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## Article

# The effect of osmotic shock on fouling bryozoans at the Balakovo Nuclear Power Plant in the experiment

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**Abstract.** Bryozoan fouling is a problem for the functioning of the technical water supply systems of the Balakovo Nuclear Power Plant. The effect of osmotic shock on bryozoan mortality was assessed in two consecutive experiments. *Fredericella sultana* and *Paludicella articulata* colonized metal plates installed into the observation water-filled chambers connected to the industrial water supply. The use of an osmotic solution may be a less hazardous alternative to biocides used to combat bryozoan fouling. The plates were set into the chambers 20 days before the first experiment and 32 days before the second one. Exposure to a NaCl solution (3 g/L) for 5 minutes had no negative effect on the bryozoans. Exposure to a higher concentration of NaCl (7 g/L) for 15 minutes significantly reduced the number of living colonies compared to the control. The osmotic shock may help clearing water equipment from fouling bryozoans.

**Keywords:** periphyton, biofouling, water equipment, Bryozoa, *Fredericella*, *Paludicella*

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### Научная статья

## Действие осмотического шока на мшанок в эксперименте на Балаковской АЭС

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**Аннотация.** Мшанковое обрастание является проблемой для функционирования систем технического водоснабжения Балаковской АЭС. В ходе двух последовательных экспериментов было исследовано влияние осмотического шока на смертность мшанок *Fredericella sultana* и *Paludicella articulata*, поселившихся на пластинах наблюдательных гидробоксов, запитанных в систему технического водоснабжения. Использование осмотически сильного раствора может быть менее опасной для окружающей среды альтернативой применяемым для борьбы с мшанковым обрастанием биоцидам. Время формирования обрастаний на пластинах до начала первого эксперимента составило 20 суток, до второго – 32. Воздействие раствора NaCl (3 г/л) в течение 5 минут не оказало негативного эффекта на мшанок. Под действием раствора NaCl большей концентрации (7 г/л) в течение 15 минут количество живых колоний достоверно сократилось по сравнению с контролем. Таким образом, действие осмотического шока позволяет очистить водонесущее оборудование от биообрастателей – мшанок.

**Ключевые слова:** перифитон, биопомехи, водонесущее оборудование, Bryozoa, *Fredericella*, *Paludicella*

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## Introduction

Most modern power plants (including all nuclear power plants) use the steam circulating in a closed loop to generate electricity. Excess heat must be removed to condense the steam, using water as a coolant, which is then cooled down in the cooling towers or open water bodies called cooling ponds. Since the latter are the ecosystems open to the local environment, a number of organisms, including fouling ones, colonize these areas. This facilitates introducing of their dispersal stages into the power plant's service water supply system (SWS). Subsequently, these organisms colonize water pipes and heat exchange elements, leading to reduced equipment efficiency and emergency situations. In freshwater ecosystems, the normal operation of SWS is threatened by the growth of bryozoans in the water equipment, although no standard methods exist to combat this phenomenon currently.

The bryozoan fouling is periodically recorded in the wastewater treatment plant of the Balakovo Nuclear Power Plant (BNPP). There is a direct correlation between the sediment load on the equipment surfaces and the degree of bryozoan community development. Their colonies retain detritus particles, facilitating the accumulation of matter; as the sediment layer increases, this facilitates successful settling of newcomers bryozoans and other fouling organisms (Mukhin et al., 2023).

The BNPP is located in the Saratov Oblast (Russia) on the left bank of the dam section of the Saratov Reservoir of the Volga River cascade. The cooling pond, formed by isolating a part of the Saratov Reservoir, is a key element of the plant's cooling system. To compensate for the water losses due to BNPP operating, water is pumped into the cooling pond from the estuary of the Berezovka River. This shallow, lentic tributary of the Volga River is heavily overgrown with higher aquatic vegetation, providing favorable environment to various fouling species, including bryozoans. Depending on the pump operating mode, water salinity varies as 0.25–0.50 g/L at the BNPP water intake site, located 3.6 km upstream the river mouth. When the cooling pond pumps are operating, low-mineralized water flows from the Saratov Reservoir through the estuary back into the Berezovka River. When the pumps are turned off, saline water from the upper reaches of the Berezovka River accumulates in SWS. Consequently, the bryozoans inhabiting the BNPP recharge system are constantly experiencing osmotic stress; however, this does not cause their death.

