

UDC 591.5: 594.32
<https://doi.org/10.33989/2414-9810.2019.5.1.195127>

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THE INFLUENCE OF DETERGENTS ON THE CILIA LOCOMOTION ACTIVITY OF EPITHELIUM IN SINANODONTA WOODIANA (MOLLUSCA, UNIONIDAE)

The influence of one of the most popular detergent «Ushasty nian» («Vinnitsapobutkhim», Ukraine) in concentrations of 3, 6, 12, 24, and 48 mg/dm³ on the duration and beat frequency of the glimmeral epithelium cilia of the gill apparatus of male, female and hermaphrodite *Sinanodonta woodiana* (Lea, 1834) specimens was investigated. The damaging effect on the viability of these mollusks, including the locomotive activity of the frontal cilia of their gill glimmeral epithelium depends on the toxic factor levels. In the toxicological study, the pathological process in mollusks shortly developed in consequent five stages in two days. At 3 and 6 mg/dm³ concentrations of detergent, the signs observed in all three categories of molluscs included *in vitro* increased duration of locomotion and beat frequency of the glimmeral epithelium cilia. That state of experimental molluscs corresponds with the stimulation stage of pathological process induced by toxicological poisoning. Aforementioned changes are the signs of adaptive protection process aimed to counteract the damage inflicted by the toxic substance. At 12 and 24 mg/dm³ of detergent, the molluscs quickly entered the depression stage of the pathological process. It manifests as a statistically significant sharp decrease of duration of activity and beat frequency of glimmeral epithelium cilia. At 24 mg/dm³ of toxicant, towards the end of exposure that stage was superseded by the sublethal stage. The latter is characterized by complete destruction of respiratory epithelium, maximum oedema of body tissues (especially in the foot), loss of locomotion, and almost total loss of tactile sensitivity. At 48 mg/dm³ of toxicant, the sequence of depression, sublethal and lethal stages is realized quickly.

Key words: *Sinanodonta woodiana*, detergent, gills, glimmeral epithelium, activity.

Introduction. The human impact on the biosphere significantly grew to the 21st century. One of its facets is aquatic pollution by various matters contained in industrial and domestic waste waters. Among the different components there are detergents or surface-active substances (SAS) which target the respiratory glimmeral epithelium of the gill apparatus of all gill-breathing organisms, affecting both salt- (Frid, & Caswell, 2017) and freshwater (Дудник, & Євтушенко, 2013; Метелев, Канаев, & Дзасохова, 1971) animals. The SAS-induced damage level is conditioned by the toxicant concentration and chemical structure, and by the tolerance and physiological state of the exposed animals. Already there are several reports of SAS inhibiting the filtration activity of bivalve (primarily saltwater) molluscs (Marin et al., 1994; Ostroumov, 2009; Ostroumov, 2006).

First decades of the 21st century were marked by the occurrence and rapid expansion in Ukraine (northern wards region from Black Sea, Transcarpathia) of the chinese pond mussel *Sinanodonta woodiana* (Lea, 1834). This species most probably occurred in Ukraine via Danube invasive corridor from the water network (Сон, 2007; Янович, & Пампура, 2011). This mollusk has a wide ecological valence which allowed it the fast expansion in the European continental waters and worldwide. The successful persistence of *S. woodiana* populations in Ukraine will, supposedly, largely depend on the species resistance to the impact of pollutants in its natural waters, including various surfactants. The significance of this danger is evidenced by the fact that, despite the current MPC standards for surfactants 0,1 mg/dm³ for anion-active and 0,012 mg/dm³ for cation ionic pollutants, their content in natural waters of several Ukrainian regions at the beginning of the 21st century became several times higher than that (Ситник та ін., 2001).

The aim of the present work was to find out the peculiarities of the detergent's effect on the duration of activity and beat frequency of the frontal glimmeral epithelium cilia of *S. woodiana* *in vitro* in norm and under exposure to high SAS concentrations. Solving this problem is important because the functionality of respiratory gill epithelium in *S. woodiana* and other Bivalvia conditions, to some degree, all their basic life support functions: breathing, feeding, excretion, reproduction. The epithelial functionality is regulated by a mechanism determining the organism's general reaction. Until now, *S. woodiana* was not studied in this aspect.

