрастания [3, 5])

(3) Возможность использования в системах охлаждения с большими расходами воды на электростанциях	+ (Оборудование для обработки воды в системах с расходом воды 900 м^3/час, прошедшее промышленные испытания, имеется в продаже)
(4) Наличие автоматизированных систем поддержания постоянства режима обработки (дозы и периодичности воздействия) при нестабильности основных параметров обрабатываемой воды	+ (On-line автоматизированные поддержание и коррекция дозы УФ в соответствии с результатами измерения параметров среды, передача данных потребителю)
(5) Воздействие эффективно независимо от не поддающихся учету колебаний физических и химических параметров воды.	+ (достигается посредством (4))
(6) Отсутствуют побочные продукты и их накопление в окружающей среде и живых организмах. Нетоксично.	+ (см. (2))
(7) Эффективность против нескольких мишеней: разрушает сформировавшуюся биопленку и предотвращает оседание обрастателей	–/+ (Не соответствует: оказывая воздействие на ограниченный сегмент водного потока, не разрушает сформировавшуюся биопленку ниже точки воздействия; Соответствует: предотвращает (или существенно снижает) формирование биопленки и оседания личинок за счет воздействия на споры и активные микроорганизмы, а также на личинки, проходящие (дрейфующие) с потоком воды через зону обработки)
(8) Контролирует не только расселительные стадии многоклеточных обрастателей, но также осевшую молодь и состоявшееся (зрелое) обрастание	– (Не соответствует: действует только на расселительные стадии обрастателей и другие биологические объекты, находящиеся в дрифте в облучаемом сегменте водного потока)
(9) В идеале: эффективно против покоящихся стадий водных беспозвоночных	***+/? (Необходимо проведение лабораторного первичного тестирования (шаг 1, данная статья) и последующие производственные испытания (шаг 2)***
(10) Экономическая целесообразность	?
(11) Не вызывает повреждений конструктивных материалов (например, коррозии металла)	+
(12) Может быть использовано как монометод и в комбинации с другими методами	+
Дополнительные критерии и требования	
(13) Применимо в режиме непрерывных обработок	+ (см. 2–6 и 11)

It was anticipated that these groups would arrive in the lab. either frozen or enough cool to remain dormant. Subsequently both these groups (A, U) were gradually brought from sub-zero temperature to room temperature in small aquaria with access of air. The period of such acclimation was 3 days in Group A (from mid — 4th till mid 7th June), in Group U it was one day longer (totally 4 days — from mid 4th till mid 8th June). *This regime imitated gradual changes in environmental conditions such as those experienced during "favorable spring" and slow beginning of summer. Such conditions are expected to predispose statoblasts to more effective germination and hatching and possibly to greater resistance to UV treatment.*

Factor 2 — regime of treatment. We used Medium pressure (M) and Low pressure (L) lamps and 4 doses (mJ/cm^2) for medium UV (M25, M50, M 100, M200) and also the same 4 doses for low pressure UV (L25, L50, L100, L200) (Table 2). All these doses, at least those of medium pressure, are reproducible in industrial water flows.

Factor 3 — time. We examined all samples treated by UV and all or part of the control samples several times during 7 days following the exposure. We looked for specific criteria (morphological, quantitative and semi-quantitative). These criteria are not discussed in detail here, as they can be found further in "Result and Discussion" section below and in companion paper's Tables 1 and 2 and in the text of "Results and Discussion" para 2 there [1].

3.2. Experiment 2.

Only zooids from Group U hatched from non-illuminated statoblasts were used in this experiment.

Factor 1 — regime of treatment. Total of 10 regimes were tested (Table 2) — doses tested were the same as in Experiment 1. However, in addition, doses M25 and M50 were also applied for 20–25 min (ZUM 25X2, ZUM 50X2) (Table 2).

Factor 2 — time. We examined treated and control zooids four times during the 60 hours following exposure to UV.

Table 2

Types of treatments, types of experiments, groups of statoblasts/zooids and codes for experimental and control samples (7–14th June 2017) used then in Figs. 2–5

Таблица 2

Типы обработок, типы экспериментов, группы статобластов/зооидов и шифры для опытных (обработанных) и контрольных проб (7–14 июня 2017), использованные затем на рисунках 2–5

