https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723
http://zoobank.org/References/7EDFB492-33F5-4F6F-BB32-FEC4BB86098F

УДК 595.122.2

Experimental study of the effect of *Echinostoma caproni* metacercariae on the survival of *Biomphalaria pfeifferi* molluscs

G. L. Ataev

Herzen State Pedagogical University of Russia, 48 Moika Emb., 191186, Saint Petersburg, Russia

Author
Gennady L. Ataev
E-mail: ataev.gennady@gmail.com
SPIN: 6944-3950
Scopus Author ID: 6602555237
ResearcherID: Q-7655-2016
ORCID: 0000-0002-4740-2117

Abstract. This study experimentally proves the pathogenicity of *Echinostoma caproni* metacercariae for *Biomphalaria pfeifferi* molluscs. The study investigated snails infected and uninfected with *E. caproni* redia. To slow down the rate of accumulation of metacercariae in aquaria, the density of cercariae was artificially reduced. The study suggests that the high pathogenicity of metacercariae in the experiment might be attributed to the laboratory conditions, while this effect is less pronounced in natural habitats.

Keywords: trematodes, *Echinostoma caproni*, metacercariae, pathogenicity, *Biomphalaria pfeifferi,* molluscs

Экспериментальное изучение влияния метацеркарий *Echinostoma caproni* на выживаемость моллюсков *Biomphalaria pfeifferi*

Г. Л. Атаев

Российский государственный педагогический университет им. А. И. Герцена, наб. реки Мойки, д. 48, 191186, г. Санкт-Петербург, Россия

Сведения об авторе
Атаев Геннадий Леонидович
E-mail: ataev.gennady@gmail.com
SPIN-код: 6944-3950
Scopus Author ID: 6602555237
ResearcherID: Q-7655-2016
ORCID: 0000-0002-4740-2117

Аннотация. Экспериментально доказана патогенность метацеркарий *Echinostoma caproni* для моллюсков *Biomphalaria pfeifferi.* Изучались незараженные и зараженные редиями *E. caproni* улитки. Для замедления скорости аккумуляции метацеркарий в аквариумах искусственно снижалась концентрация церкарий. Высказано предположение, что высокая патогенность метацеркарий в эксперименте обусловлена условиями лабораторного содержания улиток, а в природе этот эффект носит более сглаженный характер.

Ключевые слова: трематоды, *Echinostoma caproni*, метацеркарии, патогенность, *Biomphalaria pfeifferi*, моллюски

Introduction

The snails belonging to the genus *Biomphalaria* are commonly used in parasitological research as intermediary hosts for a variety of trematodes, primarily, from the genera *Schistosoma* and *Echinostoma*. Among *Echinostoma, E. caproni* has been the most extensively studied. The studeis have produced considerable data on the development of *E. caproni* parthenitae and the immune response of snail hosts (Irvin, Fried 1990; Krejci, Fried 1994; Fried, Huffman 1996).

The infrapopulation of *E. caproni* rediae develops at a relatively slow rate. The release of the first cercariae from snails occurs approximately 3 weeks post-infection (PI), while the mass release of larvae from snails begins only a month PI. In addition to this, each daughter redia (DR) does not produce more than 30 cercariae (Ataev, Tokmakova 2018). Generally, such low fecundity of rediae in trematode parthenitae is compensated by the overall quantity of produced rediae. These longer-living infrapopulations of parthenitae follow the strategy of prolonged development (Ataev 2017). Yet, under laboratory conditions, snails infected with *E. caproni* reportedly start dying in large numbers as early as 2–3 weeks after the start of cercarial release.

Previously, this phenomenon among *Biomphalaria* was attributed to autoinfection by *Echinostoma caproni* cercariae (Kuris, Warren 1980; Ataev 2010). As we know, this species of trematodes can use *Biomphalaria* snails both as the first and the second intermediary host within their life span to ensure successful development of metacercariae. Consequently, the deaths of host snails were suggested to be caused by the accumulation of metacercariae in their bodies.

However, it was not clear what attributes to snail mortality the most: a gradual accumulation of a certain number of metacercariae or a one-time accumulation of a lethal number of cysts? To answer this question, we carried out additional experiments by infecting *Biomphalaria pfeifferi* with metacercariae and conducted statistical analysis of the results thereafter.

Materials and methods

This study was conducted at the Herzen Laboratory of Experimental Zoology (Saint Petersburg, Russia) and the Laboratory of Host-Pathogen-Environment Interactions of the University of Perpignan (France). The objects of the research were *Echinostoma caproni* trematodes (Richard, 1964), whose miracidia were used to infect *Biomphalaria pfeifferi* snails (Krauss, 1848).

During the experiments, the snails were maintained in several aquaria in a refrigerated circulator (t=26°C, photoperiod of 12L: 12D) and fed on lettuce leaves. All snails that died during the experiment were then dissected to determine the number of metacercariae and DR in their bodies.

In total, the experiment involved 250 specimens of *B. pfeifferi* (6–8 mm in shell diameter) with 150 of them simultaneously infected with 3 *Echinostoma caproni* miracidia per snail. The snails were divided into 5 experimental groups depending on the maintenance conditions.

Group 1: 50 infected snails were held in a 5-liter aquarium.

Group 2: 50 infected snails were held in a 5-liter aquarium, with the water filtered every 1–2 hours during the photoperiod to lower the density of cercariae in the aquarium.

Group 3–4: 50 infected snails (Group 3) and 50 uninfected snails (Group 4) were held together in a 10-liter aquarium. The uninfected snails were supposed to act as a biological filter (since a portion of *E. caproni* cercariae released from the infected snails would spread to the uninfected snails of Group 4).

Group 5 (control group): 50 uninfected *B. pfeifferi* snails were placed in a 5-liter aquarium.