Material and methods. Material is 342 specimens of *S. woodiana* (shell length 12,9–16,1 cm, shell height 8,6–9,7 cm), collected in the Danube lower reaches (Vilkovo, Odessa region) in June, 2011. In the laboratory the animals were acclimated for 15 days. They were kept in aquariums (50 and 100 liters, 1 specimen per 10 liters). The keeping conditions were as follows: water temperature 18–20°C; pH 7,4–8,6; oxygenation 8,5–9,3 mg O₂/dm³. The culture medium was changed daily. The mollusks were regularly fed (crushed dry fish food, ground yolk of steeply cooked chicken eggs).

The acclimated specimens were used in a two-step toxicological essay according to the standard technique (Алексеєв, 1981). The toxicant was modeled by the detergent «Ushasty nian» («Vinnitsapobutkhim»). In a preliminary experiment it was established that the main toxicological indicators for *S. woodiana* are LC₀ = 0,1 and LC₁₀₀ = 100 mg/dm³. Then graphically by the sliding curve method LC₅₀ was estimated to be 50 mg/dm³. In the LC₀–LC₅₀ range, five concentrations were chosen for the main experiment. The duration of each of the two experiments was two days. Each experiment was controlled.

The duration and frequency of ciliary beats of the frontal glimmeral epithelium of the *S. woodiana* gill apparatus were determined at temporary micropreparations done according to E. A. Veselov (1952), using the BIOLAM microscope (×203 and ×450) in triplicate, closing the iris diaphragm to weak light. Mollusk sex was determined according to (Шкорбатова, & Староборатова, 1990).

The quantitative results of the experiments were processed by the methods of basic variation statistics (Крамаренко, 2003).

Results and discussion. The results of the main toxicological essay are given in Table. According to them, under 3 mg/dm³ SAS in the medium, the male, female and hermaphrodite *S. woodiana* specimens are characterized with increasing activity of the gill glimmeral epithelium and its ciliary beat frequency. Under 6 mg/dm³ SAS these changes become statistically significant ($p < 0,01$). The increased ciliary activity is a manifestation of the physiological-biochemical protective-adaptive process aimed at counteracting the damaging effect of the toxicant. It is known (Веселов, 1968) that the animal hydrobionts exposed to pollutants, depending on the toxicants' nature and concentration in the medium, duration of exposure, and resistance levels, suffer the phasic pathological process of poisoning. Each phase is characterized by specific complex of symptoms.

Physiological state of *S. woodiana* under 3 and 6 mg/dm³ SAS in the medium corresponds to that which is characteristic for the stage of stimulation. This follows from the aforementioned increased activity of their respiratory glimmeral epithelium, and from the weak mucus secretion by the frontal gill epithelium, which is a fast protective reaction demonstrated by the majority of the specimens. Even a thin mucus layer to some degree obstructs the SAS diffusion to the animal organisms and protects their gill respiratory epithelium from damage.

In the solution containing 12 mg/dm³ SAS, the experimental animals enter the depression phase of poisoning. This so-called "early" depression manifests by statistically significant ($p < 0,01$) slowed ciliary activity of glimmeral epithelium: the beat duration and frequency are reduced by 20 and 10%, respectively.

This signifies a certain decrease of overall metabolism of *S. woodiana* which is however not lethal for them (100 % survival of exposed specimens). The levels of mucus secretion by skin integument and gill apparatus rise under these conditions. There are areas of fine sparse pastiness indicating the started swelling of body tissues.

Table

The detergent effect on locomotive ciliary activity of the glimmeral epithelium of *S. woodiana* gill apparatus

Material	Locomotion duration (hr)			Beat frequency (number per minute)		
	n	lim	M±m CV	n	lim	M±m CV
Control						
Males	10	109 – 333	240.1±11.1 7.1	11	241 – 330	316.2±2.4 5.6
Females	10	121 – 340	229.2±9.3 4.9	12	238 – 341	325.1±3.7 4.8
Hermaphrodites	6	116 – 329	231.0±8.17 9.9	7	222 – 339	320.4±2.2 2.5
3 mg/dm³						
Males	11	117 – 345	258.3±12.15 11.7	11	237 – 340	329.1±3.6 3.7
Females	10	122 – 361	261.4±11.1 11.1	11	243 – 359	359.6±9.3 4.1
Hermaphrodites	6	129 – 351	250.1±18.1 5.2	6	228 – 361	348.3±5.0 4.4
6 mg/dm³						
Males	11	122 – 353	299.2±20.3 10.2	13	251 – 419	399.3±3.2 4.6
Females	12	130 – 359	311.1±18.2 13.1	14	236 – 432	401.4±4.7 8.1