Type of treatment (lamps and doses (mJ/cm^2))	Experiment 1 (experiment ***with statoblasts***)			Experiment 2 (experiment ***with zooids***)
	Group			
	A	U	T	U
Experimental samples (illuminated)				
Medium pressure				
M25	AM25	UM25	TM25	ZUM25
M25X2	no	no	no	ZUM25X2
M50	AM50	UM50	TM50	ZUM50
M50X2	no	no	no	ZUM50X2
M100	AM100	UM100	TM100	ZUM100
M200	AM200	UM200	TM200	ZUM200
Low pressure				
L25	AL25	UL25	TL25	ZUL25
L50	AL50	UL50	TL50	ZUL50
L100	AL100	UL100	TL100	ZUL100
L200	AL200	UL200	TL200	ZUL200
Control samples				
1	A-1	U-1	T-1	ZUC
2	A-2	U-2	T-2	no
3	A-3	U-3	T-3	no
L contr	AL contr	UL contr	TL contr	no
ini-1	Aini-1	Uini-1	Tini-1	no
ini-2	Aini-2	Uini-2	Tini-2	no
ini-3	Aini-3	Uini-3	Tini-3	no
1 mag	A mag 1	no	no	no
2 mag	A mag 2	no	no	no
3 mag	A mag 3	no	no	no

Тип обработки (лампы и дозы (mДж/см²))	Эксперимент 1 (эксперимент ***со статобластами***)			Эксперимент 2 (эксперимент ***с зооидами***)
	Группа			
	A	U	T	U
Экспериментальные пробы (облученные)				
Лампы среднего давления (M)				
M25	AM25	UM25	TM25	ZUM25
M25X2	no	no	no	ZUM25X2
M50	AM50	UM50	TM50	ZUM50
M50X2	no	no	no	ZUM50X2
M100	AM100	UM100	TM100	ZUM100
M200	AM200	UM200	TM200	ZUM200
Лампы низкого давления (L)				
L25	AL25	UL25	TL25	ZUL25
L50	AL50	UL50	TL50	ZUL50
L100	AL100	UL100	TL100	ZUL100
L200	AL200	UL200	TL200	ZUL200
Контрольные пробы				
1	A-1	U-1	T-1	ZUC
2	A-2	U-2	T-2	нет
3	A-3	U-3	T-3	нет
L contr	AL contr	UL contr	TL contr	нет
ini-1	Aini-1	Uini-1	Tini-1	нет
ini-2	Aini-2	Uini-2	Tini-2	нет
ini-3	Aini-3	Uini-3	Tini-3	нет
1 mag	A mag 1	нет	нет	нет
2 mag	A mag 2	нет	нет	нет
3 mag	A mag 3	нет	нет	нет

We estimated their capacity to survive the UV treatments using the criterion of the number of active *z*ooids within the total number of zooids and intact statoblasts.

Results and discussion

1. Experiment 1

1.1. Direct and side effects of UV during germination of statoblasts.

On the 4th day after the treatment (1) the direct effect of different doses and types of UV (Factor 2) was the most visible. The contribution of Factor 1 (belonging to the A, U, T groups) as well as some (2) side effects expressed in absence of biofilm on statoblasts and ciliates in treated samples became evident.

(1) Differences in % of closed and opened, intact and damaged statoblasts are contrasted between Groups and between doses within Groups (Fig. 2 with quantitative data[2)]).

(2) Surprisingly damaged looking statoblasts in some control samples also appeared by the 4th day. The (2) is explained by natural formation of

[2)] **Comment to quantitative data (in Fig. 2)**. ***Group T.*** Experimental samples contained more damaged statoblasts as compared with preceding observations (2 days) and with control samples. Thus we can suggest negative biocidal or biostatic effect of both Medium (especially) and Low UV in all doses applied to this group. Negative effect becomes more apparent over time after exposure. Group U, which passed through the most favorable regime of acclimation to room temperature, looks as the most resistant to UV comparatively to other groups (T, A). It is possible to suggest that both types of UV are ineffective in low doses for this group. Higher doses appear to decelerate dormancy release. Group A (intermediate by regime of acclimation) manifested sufficient difference in impacts from Medium and Low pressure UV. The former seems to stimulate germination (in terms of opening statoblasts) inversely proportional to the dose applied, while Low pressure UV depresses germination directly proportional the dose.

