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# Biological and biomechanical principles of the controlling molluscs *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822) in reservoirs of strategic importance

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**ABSTRACT**

The article presents the results of complex laboratory investigations on the biological and biomechanical ways of control of *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822) molluscs in simulated conditions close to the conditions of the cooling pond of the Zaporizhia Nuclear Power Plant. It was determined that molluscs have naturalized in the Zaporizhia Nuclear Power Plant cooling pond, quickly increased their number and created a threat to hydraulic structures. Taking into account biological features of *Thiaridae* mollusks and technical and ecological features of Zaporizhia NPP, we carried out a series of experiments using biological control measures (the use of predatory species of hydrobionts) and mechanical means for controlling mollusks. Representatives of different taxons of the Animalia Kingdom were selected as predatory species of hydrobionts, which potentially can consume gastropods: Mollusca, Crustaceans and Fish. It has been found experimentally that the use of marbled crayfish *Procambarus virginalis* (Lyko, 2017), pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) and *Botia lohachata* Chaudhuri, 1912 has not given positive results in the development of measures to control the number of molluscs. Positive results were obtained in a series of experiments with predatory mollusc assassin snail *Clea helena* (von dem Busch, 1847), but it was noted that in the presence of more accessible feeds, assassin snail *Clea helena* (von dem Busch, 1847) consumes smaller quantities of *Thiaridae* mollusks. The most successful results we obtained in experiments with traps for molluscs. We have developed experimental constructions of traps with lower and upper inlets that act as mollusk accumulator and can be installed

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in the coastal zone of the reservoir and Zaporizhia NPP cooling system channels for reducing the number of reproductive individuals of *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822). The most effective were the traps with the lower inlet to which the mollusks could get faster. In order to attract mollusks to traps, we have conducted studies on the use of feed baits for molluscs. Most effectively, molluscs fell into traps that contained lime feed, feedstock sunflower oil and anise oil. The most effective among mollusks was the bait with the addition of anise oil. During the exposure, traps with anise bait traps accumulated 14.1% of molluscs. The conducted researches can serve as the basis for the development of biomelioration measures aimed at reducing the negative impact of accidental introduction of new species of molluscs into technical reservoirs of strategic importance.

***Keywords*****:** assassin snail, red-rimmed melania, thiarids, *Clea helena*, *Melanoides tuberculata, Tarebia granifera*, Zaporizhia Nuclear Power Plant, cooling pond, biological invasion

## 1. INTRODUCTION

Currently, the issue of biological fouling of underwater industrial facilities remains relevant for the whole world. Exploitation of technical water of a complex purpose is often complicated because of the appearance of biological obstructions of animal and plant origin. This fouling of hydro-technical utilities creates problems for their safe operation [1-3].

For the cooling pond of energy objects, this problem is of particular importance, since the increased temperature regime in such reservoirs creates favorable conditions for the active development of certain species of hydrobionts, as it has been repeatedly pointed out in scientific and special literature [2, 3].

In addition to ordinary objects that form the biological obstructions in freshwater hydraulic systems (diatoms and blue-green algae, *Dreissena* molluscs, sponges), appearance and spread of tropical molluscs in waters of Ukraine was recently noted [4]. The main cause of the spread of exotic molluscs in the waters of our country is an aquarium industry [5-7]. Quite often, there is a drain of water from aquariums into the open water. In this case, the most heat-loving species, in general, are able to survive in reservoirs where the discharge of heated water is carried out [8, 9].

For the first time, molluscs *Melanoides tuberculata* (Müller 1774) were registered in the South-Ukrainian NPP cooling pond in 1997 [10]. In November 2005 with a biomass of 6–35 g/m$^2$.Since that time, it has completely naturalized in the reservoir and constantly dominates in the zoobenthos of the middle and upper part of the cooling pond, reaching 99% of the total number and biomass of benthos organisms [11]. Two new species of freshwater molluscs of the family Thiaridae: *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822) were registered in the cooling pond of the Zaporizhzhya NPP in 2013, causing obstructions to the operation of the hydraulic structures.

The purpose of our work was to investigate and analyze possible ways to overcome the problem of spread of molluscs of the family Thiaridae using biological and biomechanical methods.