The examination of metacercariae and rediae was done via histological sections. The material was fixed in Bouin's fluid. The paraffin sections (5 mcm in thickness) were then stained in Ehrlich's hematoxylin and eosin (water solution). The preparations were examined on a Leica DM 5000 microscope.

Prior to SEM analysis, the material was rinsed in the Chernin's solution (Chernin 1968) and fixed in a 3% glutaraldehyde solution based on 0.1 M phosphate buffer. The preparations were studied on a Zeiss EVO 40 microscope.

The statistical analysis of the results included the Spearman's rank correlation coefficient and regression analysis.

Results

The development of infrapopulations of Echinostoma caproni parthenitae

The reproduction of mother sporocysts begins 8 days PI and lasts for about a week. During this time, they produce up to 30 mother rediae (Fig. 1: *A*) which, in turn, only produce DR. The latter then penetrate *B. pfeifferi* snails and start reproducing. At first, DR form redial embryos and then irreversibly transition to producing cercariae (Fig. 1: *B*). The release of the latter begins 3 weeks PI.

Approximately a month PI, the size of an infrapopulation stabilizes, reaching about 150 rediae. In this research, we consider a parthenita population of this size as mature. The Spearman's rank correlation coefficient confirms that the growth of a population really plateaued. Additionally, we found no significant correlation between the age of a mature infrapopulation and the number of rediae comprising it ($r = 0.24$, $p < 0.31$, $n = 19$).

Under laboratory conditions, the release of rediae lasts for 1–2 weeks, after which we observed the mass mortality of infected *B. pfeifferi* snails.

Exposure of B. pfeifferi to metacercariae

E. caproni cercariae rarely penetrate *B. pfeifferi* through their external shells. Instead, they mostly infect the snails through the epithelial tissue of their mantle collars. Generally, cercariae penetrate snails directly from their mantle cavity, which they infiltrate through the pneumostome. They can also penetrate the dorsal part of a snail through the epithelial tissue, but most of the time they move from the mantle cavity to the kidney via the excretory pore. Some cercariae start encysting here, but most of them migrate into the pericardial cavity through the renopericardial canal. This is where the majority of metacercariae localize.

Before penetrating a snail host, cercariae tend to discard their tails and move by rapidly contracting their bodies (Fig. 1: *C*). We have encountered only one case of a cercariae retaining its tail even after penetration. (Ataev 2010). During the encystment, the body of a cercariae twists on its ventral side and then quickly gets covered with a three-layer cyst wall formed by the secretion of cystogenous glands. The entire encystment process takes no more than 4 hours.

Afterwards, metacercariae get encapsulated in hemocyte capsules (Fig. 1: *D*). Hemocyte also creates a fibrous tissue, which makes separate cysts stick together into large conglomerations. Metacercariae have a spherical form with a diameter of 155 ± 1.9 mcm ($n = 100$). At first, the outer wall of metacercariae is transparent, but turns opaque after a week. These metacercariae are infective and stay that way for more than a month. Larvae start dying 50 days after. Their size shrinks by half and the surface of their capsule gets loose. Thus, our results did not confirm the findings of N. O. Christensen et al. (1980) who determined the lifespan of metacercariae as 4 months.

Mortality of B. pfeifferi due to metacercariae autoinfection

The first deaths of *B. pfeifferi* snails from Group 1 were registered 3 weeks PI. This is when the release of cercariae first began. More than half of the snails died a month PI (Fig. 2). The last snail from Group 1 died 38 days PI. By contrast, only 3 uninfected snails (6%) from the control group (Group 5) died during the experiment.

As we mentioned earlier, the density of cercariae in the aquaria of Groups 2 and 4 was lowered artificially by either frequent filtration of water (Group 2) or by using uninfected snails (Group 4) as a biological cercarial filter in the aquarium with infected snails (Group 3).