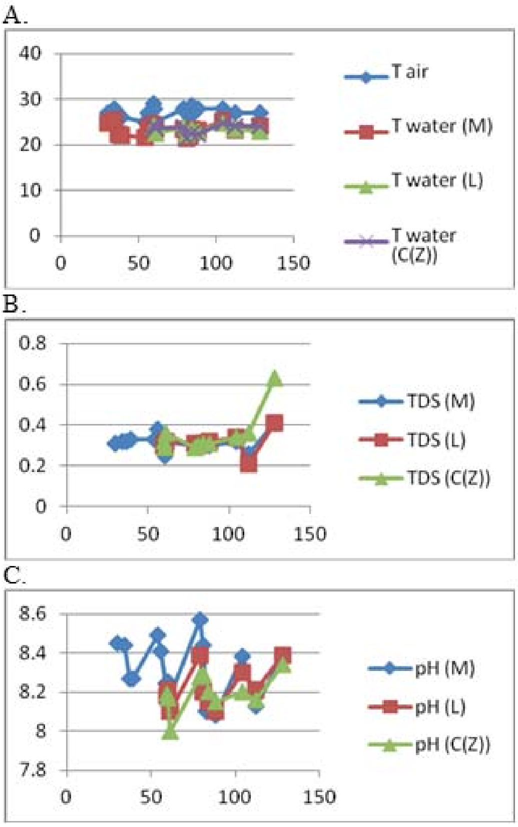


Fig. 1. Abiotic conditions in three cultivation vessels (coded by letters — M, L, C(Z)) during post-treatment cultivation of treated and control samples (8–14th June, 2017). A — Air temperature and temperature of water in three vessels (T ºC); B — TDS (ppt); C — pH. Axes: OX — Time (hours from the start of post-experimental cultivation); OY — values for each parameter (T — ºC; TDS — ppt; pH — no units).

Рис. 1. Абиотические условия в трех емкостях для культивирования обработанных и контрольных проб (буквенные обозначения емкостей — M, L, C(Z)) после обработки (в течение 8–14 июня, 2017). A — Температура воздуха и температура воды в трех емкостях (TºC); B — TDS (минерализация) (ppt); C — pH. Оси: OX — Время (в часах от момента пост-экспериментального культивирования); OY — значения каждого параметра (T — ºC; TDS — ppt; pH).

microbial communities therein (Fig. 3 with semi-quantitative Data[3)]), which were not eliminated by the UV. Thus the potential user of the UV control methods as well as the designer of the treatment strategy and those who will carry out industrial testing should take into account this disinfection effect. On the one hand this observation is considered evidence for basic criterion 7 from Table 1 (multiple effect of the method on both targets — dormant stages and microorganisms) and on the other hand it can be a source of misunderstanding of the method's efficiency against major target — statoblasts, zooids and aggregates as control samples will show worse values of analyzed parameters (germination, hatching, survivorship of zooids, etc.) due to natural destructive activity of microorganisms (bacteria, ciliates, etc.) in culture of statoblasts (zooids). In other words, the "long term" survival of controls individuals is being compromised by the presence of microorganisms absent in the treated samples.

Comparing observations on germination after a few hours, after 2 and 4 days shows that Factor 3 (Time) is driving factor for the state of both control and experimental samples and for divergence between Groups.

1.2. Hatching (5th and 7th days of observation). Divergence between Groups becomes more evident during hatching. Effects of UV vary by type of UV and dose (Fig. 4). The most effective treatments were high doses of Medium pressure UV (70 % decrease of hatched zooids when compared to controls on 5th to 7th day). Low pressure UV appears to always negatively affect hatching, although patterns are different between groups A and U. Surprisingly, the higher doses seem not to be as effective as lower doses[4)].

Therefore, exposure to UV does not prevent hatching of zooids completely BUT it does result in transformation of *inactive (resistant) statoblasts to zooids (sensitive stage of life cycle)*. All treatment regimes with one exception (UM25 in Group A) resulted in a decrease in the abundance of hatched zooids-founders of colonies over time (Fig. 4) and therefore the treatment appears to *depress Plumatella emarginata's potential to formation of fouling.*

[3)] **Comment to semi-quantitative data (in Fig. 3).** *The degree of consolidation/aggregation* (see Fig. 5 in [1] under number 5 and comments in the text [1] about formation of raft-looking floating aggregates of statoblasts and zooids) seems to be dependent on exposure; it decreases in Groups A and U inversely as the dose and it is stable (medium level) in controls and rather low in Group T, already affected by transportation conditions and by UV treatment (presence of numerous aborted zooids in experimental groups and relatively moderate in controls). *Disinfection effect* — there was no visual signs of development of bacteria or ciliates in the experimental samples, control samples demonstrated some damage and biofilm-looking overgrowth on statoblasts as well as presence of ciliates was noted.

[4)] At this point we do not understand the underlying mechanisms. However we can propose that it is analogous to an isolation reflex present in many other invertebrates with shell. The isolation reflex exists to minimize contact of sensitive soft tissues with the environment, when the environment becomes extremely hostile (above the barrier of tolerance). The higher the level of harmful impact the higher the probability of triggering isolation reflex if the organism is not instantly killed.