## 2. MATERIALS AND METHODS

Special aspects of the nutrition of *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822) were studied in experimental aquariums. To make conditions in aquariums close to the natural environment, mollusks, water, bottom sediments and substrates (wood and concrete) were selected from the Zaporizhia Nuclear Power Plant cooling pond the autumn of 2017and brought to the Department of General Biology and Water Bioresources of the Oles Honchar Dnipro National University. In the Laboratory of Hydrobiology, Ichthyology and Radiobiology of the Institute of Biology, a series of model aquarium experiments was conducted to find hydrobionts, which would be potential consumers of molluscs. The following predators have been selected: Marbled crayfish *Procambarus virginalis* (Lyko, 2017), assassin snail *Clea helena* (von dem Busch, 1847), fish – pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) and Reticulate loach *Botia lohachata* Chaudhuri, 1912.

Experiments were conducted in several series in aquariums. Experiments with pumpkinseed were carried out in aquariums with volume of 60 liters. Experiments with marbled crayfish were carried out in aquariums with a working volume of 40 liters. Experiment with Reticulate loach was carried out in aquariums with a volume of 25 liters. Experiments with assassin snails were carried out in model aquariums with a volume of 14 liters. The water temperature was maintained by the temperature regulator at 20 °C. The water was aerated using a compressor, the oxygen content in water was 5–6 mg/L, which corresponds to the optimal value for experimental species of hydrobionts.

## 3. RESULTS AND DISCUSSION

**Features of biology and distribution of mollusks of the *Thiaridae* family.** The basis of the nutrition of *Melanoides tuberculata* and *Tarebia granifera* is lower algae (mainly diatoms), semi-decomposed organic food [12]. Thiaridae species are livebearers. The mollusk carries an egg, from which completely formed small individuals appear and immediately burrow into the soil. The number of newborn *M. tuberculata* and *T. granifera* molluscs may vary depending on the size of the mollusk itself and range from 10 to 60 individuals. In order to achieve a high population density, the species *Melanoides tuberculata* and *Tarebia granifera* require at least 6–8 months. They breed, mainly, by parthenogenesis.

*M. tuberculata* and *T. granifera* are thermophilic, they die at a water temperature below 7–10 °C. The natural resettlement of molluscs in the reservoir may be caused by birds that feed on their juveniles, as well as by water currents. The main reason for anthropogenic resettlement of molluscs is their release from aquariums.

The analysis of national and foreign literature on biomarkers showed that there are currently no universal effective methods to control mollusks of the *Thiaridae* family. Taking into account biological features of molluscs and technical and ecological features of the Zaporizhia NPP, we have carried out a series of experiments using biological control measures (the use of predatory species of hydrobionts) and mechanical means for controlling molluscs.

**Experiment with marbled crayfish *Procambarus virginalis* (Lyko, 2017) (Decapoda).** As it known, crayfish can consume molluscs. They hunt molluscs and can destroy their shells and get an edible partfrom there [13-16]. This fact formed the basis of an aquarium model experiment. 300 individuals of *M. tuberculata* and *T. granifera* molluscs and 10 marbled crayfish with length from 7 to 10 cm and weighing from 6 to 15 g were put to the aquarium.

The experiment lasted 10 days. Crayfish were not fed in this period. Marbled crayfish grabbed mollusks and tried to eat them. The molluscs have hidden in the shell and covered with a protective shutter (lid). During the experiment, the crayfish could not reach the nutritional part of the mollusk, since it was tightly closed in the shell. The visual inspection of mollusks showed that the crayfish were trying to break the shell, they have violated the zone of growth of the shell with their mouths, but complete destruction of the shells was not noted. Molluscs were closed and for a long time were in a state of rest. Thus, an experiment with marbled crayfish showed a negative result in terms of consumption of *M. tuberculata* and *T. granifera* molluscs.

**Experiment with pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758)**. In the pumpkinseed supply spectrum, there are mollusks of the genus Dreissena [17-20], so it is considered that it could be a potential consumer of *M. tuberculata* and *T. granifera* molluscs under the conditions of the ZNPP. Since the pumpkinseedis present in the ZNPP cooling pond, it can adapt to the conditions of the reservoir and take its trophic niche.