https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723

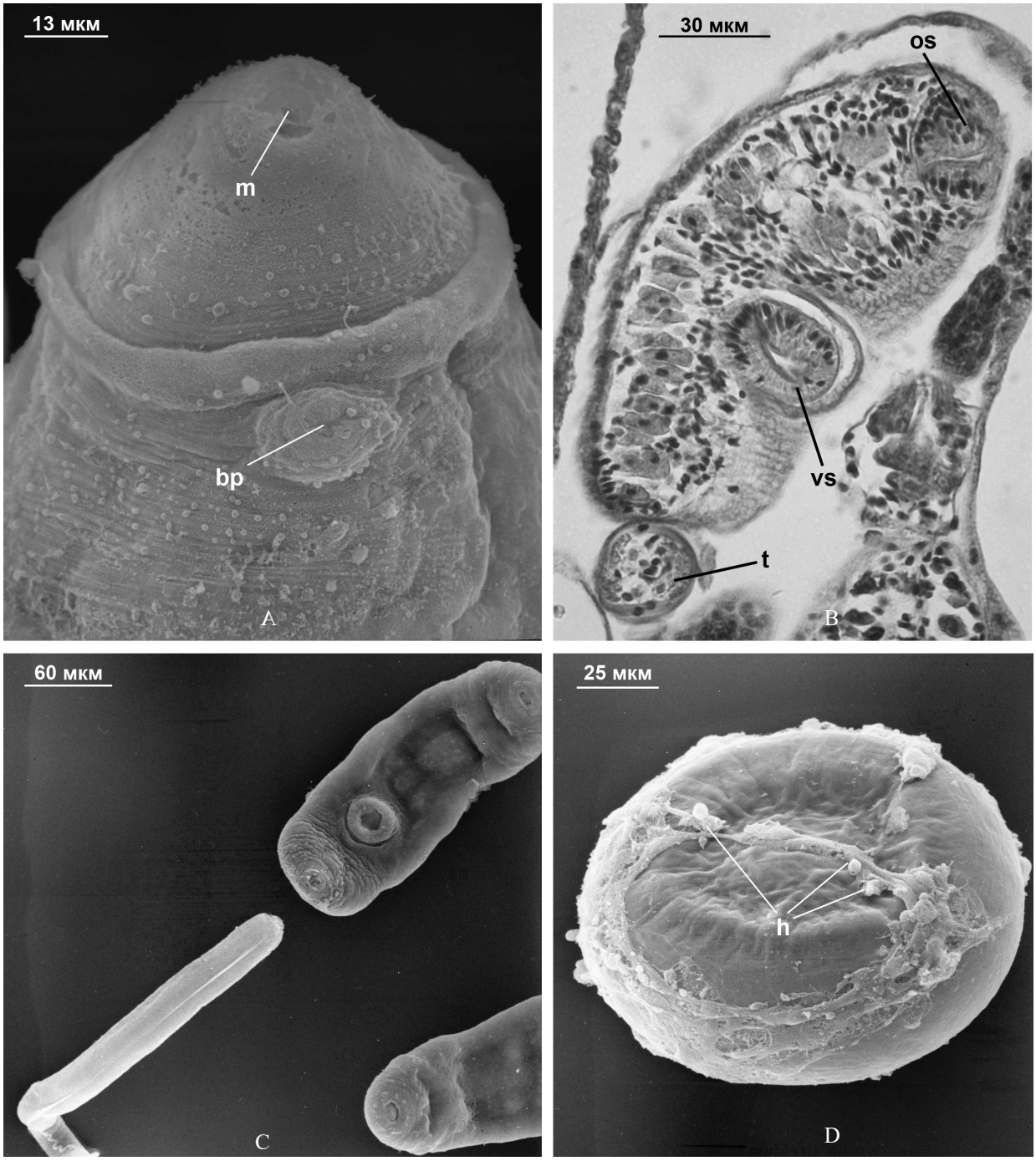

Fig. 1. Stages of development of an *Echinostoma caproni* trematode (*A–D*): *A* — SEM microphotograph of a mother redia; *B* — histological section of a cercaria embryo in a daughter redia; *C* — SEM micrograph of a cercaria with a discarded tail; *D* — SEM micrograph of a metacercaria. Legend: *bp* — birth pore, *h* — hemocytes, *m* — mouth, *os* — oral sucker, *t* — tail, *vs* — ventral sucker

Рис. 1. Стадии развития трематод *Echinostoma caproni* (*A–D*): *A* — SEM-микрофотография материнской редии; *B* — гистологический срез эмбриона церкарии в дочерней редии; *C* — SEM-микрофотография церкарии с отброшенным хвостом; *D* — SEM-микрофотография метацеркарии. Условные обозначения: *bp* — родильная пора; *h* — гемоциты; *m* — ротовое отверстие; *os* — ротовая присоска; *t* — хвост; *vs* — брюшная присоска

This artificial decrease of cercarial density led to the extension of the lifespan of infected snails (Group 2 — up to 56 days, Group 3 — up to 60 days). The experiment also confirmed the pathogenicity of metacercariae since snails from Group 4 died within 58 days. Therefore, the results of the experiments on Groups 2, 3, and 4 indicate that the mortality of snails is attributed to metacercarial infection. The analysis of the survivorship curves of different experimental groups confirms this assumption (Fig. 2). The graph shows that snails from Group 1 started dying sooner and faster than snails from the other groups. At the same time, survivorship curves for Groups 2–4 look very similar.

These findings are also confirmed by statistical analysis. According to the Friedman test, the conditions of the experiment significantly affect the mortality dynamics of *B. pfeifferi* snails. A pairwise comparison of death times between Groups 1–4 (Wilcoxon test, including the Bonferroni correction) shows that the findings on Group 1 are different from the findings on Groups 2, 3, and 4 ($p < 0.001$). At the same time, survivorship curves for Groups 2, 3 and 4 are statistically indistinguishable ($p > 0.05$).

Thus, the analysis of survivorship curves of *B. pfeifferi* snails from different groups confirmed our assumption that *E. caproni* metacercariae are pathogenic to snails regardless of whether they were initially exposed to rediae or not.

The dynamics of metacercariae accumulation in B. pfeifferi

According to our experiments, a month PI, the number of accumulated metacercariae in snails from all experimental groups reaches several hundred. The initially uninfected snails from Group 4 contained 250 cysts, while the infected snails from Group 3 contained 500 cysts. The maximum number of accumulated cercariae in snails was observed on day 45 when Groups 2, 3, 4 were found to contain 1,100, 1,580, and 1,050 cysts, respectively.

The Spearman's rank correlation coefficient showed a statistically significant correlation between the time (in days) snails were exposed to cercarial infection and the quantity of metacercariae contained in their bodies ($r = 0.70$; $p<0.001$).