The study aims to determine the salinity fluctuations, critical for bryozoans inhabiting BNPP SWS, and to search for the working concentrations of salt that can be used to combat bryozoan fouling.

## Materials and methods

The studied section of the industrial water supply system represents the beginning of the industrial water flow, specifically, the site where the latter enters the cooling pond. Therefore, it is characterized by natural hydrophysical and hydrochemical conditions consistent with its source, the Berezovka River. According to our observations lasted for over three years, bryozoan colonies have been detected in the observation chambers installed in this section, organic sediments accumulated here quite rapidly. This indicates a high saturation of the water with suspended organic matter and the maintenance of conditions favorable for bryozoan feeding.

The experiment was conducted directly in the supply water system of Balakovo Nuclear Power Plant. Two 35-L tanks (i.e., observation water-filled chambers) were used, connected in parallel to the water supply system of the pumping station. The latter pumps water from the Berezovka River into the cooling pond. The observation chambers simulated the station's water equipment, where constant water flow rate of 10 L/min is maintained. In both observation chambers, 15×15-cm metal steel plates (St3 steel class) were placed vertically on a tripod. Each observation chamber contained 12 plates organized into three tiers, four plates at each. The observation chambers were connected to the water

supply system on August 2, 2023; the experiment began on August 22, 2023. Therefore, the plates were exposed to the water flow for 20 days before the experiment has started. During this period, a multi-species periphyton community, natural for the given conditions, has formed on the plates. The water temperature was measured using a Hanna HI98130 pocket multiparameter analyzer (China); it varied from 24.6 °C at the beginning of the experiment to 18.7 °C at the end.

Control and experimental plates were removed from the observation box, and a complete count of bryozoan colonies was performed on one randomly selected side of the plate. The study was conducted directly at the plate installation site, without transporting the plates. Colonies were counted in a water-filled cuvette under a YA XUN YX-AK36 binocular microscope (China) at 8×50 magnification. The number of live and dead bryozoan colonies was recorded. Colony status was assessed visually: it was considered alive if at least one zooid emerged from the cystid (which is normal for bryozoans). In case of danger or unfavorable conditions, the zooid can retract into the cystid, but does not remain there for long. The cystid and polypide of the bryozoan are clearly visible visually at a microscope magnification of 10×15. Bryozoan species were identified using the taxonomic key (Gontar, 2016).

In the experiment, water salinity in the observation chamber was increased by adding a sodium chloride brine. The observation chamber was disconnected from the water supply by turning off the inlet and outlet valves, then the prepared brine was added, creating the desired salt concentration.

Bryozoans were determined to be dead after a significant interval (48 and 72 hours) to eliminate the possibility of false positives. A bryozoan may show no signs of life for some time in osmotic shock, but it resumes normal activity after its salt balance is restored through the contact with running fresh water. Two consecutive series of experiments were conducted with different exposure times in the abnormal saline environment and different control observation times.

During the first experiment, conducted on August 21–22, 2023, all plates were removed from one observation chamber as controls. The ratio of live to dead colonies was determined, after which they were not returned to the observation box. Brine was then added to the second observation chamber to create a salt concentration of 3 g/L. The plates were exposed to the solution for 5 minutes, after which normal water flow was restored in the observation chamber. Two days later (August 24, 2023), nine experimental plates from the second observation chamber (three plates from each tier) were removed and analyzed. At the end of the experiment, the plates were washed and returned to the observation chambers.

The second series of experiments was conducted in the same observation chambers. The plates were exposed for 32 days before the start of the second experiment. Six plates (two from each tier) were removed from the observation chambers as a control on September 29, 2023. They were not returned to the observation chambers. Sodium chloride brine was then added to the observation chamber to create a salt concentration of 7 g/L. The plates were exposed for 15 minutes, after which normal water flow was restored. After 72 hours, six experimental plates exposed to the osmotically strong solution were removed from the observation chamber and analyzed (Table 1).

Regard must be paid to the plate position (tier) in the observation chamber, which may influence the fouling development. Since an equal proportion of plates from each tier was used in each experiment, the live to dead bryozoan colonies' ratio before and after exposure to osmotic shock was compared for the pooled sample of plates from the upper, middle, and lower tiers. Statistically significant differences were assessed using Fisher's *F*-test.

**Table 1.** Scheme of experiments aimed to study the effect of osmotic shock on bryozoans at the Balakovo Nuclear Power Plant.