In the experiment involved 10 individuals of pumpkinseed, which were placed in an aquarium with *M. tuberculata* and *T. granifera* molluscs (Fig. 1). The length of the individuals of pumpkinseed ranged from 6.7 to 10.1 cm, and the weight ranged from 3.9 to 16.3 g. The experiment involved molluscs of 3 dimensional groups: I – up to 10 mm (n=100), II – 10.1–15 mm (n=100), III – over 15 mm (n=100). The total number of mollusks was 300.

**Figure 1.** *M. tuberculata* and *T. granifera* molluscs in an aquarium with pumpkinseed (photo by Oleh Marenkov, 2017).

During the 10-day experiment fish were not fed. Sometimes the pumpkinseeds swam to the mollusks and tried to grab them but the mollusks closed in the shell by the shutter and fell to the bottom, after that they were not of food interest for the pumpkinseed. Thus, under the conditions of the aquarium experiment, the pumpkinseed did not consume experimental mollusks.

**Experiment with the Reticulate loach *Botia lohachata* Chaudhuri, 1912**. In the supply spectrum of the Reticulate loach, there are gastropods [21], their eggs and juveniles, therefore one of the hypotheses was the use of Reticulate loach as a biomeliorator for consuming *M. tuberculata* and *T. granifera* molluscs (Fig. 2). In the experiment, 4 individuals of Reticulate loach were used, which were put into a 25-liter aquarium. The sizes of fish varied in the range from 6.8 to 7.8 cm, and the mass was in the rangeof 2.7–3.9 g.

**Figure 2.** Experiment with the Reticulate loach *Botia lohachata* Chaudhuri, 1912 (photo by Oleh Marenkov, 2017).

150 *M. tuberculata* and *T. granifera* molluscs of three size groups I – up to 10 mm (n=50), II – 10.1–15 mm (n=50), III–over 15 mm (n=50) were placed to the fish. The experiment lasted 10 days. During the experiment fish were not fed. Reticulate loach in the search for food actively swam across the aquarium, tried to consume mollusks, but no case of eating mollusks was observed. Thus, an experiment with Reticulate loach has not given a positive result.

**Experiment with *Clea helena* (von dem Busch, 1847) (Gastropoda: Buccinidae).** Like all snails in the clade Neogastropoda, *Clea helena* (von dem Busch, 1847) is carnivorous. It feeds on worms and gastropods, and is often known as the "assassin snail" for its habit of eating other snails [22]. *Clea helena* may be a serious threat to native freshwater gastropods in countries, when introduced [23]. The experiment with *Clea helena* involved 10 adult predatory mollusks, which were planted in an aquarium, where the conditions of the ZNPP were modeled. 100 mollusks of *M. tuberculata* and *T. granifera* molluscs were inhabited to the aquarium. Experiment lasted 35 days. In order to test the range of nutrition for assassin snails and *M. tuberculata* and *T. granifera* molluscs, after the first week of experiment, artificial and natural forages were added to the model aquarium. Therefore, with the course of the experiment, the amount of organic matter in the aquarium increased and at the end of the experiment it was the largest. Every week mollusks in an aquarium were recalculated, the deaths of mollusks was marked. The shells of dead mollusks were removed, and new molluscs were added to the aquarium so that the total number of *M. tuberculata* and *T. granifera* molluscs was 100 specimen.

*Clea helena* moved on substrates (concrete, sand, wood) in search of *M. tuberculata* and *T. granifera* molluscs. The molluscs used a siphon that is located on the front of the mollusc's body for searching the prey (Fig. 3).

**Figure 3.** Assassin snail in search of *M. tuberculata* and *T. granifera* molluscs (photo by Oleh Marenkov, 2017).

*M. tuberculata* and *T. granifera* molluscs concentrated on a concrete slab, buried in sand, often gathered near a water heater. When assassin snailsfound their "victim", they attacked *M. tuberculata* and *T. granifera* molluscs. It was noticed that groups of assassin snails often "attacked" *M. tuberculata* and *T. granifera* molluscs (Fig. 4).

**Figure 4.** A group of assassin snails "attacks" *M. tuberculata* and *T. granifera* molluscs (photo by Oleh Marenkov, 2017).