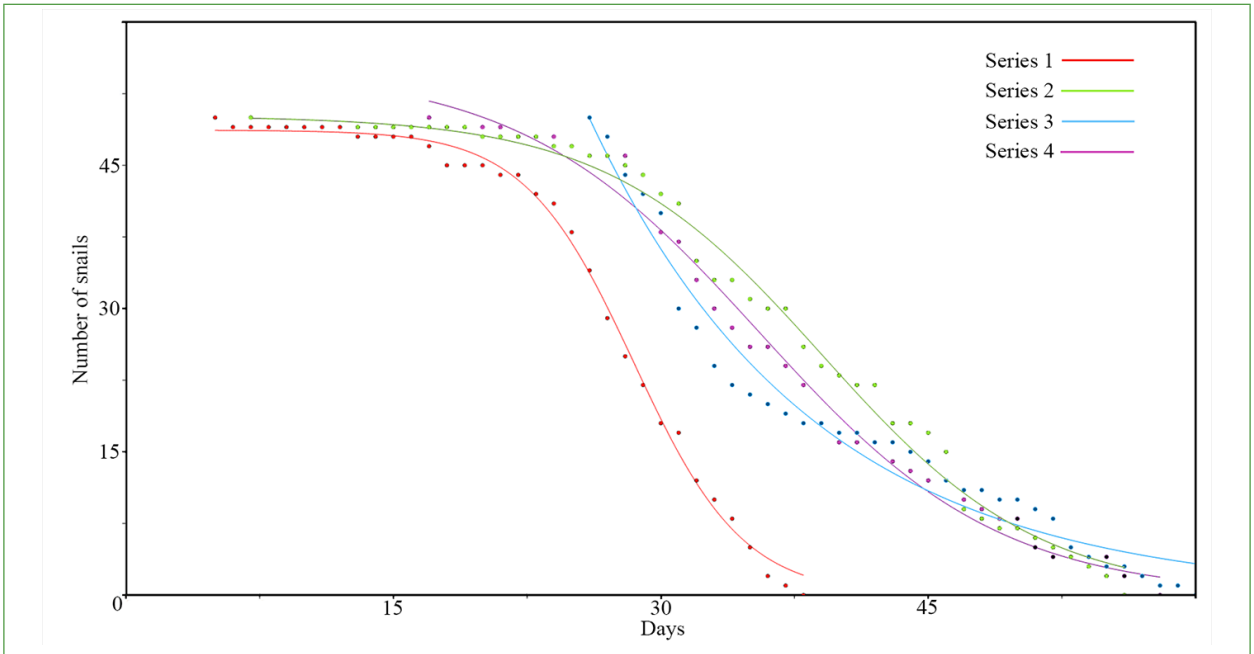

Fig. 2. Dynamics of mortality of *Biomphalaria pfeifferi* molluscs. Group *1** — red line / 38 days PI; Group *2** — green line / 56 days PI; Group *3** — blue line / 60 days PI; Group *4* — lilac line (magenta) / 58 days PI

Рис. 2. Динамика смертности моллюсков *Biomphalaria pfeifferi*. *Серия 1** — красная линия /38 дней Р. I.; *Серия 2** — зелёная линия / 56 дней Р. I.; *Серия 3** — голубая линия / 60 дней Р. I.; *Серия 4* — лиловая линия (magenta) / 58 дней Р. I.

https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723

However, this correlation becomes weaker the farther from the initial infection we move with our analysis. For example, by the end of our experiment (about 2 months PI), the maximum number of accumulated cercariae in *B. pfeifferi* snails from Groups 2, 3, 4 fell to 630, 610, and 820, respectively.

Apart from that, while the correlation coefficient was high, the number of metacercariae could vary even in snails that belonged to the same experimental group and died on the same day. Evidently, cercarial infection in different snails does not progress at the same rate, which is particularly obvious if we look at the data from the beginning and the end of our experiment.

We studied the size of a parthenita infrapopulation and the size of a snail as potential factors affecting the accumulation of cercariae in snails. The Spearman's rank correlation coefficient (n = 61) helped us discover a significant correlation between the diameter of a snail shell and the number of rediae ($r = 0.71$; $p < 0.001$).

Additionally, when it comes to snails initially exposed to rediae, we looked into potential correlation between the diameter of their shells and the number of metacercariae ($r = 0.40$; $p < 0.001$) and correlation between the number of rediae and metacercariae ($r = 0.33$, $p < 0.01$). However, we did not find any significant correlations between these parameters.

Using the *t*-test to compare the correlation coefficients confirmed that the strength of the correlation between a shell diameter and the number of rediae significantly differs from the other two correlations ($p < 0.05$, including the Bonferroni correction). In turn, the latter two were determined to be indistinguishable from each other ($p > 0.05$).

At the same time, when it comes to *B. pfeifferi* from Group 4 (not exposed to rediae), the relationship between the number of metacercariae and a shell diameter can be depicted as the following equation of linear regression:

$Y = -906.471 + 208.846 * X$; $R^2 = 0.16$; $r = 0.41$ ($\alpha = 0.03$, P = 97%, n = 29)

This data allows us to assume that initial redial infection of snails affects the accumulation of metacercariae by the uninfected snails. However, this correlation becomes insignificant for snails that were initially exposed to *E. caproni* parthenitae.

Discussion

The academic literature provides numerous examples of the pathogenicity of trematode metacercariae for secondary intermediary hosts. In particular, a lot is said about metacercarial infection of fish (Szidat 1924; Timmerman 1936; Erasmus 1959; Shigin 1993 and others). The reported studies also provide data on the effects of metacercarial infection on amphibians and various invertebrates: crustaceans, insects, leeches (Stunkard 1957; Anokhin 1966; Ginetsinskaya 1988; Fried, Huffman 1996; Haas 2000).

However, not much is known on how metacercariae affect molluscs. In particular, one study examines the effects of *Echinostoma liei* (syn. *E. caproni*) cercarial infection on *Biomphalaria glabrata* snails (Kuris, Warren 1980). To prove the pathogenic nature of metacercariae, the authors conducted experiments to lower the autoinfection of snails. The snails (both exposed and unexposed to rediae) were contained in one aquarium divided by perforated screens. During the experiment, the researchers used several screens with varying perforation diameters. This allowed to regulate cercarial ability to locate snails initially unexposed to rediae. As a result, the authors determined that the survivorship of *Biomphalaria glabrata* snails is directly contingent on the intensity of cercarial infection and the size of a snail diameter.

A. M. Kuris and J. Warren suggested using the metacercarial pathogenicity factor to biologically control *Biomphalaria* population since many of them act as natural hosts for shistosomes, a trematode that can also infect humans. A later study on how *Echinostoma caproni* metacercariae affect *Biomphalaria glabrata* и *B. pfeifferi* yielded similar results (Ataev 2010).