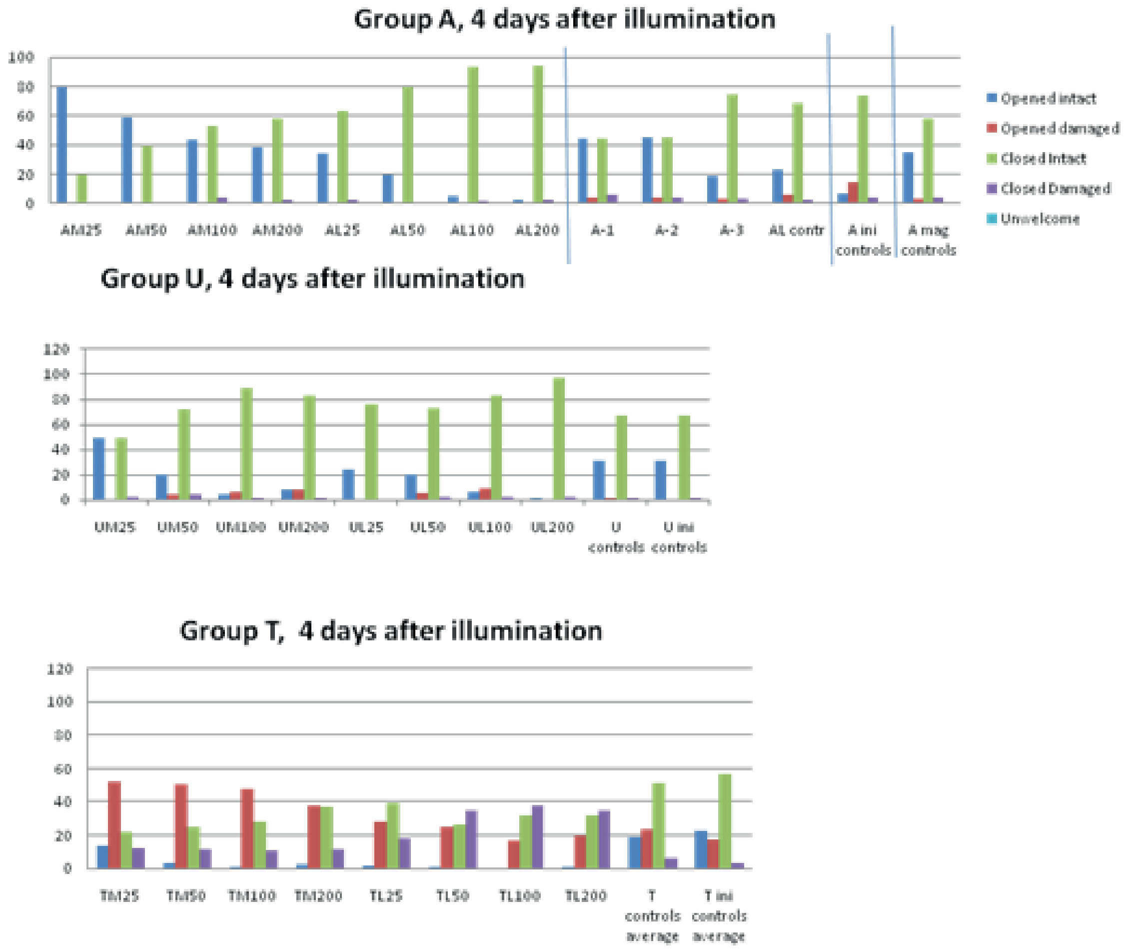


Fig. 2. State of UV treated and control samples of statoblasts in Groups A, U, T on 4th day, estimated by quantitative characteristics (codes as in Table 2). Axes: OX — type of sample (codes are in Table 1; OY — contribution of each type of statoblasts (%) into total number of statoblasts checked in 2 fields of vision of binocular microscope.

Рис. 2. Состояние облученных УФ и контрольных статобластов Групп A, U, T на 4-й день пост-экспериментального культивирования, оцененное по количественным характеристикам (см. шифры в Таблице 2). Оси: OX — тип пробы (шифр в Таблице 1); OY — доля (%) статобластов каждого типа в их общей численности, учтённой в двух полях зрения бинокулярного микроскопа.

2. Experiment 2

All doses were effective in temporarily decreasing the percentage of active zooids, 70 % decrease compared to controls, despite evidence of ongoing processes of reparation (upper diagram in Fig. 5).

3. Correspondence between results from Experiment 1 and Experiment 2

Observed diversity in zooids' responses to the UV treatment is enrooted, in particular, in diversity of UV effects registered at phase of germination – biostatic (depressing germination) or stimulating (accelerating germination) (Fig. 5). For visualization of this suggestion we combined results of observations for Group U during both experiments: on zooids dynamics (upper diagram in Fig. 5, Experiment 2), on the late germination (4th day) (second diagram from Fig. 2, Experiment 1), on hatching (days 5 and 7, right diagram from Fig. 4, Experiment 1) as a joined block of diagrams (Fig. 5). This simple graphic combination has allowed omitting some details and distinguishing FOUR major sequences of *P. emarginata* response to UV exposure at all steps of dormancy release: "germination — hatching — living as single newly born zooid".