The largest percentage of *M. tuberculata* and *T. granifera* molluscs, the 38%, was consumed by assassin snails during the first week of the experiment. With the accumulation of organic matter in the model aquarium, there was a decrease in the activity of snails` nutrition. During the second week of experiment, assassin snails consumed 25% of *M. tuberculata* and *T. granifera* molluscs, and during the third and fourth one – 23% and 5%, respectively (Fig. 5).

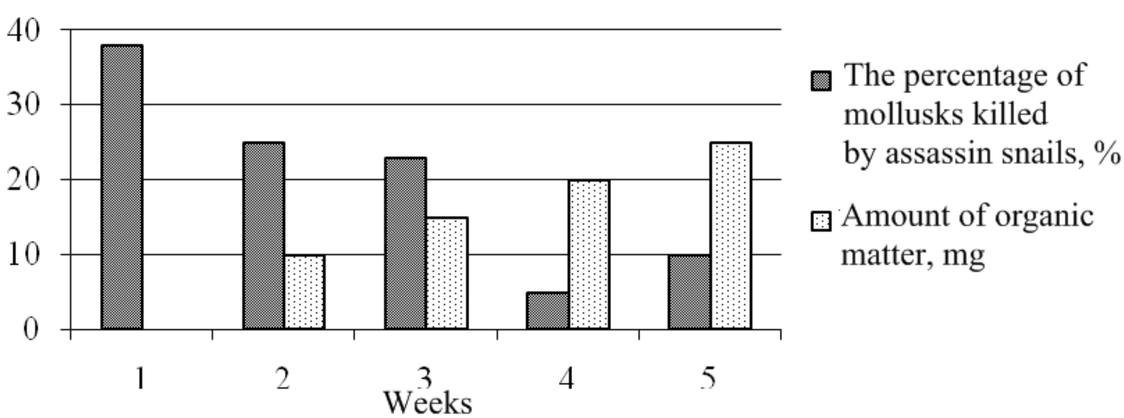


**Figure 5.** The dynamics of consumption of *M. tuberculata* and *T. granifera* molluscs by assassin snails in comparison with the amount of organic matter accumulated in the model aquarium.

Thus, assassin snails hunt on *M. tuberculata* and *T. granifera* molluscs, attack and consume them. The effect of using assassin snails is increased on plots of water with a minimum amount of organic matter and a maximum cluster of *M. tuberculata* and *T. granifera* molluscs.

**Traps for mollusks.** The spontaneous invasion of molluscs *Melanoides tuberculata* (Müller 1774) and *Tarebia granifera* (Lamarck, 1822) caused the search for ways to control the massive accumulation of these molluscs in hydraulic structures. Mechanical cleaning is rather effective, but expensive and complicated process under the conditions of significant areas of the NPP cooling pond. The molluscs have quickly increased their number and created a threat to hydraulic structures, and their further development must be restrained and suppressed by biomelorating measures. It is quite difficult to control a population of molluscs under the conditions of the ZNPP, but it is possible to use specialized traps.