Despite the studies, the pathogenic mechanism of *Echinostoma* metacercariae was still unclear. There was an assumption that high

pathogenicity of *Echinostoma caproni* metacercariae manifests only when their dispersion area is limited (e.g., an aquarium). Besides, the reported experiments did not involve alternative hosts for cercarial infection. By contrast, in their natural habitat, *E. caproni* cercariae can use up to 14 species of pulmonates and several species of amphibians as their secondary intermediary hosts (Fried, Huffman 1996).

Generally, cercariae belonging to the genus *Echinostoma* can encyst in various species of gastropods, bivalves, leeches as well as in tadpoles and fish (Haas 2000). Several experiments showcased their encystment in snail tissue and mucous (Beaver 1937; Stein, Bash 1977; Fried, Bennet 1979; Evans et al. 1981; Evans, Gordon 1983; Anderson, Fried 1987; McCarthy, Kanev 1990; Fried et al. 1997; Esteban, Muñoz-Antoli 2009).

Naturally, this ability to infect a variety of different species does not guarantee metacercariae successful development. However, it means that the density of cercariae in natural bodies of water decreases. Besides, there are other factors that might affect their density, such as getting eaten by various aquatic organisms, physical damage, dispersion in the water, etc. The dynamics of metacercarial accumulation in snails in natural habitats is likely to be more subdued than under laboratory conditions.

Our experiments on snails from Group 3 (initially exposed to rediae) and Group 4 (unexposed) confirms this assumption. During the experiment, snails from both groups ended up dying, and the mortality curves of both experimental groups did not show significant differences (Fig. 2). Moreover, they were not different from the mortality curve of Group 2 snails, which had their water artificially cleaned from cercariae via filtration. At the same time, the dynamics of mortality in Groups 2, 3, 4 exhibited significant differences from mortality patterns in snails from Group 1, whose aquarium was not equipped with any artificial means of controlling autoinfection (Fig. 2).

It is important to note that the maximum lifespan of snails from Groups 2, 3, and 4 was more or less the same since the last snails from each Group died about 2 months after the beginning of the experiment. This means that the initial exposure to rediae did not affect the maximum snail lifespan. At the same time, the maximum survivorship of snails from Group 1 was only 38 days. Therefore, by decreasing the cercarial density, we were able to prolong the life of snails from Groups 2 and 3 initially exposed to rediae and, consequently, prolong the presence of *Echinostoma caproni* parthenita infrapopulations in them.

Now, let us look at how echinostome cercariae infect host snails. As a rule, cercariae penetrate snails without damaging a host's tissues. Many echinostome species use a pneumostome to infiltrate a gastropode's mantle cavity where they begin the encystment process. In this case, these metacercariae develop without forming metabolic connections with a host. Therefore, their biology resembles that of adolescariae of trematodes belonging to the families Fasciolidae, Notocotylidae, Philophthalmidae, etc., which develop outside of a host's body. Interestingly, while the life cycle of most trematodes of the Echinostomatidae family does involve a second intermediary host, some species (e.g., from the genus *Echinochasmus*) have retained a capability to encyst in the external environment (Galaktionov, Dobrovol'skij 1987).

It should be noted that *E. caproni* cercariae can infect snails through external tissues only if they penetrate the mantle collar. The vast majority of larvae get there though a pneumostome. Some of them infiltrate the epithelial tissue of the mantle collar and start encysting in the lungs, kidney and other internal organs. The majority of cercariae, however, move on to the renopericardial canal through the excretory pore in order to get into the pericardial cavity and begin the process of encystment there.

E. caproni metacercariae are surrounded by a multilayered cyst wall, which is in turn encased in a hemocyte capsule formed from a host's hemolymph cells. This capsule protects cysts from a host's defense responses but also limits its access to a snail's energy resources

https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723

(Laurie 1974; Irwin, Fried 1990). As a result, these maritae mostly use energy resources they managed to accumulate as cercariae from their first intermediary host. Thus, while echinostome metacercariae have better developed adaptation mechanisms compared to adolescariae, they do not need to depend on the metabolic connection to their hosts for successful development, either.

There are two main reasons that may explain snail mortality from metacercarial infection. The first reason is the accumulation of a specific (lethal) number of cysts in a snail's body. Not only do the bodies of snails run out of space and energy for metacercariae to develop, they also lack necessary resources to survive themselves. The second reason behind *Biomphalaria* mortality may lie in a one-time accumulation of a lethal dose of infection within 1–2 days. In this case, it is the infection process itself that inflicts more damage to snails: if the density of cercariae in the mantle cavity is too high, they might start infiltrating a snail through its respiratory and dorsal epithelium tissues. Besides, some larvae penetrate snails through the epithelium tissue of their mantle collar. As a result, the penetration of cercariae does a lot of physical damage to a snail's body tissues, leading to its death.

It is also possible that both reasons equally contribute to snail mortality. Cercariae do not always infiltrate their host snail at a steady pace. So, a snail that accumulates several dozen metacercariae daily can live much longer than a snail that is infected with several hundred larvae over a handful of hours. The absence of a significant correlation between metacercarial population size and snail mortality confirms this assumption.

Apart from the dynamics of metacercariae accumulation, the survivorship of snails may depend on their size and whether they were initially exposed to rediae. Echinostome cercariae will try to infiltrate into any snail regardless of its size and age. For instance, cercariae of *Echinostoma trivolvis* can infect newborn *Biomphalaria glabrata* with a shell diameter of only 0.7–1.0 mm. This, however, often leads to their own death (Fried et al. 1995).