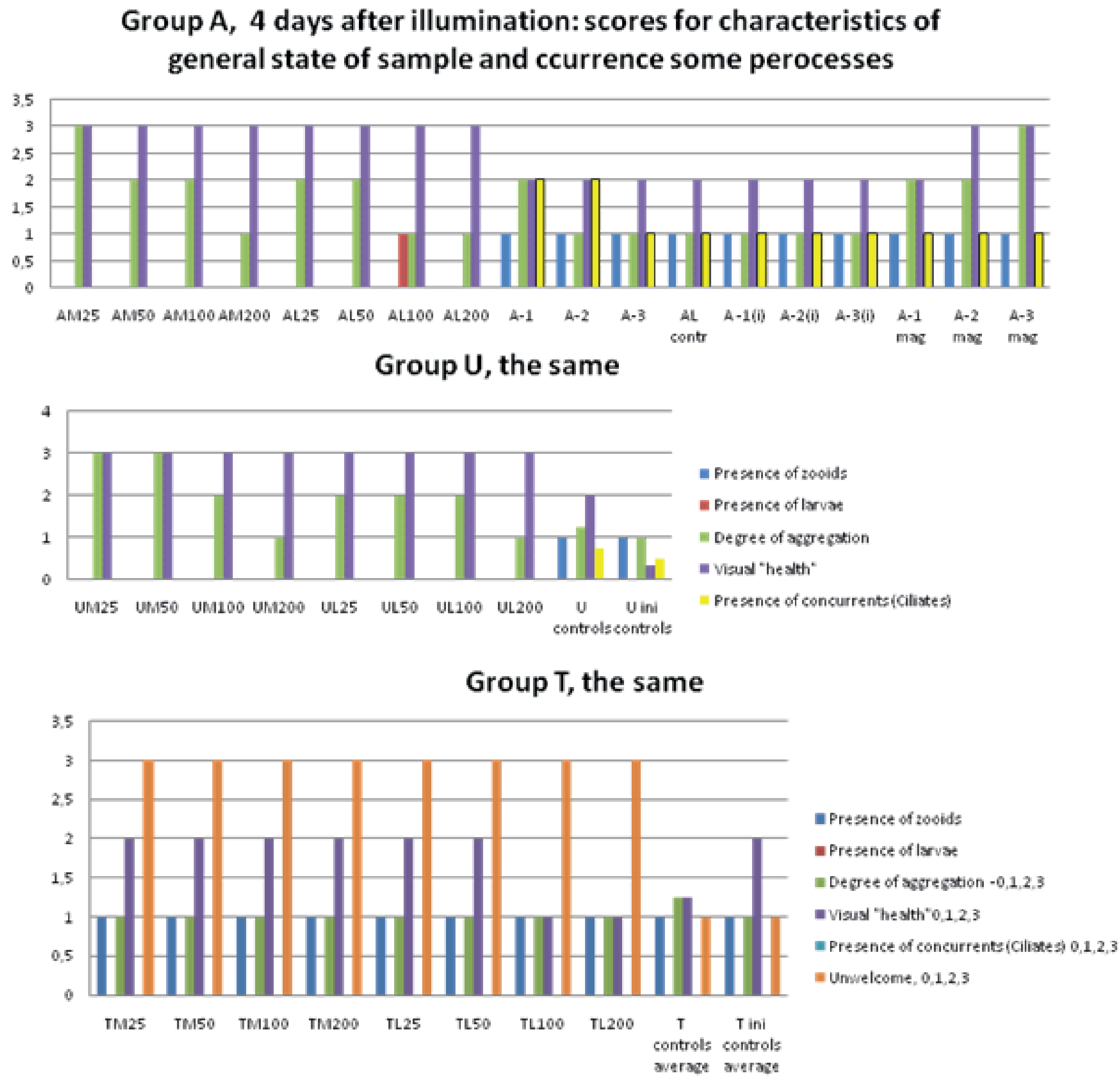


Fig. 3. Visual semi-quantitative evaluation (characteristics of consolidation processes (“degree of aggregation”), leading to formation of raft-looking aggregates, beginning of hatching and efficiency of UV dose in inhibiting development of microbial communities (“presence of competitors (Ciliates)”, “visual health”) of the appearance of experimental and control statoblasts on 4th day. Axes: OX — type of sample (codes are in Table 2); OY — scores (in 2 fields of vision of binocular microscope).

Рис. 3. Визуальная полуколичественная оценка (характеристики процесса консолидации («степень агрегированности»), ведущей к формированию флотирующих скоплений, начала вылупления зооидов и эффективности облучения УФ в подавлении развития микробиальных сообществ (“присутствие конкурентов (инфузории)”, “визуального здоровья”) внешнего вида статобластов в экспериментальных и контрольных пробах на 4-й день пост-экспериментального культивирования в баллах. Оси: OX — тип пробы (шифр в Таблице 1); OY — баллы (по результатам учета в двух полях зрения бинокулярного микроскопа).

