

Each coin has 2 sides: a positive role of alien *Potamopyrgus antipodarum* (Grey, 1843) snails in reducing the infection of native lymnaeids with trematodes

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Abstract

The change in the distribution of organisms in freshwater ecosystems due to natural or manmade processes raises the question of the impact of alien species on local communities. Although most studies indicate a negative effect, the positive one is more difficult to discern, especially in multispecies systems, including hosts and parasites. The purpose of the study was to check whether the presence of an alien host, *Potamopyrgus antipodarum*, reduces the intensity of *Echinoparyphium aconiatum* metacercariae in a native host, *Radix* spp. We additionally tested the impact of water temperature and the biomass of the alien host on the dilution effect. We experimentally studied (1) the lifespan of echinostome cercariae in different temperatures, (2) the infectivity of cercariae toward the alien host and native host, and (3) the impact of different biomass of the alien host on the intensity of metacercariae in the native host. We found that cercarial survival and infectivity were temperature dependent. However, cercarial survival decreased with increasing temperature, contrary to cercarial infectivity. Echinostome cercariae entered the renal cavity of both the native host and alien host, and successfully transformed into metacercariae. The number of metacercariae in the native host decreased with the increasing biomass of the alien host. Our results indicate that lymnaeids may benefit from the co-occurrence with *P. antipodarum*, as the presence of additional hosts of different origins may reduce the prevalence of parasites in native communities. However, the scale of the dilution effect depends not only on the increased spectrum of susceptible hosts but also on the other variables of the environment, including water temperature and host density.

Key words: biological invasion, density, dilution effect, echinostomes, *Radix* spp., temperature.

Biological invasions are key drivers of change in biodiversity and community structure (Hatcher and Dunn 2011). They are usually perceived negatively because of threats to native ecosystems (Bellard et al. 2016), public health (Hulme 2014; Schindler et al. 2015), and the economy (Perrings et al. 2002; Lovell et al. 2006). However, the ‘native good, alien bad’ narration seems to be an oversimplification (Goodenough 2010) resulting from insufficient and usually short-term research (Giasu 2016). In recent years, the approach to the role of alien species in the ecosystem has changed. It is even postulated to evaluate organisms by their effect on the environment rather than whether they are natives or aliens (Davis et al. 2011).

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843), is one of the most effective colonizers of inland waters in Europe (Alonso and Castro-Díez 2012). A wide range of tolerance to environmental factors, rapid spread, and high reproductive capacity, in combination with low pressure from natural enemies, have contributed to significant success in invading new areas (Alonso and Castro-Díez 2008). However, the invasive status of *P. antipodarum* is still debatable as it varies geographically and depends on the habitat features and the ability to form high-density populations (Múrria et al. 2008; Romero-Blanco and Alonso 2019). In

favorable habitats, *P. antipodarum* reaches high densities and competes with native macroinvertebrates for environmental resources (Kerans et al. 2005; Larson and Ross Black 2016). The complexity of interactions between alien and native snails becomes even more complicated if we include parasites in the analysis.

Alien hosts may lose their parasites during the initial stages of invasion, a hypothesis known as the enemy release (Torchin et al. 2003; Heger and Jeschke 2014). Usually, this phenomenon is related to bottleneck effects, selective pressures during introduction and establishment, and the type of parasite transmission—vertical or horizontal (Hatcher and Dunn 2011). Alien hosts may also introduce their parasites into native communities (Blackburn and Ewen 2017) or acquire new parasites from local hosts (Cichy et al. 2017). These phenomena usually alter the dynamics of host–parasite systems, apart from the impact of multifactorial habitat variables. The parasite acquisition may result in spillback when alien hosts act as reservoirs for the infection (Kelly et al. 2009) or may result in dilution, leading to a decrease in prevalence in main host communities (Johnson and Thieltges 2010). The term ‘dilution effect’ is derived from disease ecology and was initially referred to as vector-borne pathogens or protozoan parasites transmitted to humans (Schmidt and Ostfeld 2001).

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It implies that an increase in biodiversity may promote lower prevalence in hosts differing in susceptibility to infection (Khalil et al. 2016). However, there is fairly limited evidence of a dilution effect between metazoan parasites, such as trematodes and native-alien snails. Moreover, determinants of parasite transmission to alien hosts remain poorly understood (Paterson et al. 2012).