Date	The number of studied plates in the upper/middle/lower tiers of the observation chamber		NaCl concentration, g/L	Exposure time, min	Check-up, hours after exposure
	control	experiment			
22.08.23	4/4/4	3/3/3	3	5	48
29.09.23	2/2/2	2/2/2	7	15	72

## Results

Bryozoa dominate in the fouling communities on the studied plates. Iron-oxidizing bacteria also thrive on the plate surface. The iron-containing products of their metabolism form dense masses covering a significant portion of the plate surface. Crustaceans (primarily Ostracoda) are found rarely; unstructured (particulate) organic matter is present.

The representatives of two bryozoan taxa, *Fredericella sultana* (Blumenbach, 1779) and *Paludicella articulata* (Ehrenberg, 1831), were found on the control plates examined in the first experiment before exposure to NaCl. *F. sultana* dominated: 27 live and 29 dead colonies were found on the control plates (Table 2). After exposure to a weak (3 g/L) solution of NaCl, 14 live and 15 dead colonies of *F. sultana* were found. No statistically significant differences were observed when comparing proportions of live and dead colonies in the control and experimental samples using Fisher's *F*-test ( $V = 0.00$ ;  $p = 0.99$ ) (Fig. 1).

Representatives of *P. articulata* were less common: 2 live and 17 dead colonies in the control. After the experimental exposure, 1 live colony and 16 dead ones were detected. No significant differences ( $V = 0.25$ ;  $p = 0.62$ ) were found (Fig. 2).

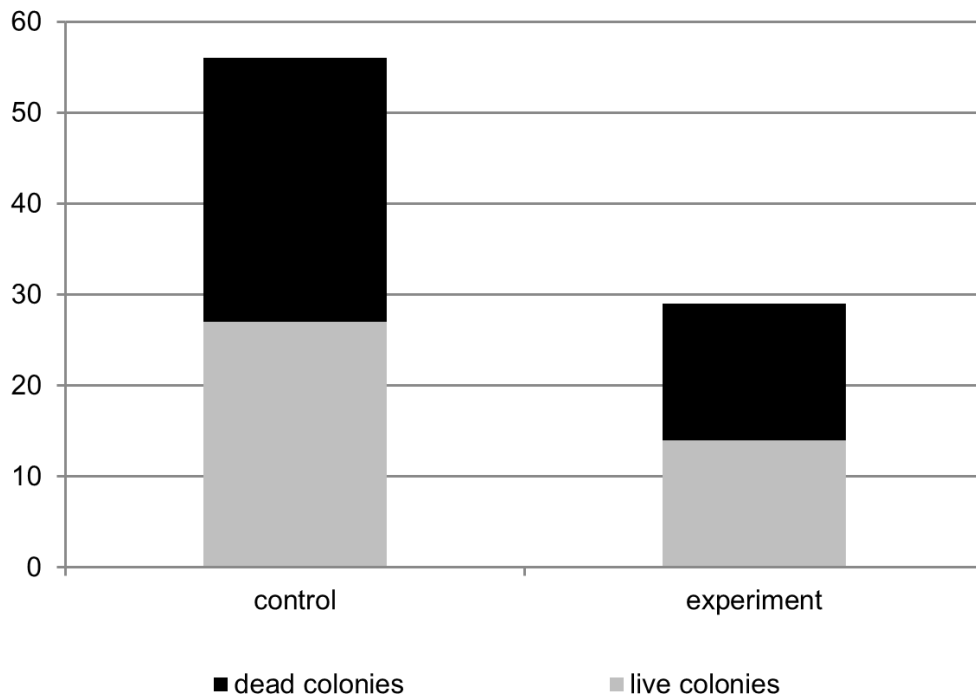
In the second experiment, both the salt concentration and the exposure time were increased, but only *Fredericella sultana* colonies were taken into account, because this species was dominant. Before exposure to the osmotically strong solution, 7 live and 4 dead colonies were registered on the experimental plates. After exposure to 0.7% NaCl solution, only one live colony and 11 dead ones were observed. The ratio of live to dead colonies of *F. sultana* differed statistically significantly from that in the control ( $V = 7.4$ ;  $p = 0.006$ ) (Fig. 3).