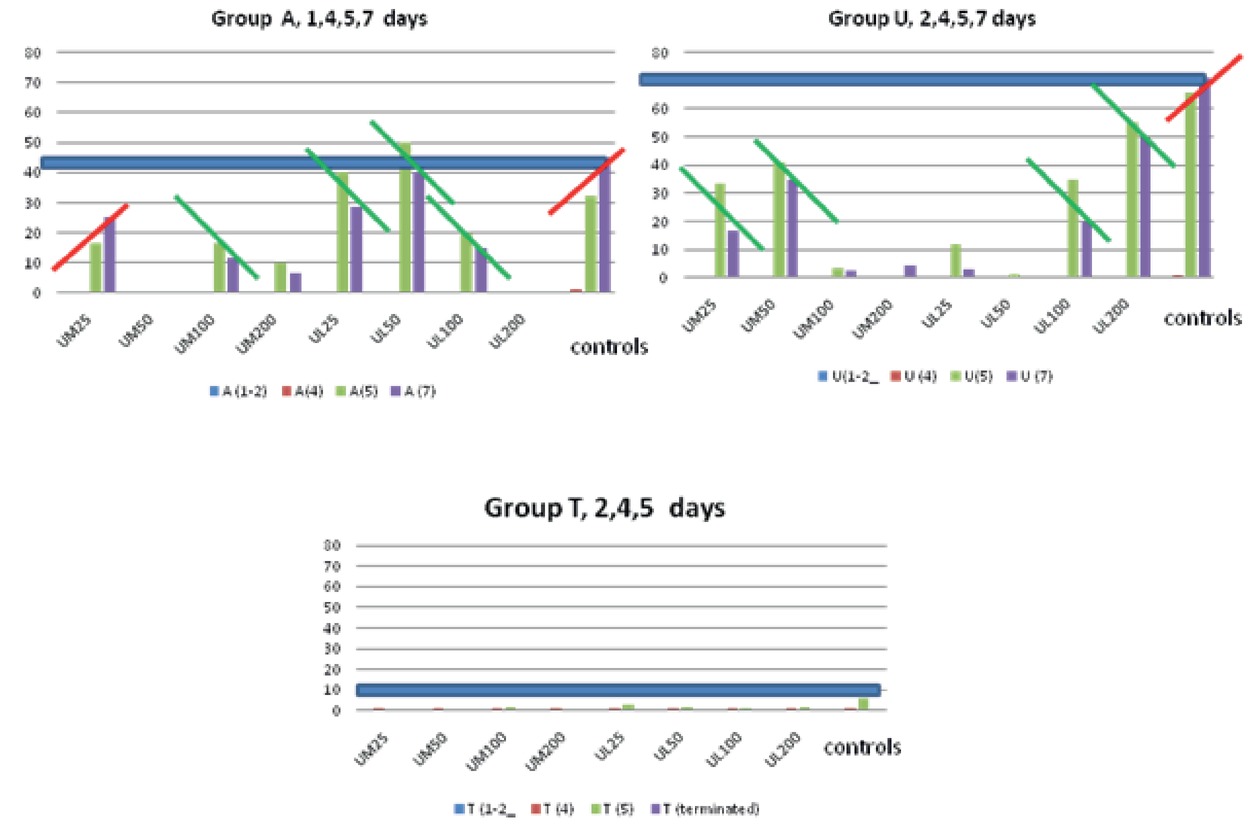


Fig. 4. Percentage of zooids in the total number of viable individuals (statoblasts and zooids) observed in 2 fields of vision of binocular microscope in experimental and control (averages) samples and the dynamics of this percentage over the period of observation (in 1–2, 4, 5, 7th days). *Legend*: Thick horizontal line shows average % of zooids in controls on 7th day; red lines indicate positive dynamics (*Factor 3, time*) in % of zooids during 5 and 7th days (*shows absence of biocidal and biostatic impact or its compensation with reparative processes or its compensation due to stimulation of "reserve" development); green lines indicate negative dynamics (evidence of gradual decrease in the population of zooids*).

Рис. 4. Процентное содержание зооидов в общем количестве жизнеспособных индивидуумов (статобластов и зооидов), учтенных в двух полях зрения бинокулярного микроскопа, в экспериментальных и в контрольных (среднее значение) пробах и его динамика за период наблюдений (в 1–2, на 4, 5, 7-й дни). *Обозначения*: Жирная горизонтальная линия на диаграммах показывает среднее (%) содержание зооидов в контрольных пробах на 7-й день культивирования; красные наклонные линии демонстрируют положительную динамику показателя (*Фактор 3, время) в период с 5 по 7-й день (то есть отсутствие биоцидного и биостатического воздействия или компенсацию такового за счет либо процессов репарации повреждения, либо за счет стимулирования к развитию «резервных» статобластов*); зеленые наклонные линии являются индикаторами негативной динамики показателя, демонстрируя постепенное снижение численности популяции зооидов.