Hermaphrodites	7	137 – 347	321.2±15.1 8.7	6	198 – 440	409.5±6.2 6.3
12 mg/dm³						
Males	10	105 – 308	205.0±15.2 10.3	13	227 – 331	301.2±5.5 4.1
Females	12	110 – 316	200.1±18.3 17.3	11	219 – 328	300.3±3.8 5.4
Hermaphrodites	6	102 – 312	198.1±11.1 13.1	7	220 – 336	298.7±4.1 3.3
24 mg/dm³						
Males	12	89 – 157	120.2±9.1 6.8	10	92 – 122	106.2±2.9 3.0
Females	13	82 – 149	116.1±10.3 12.5	10	89 – 127	99.9±1.4 6.3
Hermaphrodites	7	77 – 137	111.2±9.2 11.1	6	91 – 129	102.3±3.3 4.1
48 mg/dm³						
Males	10	38 – 51	44.0±6.1 14.1	10	28 – 51	43.0±1.1 2.2
Females	11	34 – 46	33.3±7.1 8.8	12	26 – 48	38.7±3.0 4.7
Hermaphrodites	6	29 – 39	35.1±6.2 12.3	7	23 – 44	34.3±2.1 5.2

At 24 mg/dm³ after 9–12 hours of exposure, the experimental *S. woodiana* specimens are at the “deep” depression stage. The complex of relevant symptoms includes intense mucus excretion by the gills, emerging foci of destruction of the respiratory epithelium with developing lesions and hemorrhages, increasingly swollen body tissues, and reduced tactile sensitivity. However at the end of the first day of exposure all specimens retain viability despite the failed respiratory function of the gill apparatus epithelium. It is assumed (Маляревская, & Карасина, 1983; Маляревская, 1985) that at this stage of the pathological process the mollusks usually turn from the aerobic to anaerobic method for the cleavage of glycogen, their universal energy substrate. This physiological and biochemical protective measure allows them to remain viable in conditions of anoxia for at least some time. The duration of activity of glimmeral epithelium on the areas of *S. woodiana* body where it still remains decreases twofold, and its ciliary beat frequency decreases 3.1 times compared to norm. At the second half of the first day of exposure, all *S. woodiana* specimens enter the sublethal poisoning stage. It is characterized by completely damaged frontal ciliated epithelium of the gills. Due to increasingly swelled body tissues the foot is so enlarged that it does not fit in the shell and hangs out far beyond the valve edges. The medium contains copious excrements simultaneously ejected by *S. woodiana* experimental specimens. At the second day of experiment, all specimens are completely immobile however they still retain slight tactile sensitivity. At the end of exposure, 22,8% of animals develop the last poisoning stage, lethal, manifesting in true shock and complete lack of tactile sensitivity. In mollusks, it manifests as developing true shock symptoms and full loss of the tactile sensitivity.

In the medium containing 48 mg/dm³ experimental animals experienced the three highest degrees of intoxication: depression, sublethal and lethal stages. At “deep” depression stage the duration of activity of gill glimmeral epithelium decreases 6,2 times and ciliary beat frequency in 8,3 times.

The nature of the changes in the ciliary activity indices of the frontal ciliated epithelium of *S. woodiana* gill apparatus depends on the level of surfactant content in its environment, which is consistent with the concept of G. Selye (1982). According to it, small stresses («eustresses») in contrast to overexertions («distresses») optimize the vital activity of organisms, increasing their adaptive capacity with respect to new conditions. Our results indicate that for *S. woodiana* «eustresses» are surfactants in concentrations of 3 and 6 mg/dm³, while the higher concentrations of this pollutant must be characterized as «distresses».

Conclusions. The duration of activity and ciliary beat frequency of the glimmeral epithelium of *S. woodiana* gill apparatus increase at 3 and 6 mg/dm³ of the «Ushasty nian» detergent in the environment. This protective adaptation is characteristic for the stimulation stage of the intoxication process.

The duration of activity of the gill epithelium and its beat frequency decline in experimental animals, inversely related to the growing content of the toxic substance (12, 24, 48 mg/dm³). The intoxication process in the affected specimens develops into the depression, sublethal and lethal stages.

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Рекомендовано до друку В.М. Закалюжний
Отримано 21.02.2019 р.