## Discussion

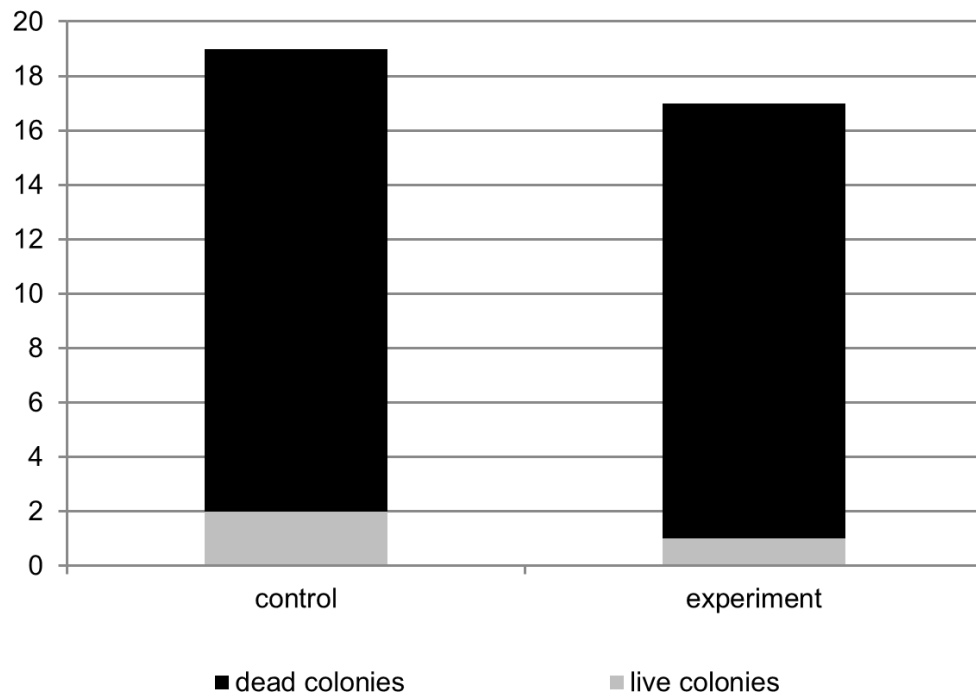
The response of aquatic organisms to acute salinity change depends on many factors. These include the salinity range they encounter in their natural habitat, the rate and range of the salinity shift, the duration of exposure, water temperature, life stage, and the organism's osmoregulatory capacity (Dietz et al., 1996; Gosling, 2015; Jørgensen et al., 1995). Salinity tolerance may vary widely among geographically separated populations of a single species or among different species of a genus. However, in general, the salinity tolerance of many aquatic organisms largely reflects the salinity range of their existing habitat (Jørgensen et al., 1995; Ram et al., 2004).

**Table 2.** Ratio of living and dead bryozoan colonies in the control and experiment at the Balakovo Nuclear Power Plant.

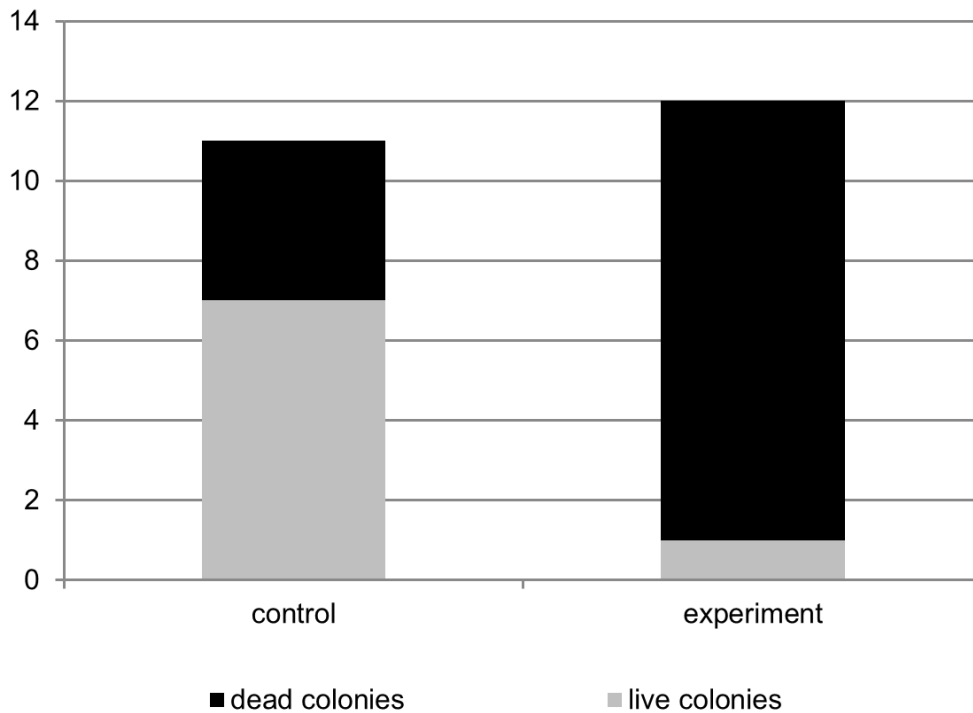
NaCl concentration, g/L	Exposure time, min	The number of live/dead colonies in the upper, middle and lower tiers of the observation chamber	
		control	experiment
<i>Paludicella articulata</i>			
3	5	$\frac{1}{4}$	$\frac{1}{2}$
		$\frac{0}{3}$	$\frac{0}{3}$
		$\frac{1}{10}$	$\frac{0}{11}$
<i>Fredericella sultana</i>			
		$\frac{5}{10}$	$\frac{2}{3}$
		$\frac{9}{6}$	$\frac{9}{10}$
		$\frac{13}{13}$	$\frac{3}{2}$
<i>Fredericella sultana</i>			
7	15	$\frac{6}{1}$	$\frac{1}{3}$
		$\frac{1}{3}$	$\frac{0}{2}$
		$\frac{0}{0}$	$\frac{0}{6}$