Summary

The results we obtained with UV exposure of *Plumattella* are all obtained in a laboratory and rely heavily on descriptive assessment. The study is useful for generating hypotheses for statistical treatment and development of further research.

From the practical point of view the study provides:

(1) insights into (1.1.) possible problems with the repeatability of the results obtained from the same dose given how important the initial state of the target organisms is. The organisms are affected by weather, seasonality, environmental conditions during the period of activation and during dormancy release. Equally difficult is (1.2.) predicting when fouling will develop in cooling systems;

(2) further understanding on how to best manage *P. emarginata* life cycle either by activating the resting stages and depressing activation through the use of either low or medium pressure UV irradiation or their combination;

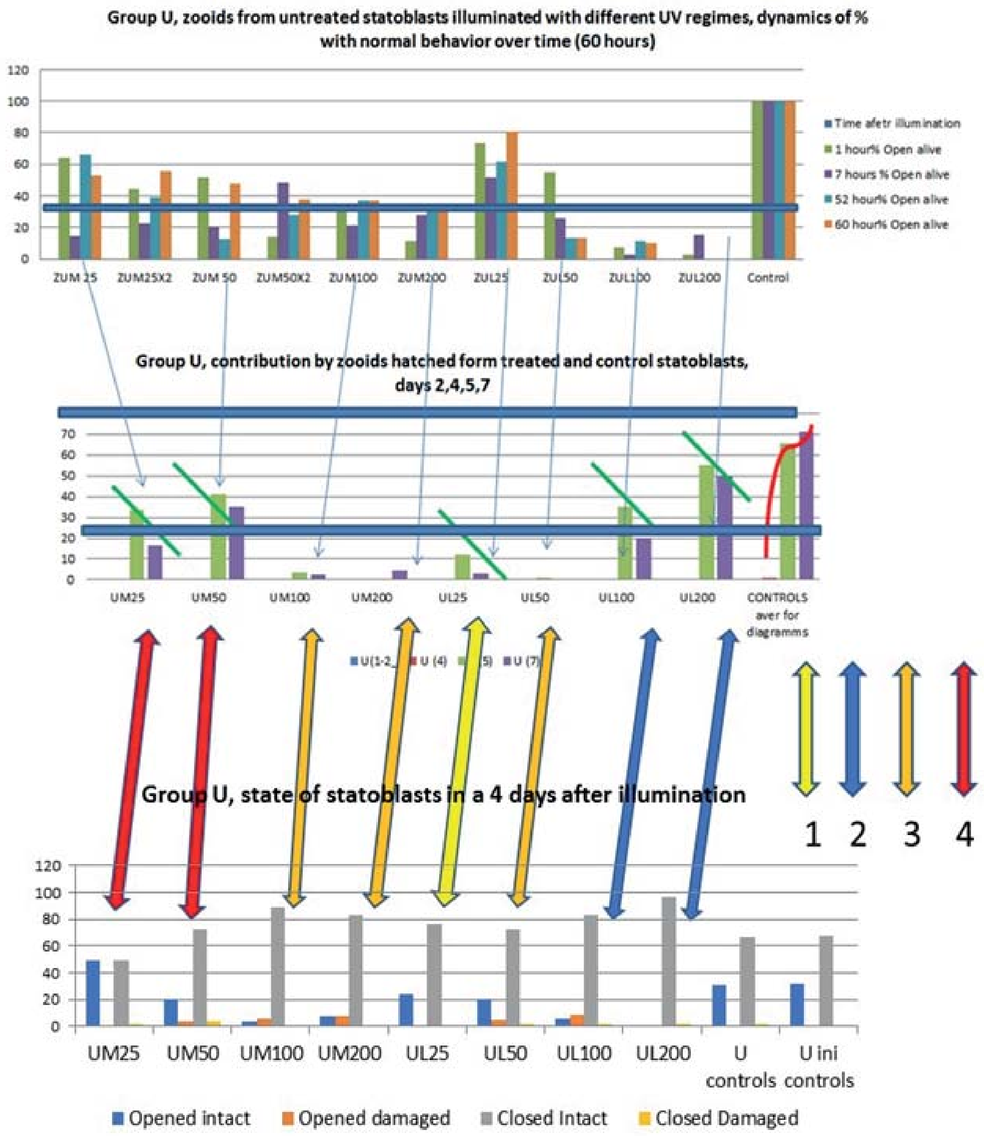


Fig. 5. Comparison of main results for 8 UV treatments of *P. emarginata* at key phases of the life cycle: germination (**Lower diagram**) and hatching occurred during Experiment 1 (***Middle diagram***), zooids, newly hatched from untreated statoblasts (***Upper diagram***) and patterns matching with arrows: thin blue arrow join corresponding regimes in diagrams, related to Experiment 2 and Experiment 1; color arrows point to suggested sequences of *P. emarginata* response onto various regimes of treatment.