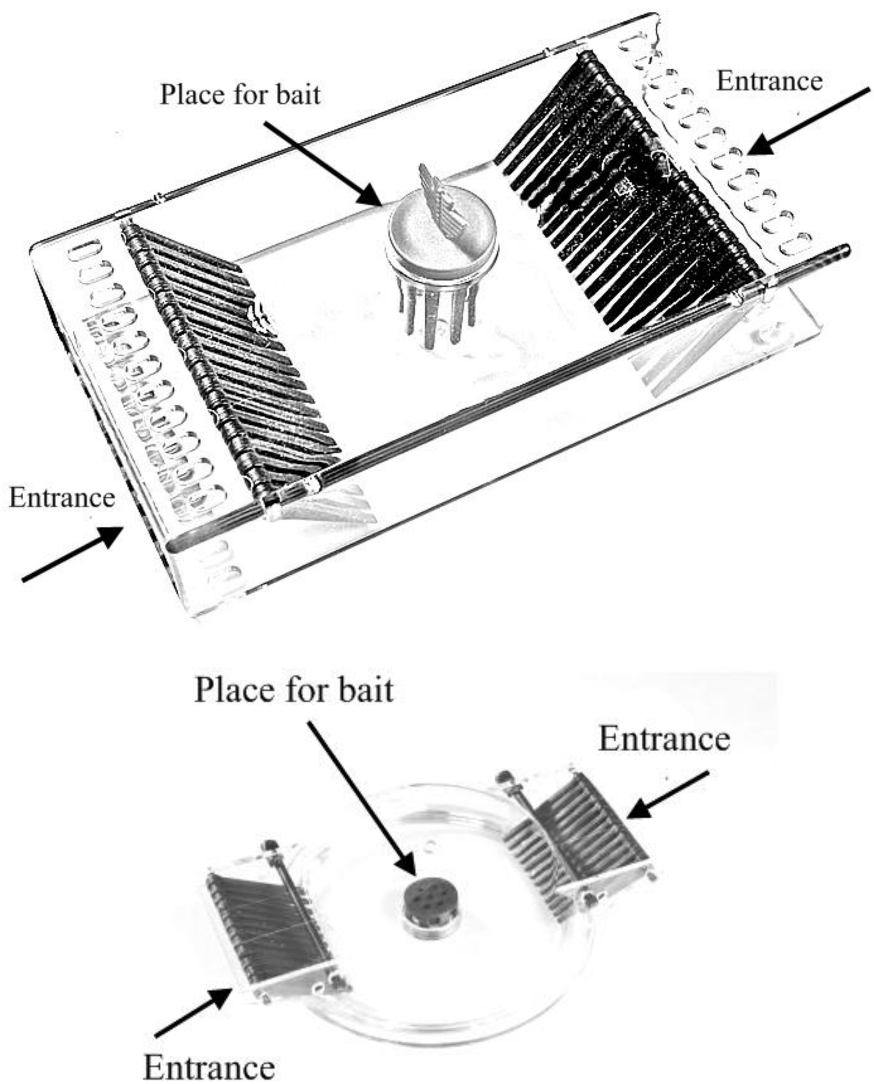


**Figure 6.** Constructions of stationary traps for mollusks with two inlets

The principle of the trap is that the mollusks are attracted by the bait (flavored food supplement), they move to the bait and fall into the trap. The design of the trap implies a one-way directional movement of mollusks to the design. In the middle of the trap, mollusks are concentrated around the food bait and cannot escape the trap.

Such traps allow catching adult sexually mature individuals, thus, removing productive individuals from the population. This, in turn, reduces the reproductive potential of the mollusc population. Artificial traps can be purchased ready-made in specialized stores or can be handmade.

Experimental work was carried out using artificial traps of various designs. It was based on two principles of getting into the trap, the upper and lower ones. Experimental studies using traps with upper and lower inlets showed that with the same food baits, mollusks chose traps with a lower inlet, since they spent less time searching for trap.

The effectiveness of using traps was checked in aquariums in a volume of 60 liters (30×40×50 cm). The aquarium contained 500 mollusks, 2 traps with the upper and lower inlets were set there (Fig. 6). As a bait, mixed fodder for carp fish was used, which was provided by employees of ZNPP biomelioration station. The area of the bottom of the trap was 150 $cm^2$, the area of the bottom of the aquarium was 1500 $cm^2$. The mollusks were located on all the walls of the aquarium, thus they populated the area of 9500 $cm^2$. Consequently, the area of the trap (by the bottom with the bait) was about 1.6% of the settlement area of mollusks. Exposure of the trap was 60 minutes.

Most effectively molluscs were concentrated in traps with a lower inlet (Fig. 7). In traps with a lower inlet on average 7% of molluscs (from 9.8% to 20.8%) concentrated in 60 minutes. At that time, only 2.7% of molluscs (from 0.6% to 5.6%) accumulated in traps with upper inlets.