**Fig. 1.** The number of living and dead colonies of *Fredericella sultana* in the control group and on the plates exposed to NaCl solution (3 g/L) in the experiment.



**Fig. 2.** The number of living and dead colonies of *Paludicella articulata* in the control group and on the plates exposed to NaCl solution (3 g/L) in the experiment.



**Fig. 3.** The number of living and dead colonies of *Fredericella sultana* in the control group and on the plates exposed to NaCl solution (7 g/L) in the experiment.

Osmoregulation has been well studied for many groups of organisms (Dietz et al., 1994; Khlebovich, 1974; Kochneva et al., 2023; Smurov et al., 2010). Most aquatic organisms are tolerant to a very limited range of environmental salinity. Freshwater organisms exposed to acute salinity increase are known to experience so-called osmotic shock, which reduces their viability. All freshwater animals are hyperosmotic regulators, i.e., they maintain an internal osmotic concentration higher comparing to that in the environment. The critical salinity for freshwater aquatic organisms is known to be 5–8‰, which is based on the biochemical and evolutionary characteristics of living organisms (Khlebovich, 1974, 2013). Bryozoans have been observed in the technical water supply system of the Balakovo Nuclear Power Plant throughout the entire history of observations of the water supply system.

In both experiments, conducted in late summer and early autumn, *Fredericella sultana* specimens were observed on the plates. The ratio of live to dead colonies of *F. sultana* in the control groups of the first and second experiments did not differ statistically significantly ( $V = 0.86$ ;  $p = 0.35$ ). This suggests that the survival rate of bryozoan colonies in the observation chambers in the BNPP SWS did not change significantly between the experiments. After the first experiment, bryozoans managed to recolonize the cleaned plates and restore the population to a state normal for the given conditions. Therefore, both experiments can be considered independent.

Exposure to a 0.3% NaCl solution for 5 minutes had no negative effect on bryozoan colonies. However, exposure to a 0.7% NaCl solution for 15 minutes caused statistically significant mortality of bryozoan colonies. Therefore, osmotic shock may be a promising method for cleaning water equipment from these fouling organisms dangerous for normal nuclear power plant operating. This applies particularly to *Fredericella sultana* and *Paludicella articulata*, which do not form flotoblasts. Their recolonization of the SWS occurs only during the dispersal period in a natural watercourse. This measure will be less effective for representatives of the Plumatellidae family, which have a flotoblast dispersal stage in their life cycle.

## Conclusions

Exposure of bryozoan colonies living on the inner surface of water equipment at the Balakovo Nuclear Power Plant to a 0.7% sodium chloride solution for 15 minutes causes their death. This reduces the spread and biomass of these dangerous biofouling organisms in the technical water supply system. However, after the colonies die, mechanical removal of the dead organisms is necessary.

Since the critical salinity for freshwater organisms is 5–8‰, further research is needed in the direction of decreasing the salinity of the solution used with increasing exposure time to find a more effective means of combating biological fouling.

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