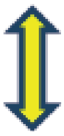
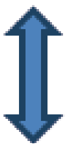
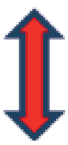
Рис. 5. Сравнение основных результатов для 8 типов обработки УФ *P. emarginata* на ключевых фазах ее жизненного цикла: всхожести (пробуждения) статобластов (***нижняя диаграмма***) и вылупления из них зооидов в ходе Эксперимента 1 (***средняя диаграмма***), zooids, на стадии новорожденных зооидов, вылупившихся из необработанных статобластов (***верхняя диаграмма***) и паттернов, обозначенных стрелками: тонкие голубые стрелки позволяют отследить одни и те же режимы обработки на разных диаграммах, относящихся к Экспериментам 1 и 2; цветные стрелки указывают на предполагаемые варианты последовательностей ответов *P. emarginata* на различные режимы обработки.

Table 3

Four major sequences of dynamic responses of *P. emarginata* to medium wavelength poly- and monochromatic UV treatment

Таблица 3

Четыре основные последовательности динамического реагирования *P. emarginata* на облучение различными дозами средневолнового широкополосного и монохроматического УФ

No of sequence as in Fig. 5	Types and doses	Brief description	Краткое описание
1 	UL25	— No detectable biostatic or stimulating effect on germination of statoblasts; — Suppressed hatching of zooids; — Moderate biocidal effect onto already developed zooids	— Биостатический или стимулирующий всхожесть статобластов эффекты не выражены; — Подавление вылупления зооидов; — Биоцидный эффект против сформировавшихся зооидов умеренный
2 	UL100 UL200	— Strong biostatic effect of high doses on germination, probably playing protective role through something analogical to isolation reflex; — High abundance of hatched zooids in 5th and 7th days can be explained as resulting from isolation reflex; — Strong biocidal effect onto already developed zooids	— Сильно выраженный биостатический эффект высоких доз препятствует всхожести, возможно имеет место реакция аналогичная изолирующему рефлексу, например, у моллюсков, т. е. играющего защитную роль; — Значительная численность зооидов на 5 и 7 дни, возможно обеспечена действием защитного изолирующего рефлекса или его аналога; — Выраженный биоцидный эффект в отношении развитых зооидов
3 	UM100 UM200 UL50	— Biostatic effect is moderate, germination is normal at the beginning with its subsequent deceleration; — Hatching is very weak; — Initially strong or moderate biocidal effect onto developed zooids does not exclude their recovery in 2-3 days	— Биостатический эффект выражен средне, сначала нормальная всхожесть статобластов, затем замирание их развития; — Вылупившихся зооидов относительно немного; — Заметный биоцидный и биостатический эффект в отношении зооидов, однако возможна репарация повреждений, полученных в результате облучения
4 	UM25 UM50	— Stimulating effect can accelerate germination; — Good beginning of hatching with subsequent negative dynamics; — Moderate biocidal effect onto developed zooids with their subsequent recovery	— Отмечен стимулирующий всхожесть эффект; — Сначала быстрое вылупление зооидов. Сменяется негативной динамикой их численности; — Умеренный биоцидный эффект в отношении развившихся зооидов с последующей репарацией повреждений

(3) evidence that UV is universal and eco-friendly control acting against resting stages of metazoans;

(4) information on antimicrobial disinfection of water to be taken into account as side effect of treatment of statoblasts by UV.

In general our experimental laboratory study has proven applicability of UV-based technologies to criterion 9 in Table 1. There have been revealed biocidal and biostatic effects of all UV doses with one exception (Group A, UM25) on statoblasts, simultaneously some regimes can also stimulate dormancy release. Further, the study demonstrated the importance of the initial conditions of statoblasts in their response to UV dose. Zooids hatched from non-illuminated statoblasts were negatively affected by exposure to all doses of UV. There were multiple "responses" of statoblasts to UV exposure: from visible suppression of development to combination of suppression and temporary delay of development accompanied by subsequent more simultaneous hatching of zooids from statoblasts which remained viable.

These experimental results present the basis for further planning of industrial testing aimed at proving applicability of the UV treatment in the field. UV can be used to either directly treat statoblasts and suppress their dormancy release or facilitate transformation of statoblasts into more vulnerable life stage, the active zooid. Already hatched zooids which would be carried in as aggregated colonies would also be susceptible to UV. Both approaches would be considered as constituents of the strategy helping manage biofouling by managing the life cycle of *Plumatella*.

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