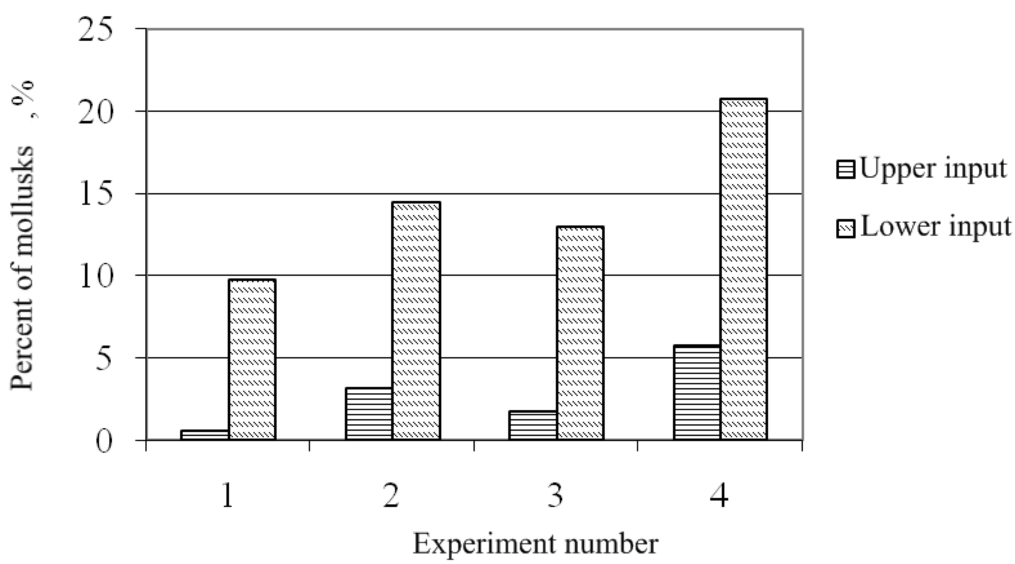


**Figure 7.** Efficiency of traps with different inlets.

Thus, for the capture of mollusks, it is more efficient to use traps with the lower inlet, which facilitates the penetration of mollusks to the trap, and reduces the time for the entry of molluscs into the traps. An important aspect for catching mollusks is the selection of optimal food baits that would be attractive to mollusks and stimulate the instinct of food search, thus accelerating the process of accumulation of molluscs in traps. Subsequent studies of flavor (aromatic) baits we conducted under aquarium conditions using traps with lower inlet.

As food baits we used the following components: 1 – fresh granulated forage for trout, 2 – fresh feed for carp, 3 – rennet feed for catfish, 4 – dough with the addition of fresh sunflower oil, 5 – dough with the addition of roasted sunflower oil, 6 – dough with anise oil added. The search for aromatic food baits can have many options, but it is necessary to adhere to the basic principles: the aromatic additive should not affect the hydrochemical state of the reservoir, the fodder component must have a fairly stable structure so that it is not washed out and not carried by the flow, feed bait should be cheap enough.

The traps with bait were mounted to the aquarium and the behavior of mollusks was marked. The effectiveness of feed baits was tested in aquariums with a volume of 60 liters – 30x40x50 cm. The aquarium contained 500 mollusks. The trap occupied 1.6% of the total area inhabited by mollusks. Experiment exposure was 60 minutes.

Positive results were obtained in all 6 variants, molluscs reacted positively on all baits and got into the trap (Fig. 8).

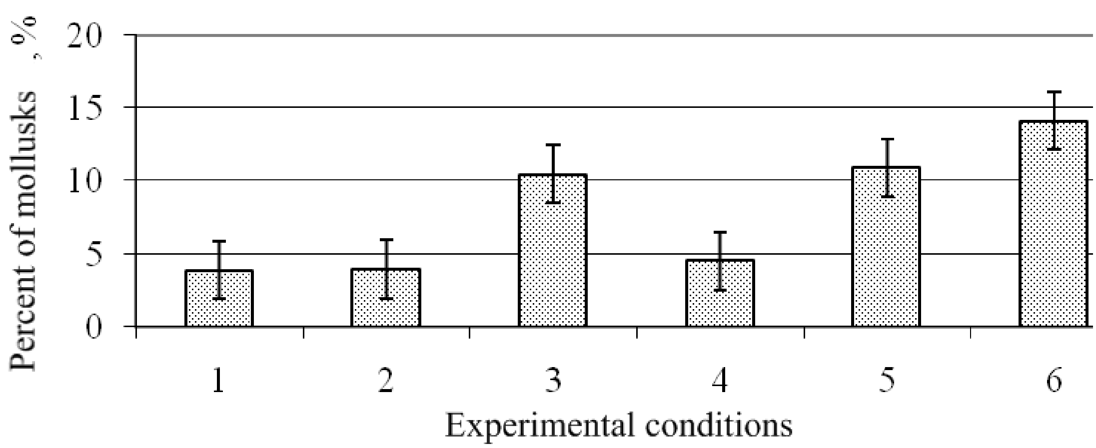


**Figure 8.** Results of feed preferences of mollusks in trap experiments. Feed ingredients in the experiment: 1 – fresh granulated forage for trout, 2 – fresh feed for carp, 3 – rennet feed for catfish, 4 – dough with the addition of fresh sunflower oil, 5 – dough with the addition of roasted sunflower oil, 6 – dough with the addition anise oil.