Comparing the data on metacercarial infection rates of *B. pfeifferi* snails (6–8 mm shell width) with that of larger *B. glabrata* snails (10–12 mm shell width) confirms that larger molluscs can accumulate a larger number of metacercariae in their bodies (Ataev 2010). The average number of metacercariae accumulated in *B. pfeifferi* at the time of their death was 136 ± 28 (n = 60), whereas for *B. glabrata*, this number reached 2398 ± 426 (n = 36). The maximum number of accumulated metacercariae in *B. pfeifferi* (7.5 mm shell width) was found to be 1,585 cysts, while *B. glabrata* (14.8 mm shell width) accumulated a maximum of 5,850 cysts. These differences between *B. pfeifferi* and *B. glabrata* snails' capacities for metacercarial accumulation substantially affected their survivorship. The maximum life span of *B. pfeifferi* snails exposed to rediae and subjected to metacercariae autoinfection was 38 days, while the lifespan of *B. glabrata* under the same conditions reached 72 days (Ataev 2010).

It is likely that this contrast is due to the differences in space available for metacercarial localization between the smaller and larger snails. For example, infected snails may die because metacercariae have obstructed vital ducts and cavities in their bodies. In particular, if a large number of metacercariae accumulates in the pericardial cavity, it may negatively affect a snail's heart performance. For example, snails infected with a large number of metacercariae experience fewer ventricular contractions: their ventricular rate slows down from normal 60 bpm to 30–40 bpm (personal observations).

There was no correlation found between the number of accumulated metacercariae and shell size recorded in *B. pfeifferi* snails exposed to rediae. However, the regression analysis helped us identify a positive correlation between the number of accumulated metacercariae and shell diameter in snails from Group 4. This indicates that the initial exposure to rediae may reduce this correlation completely. Besides, the shells of *B. pfeifferi* snails chosen for our experiment did not have any significant variety in size.

What we found unexpected is the absence of a significant correlation between survivorship of snails initially exposed to *Echinostoma caproni* rediae and that of their unexposed counterparts. Before initiating our experiment, we hypothesized that snails initially exposed to rediae would be more vulnerable to metacercarial infection. However, the experiment on combining initially exposed and unexposed *Biomphalaria pfeifferi* snails (Groups 3 and 4) in one aquarium disproved this assumption. As it turned out, the mortality curves for both snail groups look very similar, especially when it comes to level L_{50}.

Therefore, the exposure to rediae does not determine the survivorship of snails infected with metacercariae. A possible reason is that the development of *Echinostoma caproni* metacercariae is not as energy-consuming as the development of redial infrapopulations. Furthermore, the majority of metacercariae accumulate in the pericardial cavity, while rediae rarely reach that far.

There are studies indicating that echinostome cercariae can encyst before leaving their first snail host (Haseeb, Eveland 2000; Ataev 2010). However, this phenomenon is statistically rare and does not affect the survivorship of snails.

Therefore, our experiment showed that rapid death of *Biomphalaria pfeifferi* snails exposed to *Echinostoma caproni* rediae is caused by cercarial autoinfection. In theory, the lethality of metacercarial infection can depend on multiple factors, such as the size and physiological health of a snail as well as the prior exposure to rediae. However, in reality, the main factor affecting *Biomphalaria* mortality is the dynamics of cercarial infection development. The pathogenicity of metacercariae for snails contained in small aquaria was particularly prominent due to the high density of cercariae in their environment. By artificially lowering the density of cercariae, we were able to prolong the lifespan of *Biomphalaria pfeifferi* by 60%. It is evident that natural environment allows for a more even and spread-out accumulation of metacercariae in snails. For this reason, the pathogenicity of metacercariae for host snails is not that pronounced.

Funding

This study was supported by the Russian Science Foundation (project No. 22-24-20057) and Saint Petersburg Science Foundation (project No. 49/2022).

References

Anderson, R. M., Fried, B. (1987) Experimental infection of *Physa heterostropha*, *Helisoma trivolvis* and *Biomphalaria glabrata* (Gastropoda) with *Echinostoma revolutum* (Trematoda) cercariae. *Journal of Parasitology,* vol. 73, pp. 49–54.

Anohin, I. A. (1966) Sutochnyj ritm murav'ev, invazirovannyh metacerkariyami Dicrocoelium lanceatum. *Doklady AN SSSR*, vol. 166, pp. 757–759. (In Russian)

Ataev, G. L. (2010) The influence of *Echinostoma caproni* metacercariae (Trematoda) on the survival of *Biomphalaria* molluscs (Pulmonata). *Parazitologiya*, vol. 44, no. 6, pp. 481–495. (In Russian)

Ataev, G. L. (2017) *Razmnozhenie partenit trematod: Obzor osnovnyh teorij.* Saint Petersburg: Nauka Publ., 87 p. (In Russian)

Beaver, P. C. (1937) Experimental studies of *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. *Illinois biological monographs*, vol. 15, pp. 7–96.

Chernin, E. (1968) Observations on hearts explanted in vitro from the snail *Australorbis glabratus*. *Journal of Parasitology,* vol. 49, pp. 353–364. https://doi.org/10.2307/3275797

Erasmus, D. A. (1959) The migration of Cercaria X Baylis (Strigeida) within the fish intermediate host. *Parasitology*, vol. 49, no. 1-2, pp. 173–190. https://doi.org/10.1017/S0031182000026810

Esteban, J. G., Muñoz-Antoli, C. (2009) Echinostomes: systematics and life cycles. In: R. Toledo, B. Fried (eds.) *The Biology of Echinostomes. From the Molecule to the Community*. New York: Springer, pp. 1–34.