The smallest number of molluscs fell into traps with baits number 1, 2, 4 – from 3.8% to 4.5% of molluscs. The most effective was the use of traps with rancid feed, roasted sunflower oil and aniseed oil (Experiments No. 3, 5, 6). The most effective was the bait with the addition of anise oil, which attracted mollusks to traps. During the exposure, traps with anise bait have accumulated up to 14.1% of molluscs.

It is noted that the concentration of molluscs is also influenced by the lighting of the reservoir. Thus, a greater number of *M. tuberculata* and *T. granifera* molluscs was observed in the shade of cane thicket. It is also known that mollusks are better fed at night, so we conducted an experiment to investigate the effectiveness of traps during the day and at night. The experiments were carried out in 30×40×50 cm aquariums with volume of 60 liters. The aquarium contained 500 mollusks. The trap occupied 1.6% of the total area inhabited by mollusks. Exposition of the experiment was 8 hours.

Investigation of the effectiveness of traps for mollusks taking into account light allowed to establish that during the dark period, mollusks became trapped more quickly (Fig. 9). Thus, on average, at night, the trap accumulates about 22.1% of molluscs, despite 8.9% during daylight hours.

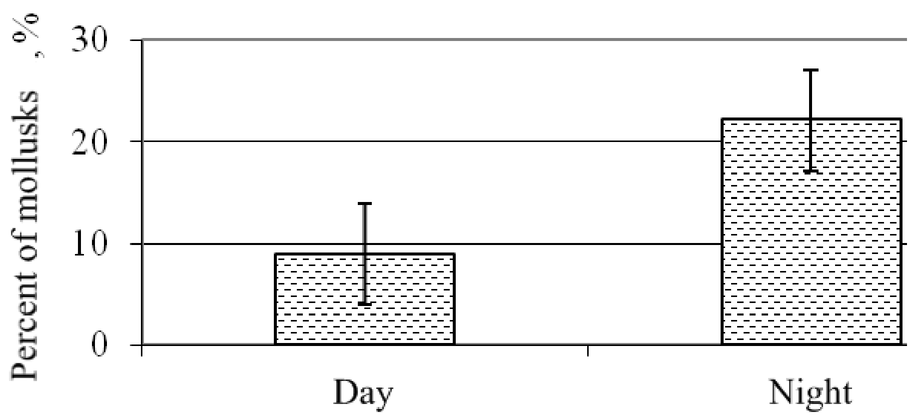


**Figure 9.** Efficiency of traps with the lower inlet at day and night.

Thus, biomeliorative methods for controlling the spread of *M. tuberculata* and *T. granifera* molluscs include the use of traps with one-way inlet. Their effectiveness depends on the design, location, feed bait, installation time and maintenance.

## 4. CONCLUSIONS

**1.** Among the proposed meliorative species, the most effective were the predatory mollusks the assassin snails, which consumed *M. tuberculata* and *T. granifera* molluscs in a fairly large amount, but in the presence of organic residues, the intensity of consumption of *M. tuberculata* and *T. granifera* molluscs by assassin snails is significantly reduced, which requires more careful and well-considered research.

**2.** For the capture of mollusks from ponds, it is recommended the introduction of special traps for molluscs, the principle of which is based on the accumulation of mollusks in the middle of the trap. For the capture of mollusks, it is more efficient to use trap-collectors with a lower inlet, which facilitates the penetration of mollusks to the trap and greatly reduces the time when molluscs hit traps.

**3.** For the efficient use of traps, it is necessary to introduce inexpensive and effective baits for mollusks. Positive results we obtained with the use of baits with the addition of anise oil.

**4.** In order to control the number of gastropods in integrated reservoirs, it is necessary to use a multilevel approach, which can be implemented through the introduction of biological and biomechanical methods for the control of molluscs.

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