Evans, N. A., Gordon, D. M. (1983) Experimental observations on the specificity of *Echinoparyphium recurvatum* toward second intermediate hosts. *Zeitschrift für Parasitenkunde*, vol. 69, pp. 217–222. https://doi.org/10.1007/BF00926956

Evans, N. A., Whitfield, P. J., Dobson, A. P. (1981) Parasite utilization of a host community: the distribution and occurence of metacercarial cysts of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) in seven species of mollusc at Harting Pond, Sussex. *Parasitology*, vol. 83, no. 1, pp. 1–12. https://doi.org/10.1017/S0031182000049982

Fried, B., Bennet, M. C. (1979) Studies on encystment of *Echinostoma revolutum* cercariae. *Journal of Parasitology*, vol. 65, no. 1, pp. 38–40. https://doi.org/10.2307/3280199

Fried, B., Huffman, J. (1996) The biology of the intestinal trematode *Echinostoma caproni*. *Advances in Parasitology*, vol. 38, pp. 311–368. https://doi.org/10.1016/S0065-308X(08)60037-8

Fried, B., Idris, N., Ohsawa, T. (1995) Experimental infection of juvenile *Biomphalaria glabrata* with cercariae of *Echinostoma trivolvis*. *Journal of Parasitology*, vol. 81, no. 2, pp. 308–310. https://doi.org/10.2307/3283941

Fried, B., Schmidt, K. A, Sorensen, R. E. (1997) In vivo and ectopic encystment of *Echinostoma revolutum* and chemical excystation of the metacercariae. *Journal of Parasitology*, vol. 83, no. 2, pp. 251–254. https://doi.org/10.2307/3284449

Galaktionov, K. V., Dobrovolskij, A. A. (1987). *Germafroditnoe pokolenie trematod.* Leningrad.: Nauka Publ., 193 p. (In Russian)

Ginetsinskaya, T. A. (1988) *Trematodes, their life cycles, biology and evolution*. Amerind Publishing Private Limited, 559 p.

Haas, W. (2000) The behavioral biology of echinostomes. In: B. Fried, T. K. Graczyk (eds.) *Echinostomes as experimental models for Biological Research*. Dordrecht: Springer, pp. 175–197.

Haseeb, M. A., Eveland, L. K. (2000) Human echinostomiasis: mechanisms of pathogenesis and host resistance. In: B. Fried, T. K. Graczyk (eds.) *Echinostomes as experimental models for Biological Research*. Dordrecht: Springer, pp. 83–98.

Irwin, S. W. B., Fried, B. (1990) Scanning and transmission electron microscopic observations on metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* during in vitro excystation. *Journal of the Helminthological Society of Washington*, vol. 57, pp. 79–83.

Krejci, K.G., Fried, B. (1994) Light and scanning electron microscopic observations of the eggs, daughter rediae, cercariae, and encysted metacercariae of *Echinostoma trivolvis* and *E. caproni*. *Parasitology Research*, vol. 80, no. 1, pp. 42–47. https://doi.org/10.1007/BF00932622

Kuris, A. M., Warren, J. (1980) Echinostome cercarial penetration and metacercarial encystment as mortality factors for a second intermediate host, *Biomphalaria glabrata*. *Journal of Parasitology*, vol. 66, no. 4, pp. 630–635. https://doi.org/10.2307/3280520

Laurie, J. S. (1974) *Himastla quissentensis*: induced in vitro encystment of cercaria and ultrastructure of the cyst. *Experimental Parasitology*, vol. 35, no. 3, pp. 350–362. https://doi.org/10.1016/0014-4894(74)90040-X

McCarthy, A. M., Kanev, I. 1990. *Pseudechinoparyphium echinatum* (Digenea: Echinostomatidae): experimental observations on cercarial specificity toward second intermediate hosts. *Parasitology*, vol. 100, no. 3, pp. 423–428. https://doi.org/10.1017/S0031182000078719

Shigin A. A. (1993) Trematody fauny Rossii i sopredel'nyh regionov. Rod Diplostomum. Moscow: Nauka Publ., 208 p. (In Russian)

Stein, P. C., Bash, P. F. (1977) Metacercarial cyst formation in vitro of *Echinostoma paraensei*. *Journal of Parasitology*, vol. 63, no. 6, pp.1031–1040. https://doi.org/10.2307/3279839

Stunkard, H. W. (1957) The morphology and life history of the digenetic trematode *Microphallus similes*. *Biological Bulletin*, vol. 112, no. 2, pp. 254–266. https://doi.org/10.2307/1539203

Szidat, L. (1924) Beitrage zur Entwicklungsgeschichte der Holostomiden. *Zoologischer Anzeiger*, vol. 61, pp. 249–266.

Timmermann, W. (1936) Zur Biologie von Cercaria C (Szidat) und *Diplostomum volvens* (von Nordmann). Inaugural Dissertation, München. 63 p.

Литература

Анохин, И. А. (1966) Суточный ритм муравьев, инвазированных метацеркариями *Dicrocoelium lanceatum*. *Доклады АН СССР*, т. 166, с. 757–759.

Атаев, Г. Л. (2010) Воздействие метацеркарий *Echinostoma caproni* (Trematoda) на выживаемость моллюсков рода *Biomphalaria* (Pulmonata). *Паразитология*, т. 44, № 6, с. 481–495.

Атаев, Г. Л. (2017) *Размножение партенит трематод: Обзор основных теорий.* СПб.: Наука, 87 с.
Галактионов, К. В., Добровольский, А. А. (1987). *Гермафродитное поколение трематод.* Л.: Наука, 193 с.
Шигин А. А. (1993) Трематоды *фауны России и сопредельных регионов. Род Diplostomum. Мариты.* М.: Наука, 208 с.
Anderson, R. M., Fried, B. (1987) Experimental infection of *Physa heterostropha*, *Helisoma trivolvis* and *Biomphalaria glabrata* (Gastropoda) with *Echinostoma revolutum* (Trematoda) cercariae. *Journal of Parasitology,* vol. 73, pp. 49–54.
Beaver, P. C. (1937) Experimental studies of *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. *Illinois biological monographs*, vol. 15, pp. 7–96.
Chernin, E. (1968) Observations on hearts explanted in vitro from the snail *Australorbis glabratus*. *Journal of Parasitology,* vol. 49, pp. 353–364. https://doi.org/10.2307/3275797
Erasmus, D. A. (1959) The migration of Cercaria X Baylis (Strigeida) within the fish intermediate host. *Parasitology*, vol. 49, no. 1-2, pp. 173–190. https://doi.org/10.1017/S0031182000026810
Esteban, J. G., Muñoz-Antoli, C. (2009) Echinostomes: systematics and life cycles. In: R. Toledo, B. Fried (eds.) *The Biology of Echinostomes. From the Molecule to the Community*. New York: Springer, pp. 1–34.
Evans, N. A., Whitfield, P. J., Dobson, A. P. (1981) Parasite utilization of a host community: the distribution and occurence of metacercarial cysts of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) in seven species of mollusc at Harting Pond, Sussex. *Parasitology*, vol. 83, no. 1, pp. 1–12. https://doi.org/10.1017/S0031182000049982
Evans, N. A., Gordon, D. M. (1983) Experimental observations on the specificity of *Echinoparyphium recurvatum* toward second intermediate hosts. *Zeitschrift für Parasitenkunde*, vol. 69, pp. 217–222. https://doi.org/10.1007/BF00926956
Fried, B., Bennet, M. C. (1979) Studies on encystment of *Echinostoma revolutum* cercariae. *Journal of Parasitology*, vol. 65, no. 1, pp. 38–40. https://doi.org/10.2307/3280199
Fried, B., Idris, N., Ohsawa, T. (1995) Experimental infection of juvenile *Biomphalaria glabrata* with cercariae of *Echinostoma trivolvis*. *Journal of Parasitology*, vol. 81, no. 2, pp. 308–310. https://doi.org/10.2307/3283941
Fried, B., Huffman, J. (1996) The biology of the intestinal trematode *Echinostoma caproni*. *Advances in Parasitology*, vol. 38, pp. 311–368. https://doi.org/10.1016/S0065-308X(08)60037-8
Fried, B., Schmidt, K. A, Sorensen, R. E. (1997) In vivo and ectopic encystment of *Echinostoma revolutum* and chemical excystation of the metacercariae. *Journal of Parasitology*, vol. 83, no. 2, pp. 251–254. https://doi.org/10.2307/3284449
Ginetsinskaya, T. A. (1988) *Trematodes, their life cycles, biology and evolution*. Amerind Publishing Private Limited, 559 p.
Haas, W. (2000) The behavioral biology of echinostomes. In: B. Fried, T. K. Graczyk (eds.) *Echinostomes as experimental models for Biological Research*. Dordrecht: Springer, pp. 175–197.
Haseeb, M. A., Eveland, L. K. (2000) Human echinostomiasis: mechanisms of pathogenesis and host resistance. In: B. Fried, T. K. Graczyk (eds.) *Echinostomes as experimental models for Biological Research*. Dordrecht: Springer, pp. 83–98.
Irwin, S. W. B., Fried, B. (1990) Scanning and transmission electron microscopic observations on metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* during in vitro excystation. *Journal of the Helminthological Society of Washington*, vol. 57, pp. 79–83.
Krejci, K.G., Fried, B. (1994) Light and scanning electron microscopic observations of the eggs, daughter rediae, cercariae, and encysted metacercariae of *Echinostoma trivolvis* and *E. caproni*. *Parasitology Research*, vol. 80, no. 1, pp. 42–47. https://doi.org/10.1007/BF00932622
Kuris, A. M., Warren, J. (1980) Echinostome cercarial penetration and metacercarial encystment as mortality factors for a second intermediate host, *Biomphalaria glabrata*. *Journal of Parasitology*, vol. 66, no. 4, pp. 630–635. https://doi.org/10.2307/3280520
Laurie, J. S. (1974) *Himastla quissentensis*: induced in vitro encystment of cercaria and ultrastructure of the cyst. *Experimental Parasitology*, vol. 35, no. 3, pp. 350–362. https://doi.org/10.1016/0014-4894(74)90040-X
McCarthy, A. M., Kanev, I. 1990. *Pseudechinoparyphium echinatum* (Digenea: Echinostomatidae): experimental observations on cercarial specificity toward second intermediate hosts. *Parasitology*, vol. 100, no. 3, pp. 423–428. https://doi.org/10.1017/S0031182000078719

Stein, P. C., Bash, P. F. (1977) Metacercarial cyst formation in vitro of *Echinostoma paraensei*. *Journal of Parasitology*, vol. 63, no. 6, pp.1031–1040. https://doi.org/10.2307/3279839

Stunkard, H. W. (1957) The morphology and life history of the digenetic trematode *Microphallus similes*. *Biological Bulletin*, vol. 112, no. 2, pp. 254–266. https://doi.org/10.2307/1539203

Szidat, L. (1924) Beitrage zur Entwicklungsgeshichte der Holostomiden. *Zoologischer Anzeiger*, vol. 61, pp. 249–266.

Timmermann, W. (1936) Zur Biologie von Cercaria C (Szidat) und *Diplostomum volvens* (von Nordmann). Inaugural Dissertation, München. 63 p.

For citation: Ataev, G. L. (2023) Experimental study of the effect of *Echinostoma caproni* metacercariae on the survival of *Biomphalaria pfeifferi* molluscs. *Amurian Zoological Journal*, vol. XV, no. 4, pp. 712–723. https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723

Received 30 May 2023; reviewed 20 September 2023; accepted 12 October 2023.

Для цитирования: Атаев, Г. Л. (2023) Экспериментальное изучение влияния метацеркарий *Echinostoma caproni* на выживаемость моллюсков *Biomphalaria pfeifferi*. *Амурский зоологический журнал*, т. XV, № 4, с. 712–723. https://www.doi.org/10.33910/2686-9519-2023-15-4-712-723

Получена 30 мая 2023; прошла рецензирование 20 сентября 2023; принята 12 октября 2023.