Echinoparyphium aconiatum Dietz, 1909 (syn. *Pseudoechinoparyphium echinatum*) is a widespread echinostome with a complex 3-host life cycle (Pantoja et al. 2021). It includes a broad range of waterfowl as definitive hosts (Huffman and Fried 2012). The great pond snail *Lymnaea stagnalis* (L.) is an obligatory first intermediate host for cercariae—larval stages developing inside the hepatopancreas (Faltýnková et al. 2007). Fully formed cercariae released from snails into the water start looking for the next host, where they transform into metacercariae. The spectrum of second intermediate hosts for metacercariae-encysted larvae infective for final hosts is diverse (Keeler and Huffman 2008). It includes other mollusks, amphibians, and fishes (Yurlova et al. 2006). Free-living stages of these parasites, for example, miracidia and cercariae penetrating the first and second intermediate hosts, respectively, are influenced by a wide range of biotic (Thieltges et al. 2008) and abiotic (Pietrock and Marcogliese 2003) factors limiting or enhancing the transmission success. For instance, water temperature affects cercariae's emergence, survival, and infectivity (Poulin 2006; Thieltges and Rick 2006). It also influences the hosts' biology, including growth, reproduction, and mortality (Kalinda et al. 2017). Specific snail–host stimuli in the water, usually macromolecular mucus compounds, stimulate miracidia to find, recognize, and penetrate the specific first intermediate host (Haas et al. 1995). On the other hand, chemical cues in the water may also affect predator–prey interactions between host and nonhost organisms, altering host–parasite dynamics (Ferrari et al. 2010). The examples above show that the transmission of parasites to hosts should be regarded as multifactorial, which is easier to investigate under controlled experimental conditions than in nature.

Despite the relatively well-known interaction between snails and echinostomes at the individual level (Toledo and Fried 2009), there is little data on the ecological impact of introducing alien species that are real and potential hosts of these parasites. This study aimed to test whether the co-occurrence of *P. antipodarum* with native lymnaeids reduces the intensity of echinostome metacercariae in native hosts and, consequently, causes the dilution effect in experimental conditions. We hypothesize that the intensity of echinostome metacercariae in lymnaeids will decrease with the increasing biomass of *P. antipodarum* and at lower temperatures. Specifically, we conducted laboratory studies using native hosts *Radix* spp., *E. aconiatum* cercariae, and alien hosts *P. antipodarum* as a research model to test the above hypothesis. Both the native host and alien host co-occur in the littoral zone of lakes (Schmidlin et al. 2012; Lewin et al. 2015) and play the role of second intermediate hosts for *E. aconiatum* metacercariae (McCarthy and Kanev 1990; Żbikowski and Żbikowska 2009). Although our research focuses on the effect of nonnative hosts on parasite transmission, we included temperature as an additional variable due to its significant impact on free-living stages of trematodes, for example, cercariae used in experiments (Leicht and Seppälä 2014). We predict that the survival of *E. aconiatum* cercariae will

decrease with the increasing temperature and the duration of the experiment, as cercarial survival is often temperature dependent (Pechenik and Fried 1995; McCarthy 1999). We expect *E. aconiatum* cercariae to penetrate both the native host and alien host due to the lack of species specificity during the chemo-orientation of cercariae toward their second intermediate hosts (Körner and Haas 1998). If echinostome cercariae penetrate both *Radix* spp. and *P. antipodarum*, we expect a dilution effect between the native host and alien host.

Materials and Methods

Snails and trematodes

We gathered *Radix* spp. from the Lake Łabędź (N 53°36'46.1", E 19°36'48.7") (Figure 1) in April and May 2019. Lymnaeids were collected from the macrophytes (depth of ca. 0.5 m) by hand and using a metal sieve. We caught only small individuals measuring <10 mm to minimize the risk of natural infection with trematodes as small, and presumably, juvenile snails are less likely to be naturally infected with parasites (Graham 2003). Additionally, snails were checked for parasites following the method of cercariae shedding. For this purpose, each *Radix* spp. individual was placed in a 30-mL glass beaker with tap water and exposed to a light source (lamps) for 2 h. Snails without patent invasion (shedding cercariae) were used for further experiments. After the experiments, we additionally verified the lack of natural infection by necropsy. In Poland, there are 4 closely related species from the *Radix* genus, namely *R. balthica* (L., 1758), *R. auricularia* (L., 1758), *R. ampla* (Hartmann, 1821), and *R. labiata* (Rossmässler, 1835) (Piechocki and Wawrzyniak-Wydrowska 2016). Only the first 2 species are common in the Polish lowlands and have the characteristics of shells corresponding to the collected snails. However, due to the high similarity of juveniles representing these 2 species (Huňová et al. 2012), the collected snails were designated *Radix* spp.

Potamopyrgus antipodarum specimens were collected in May and June 2019 from Lake Sosno (Figure 1) (N

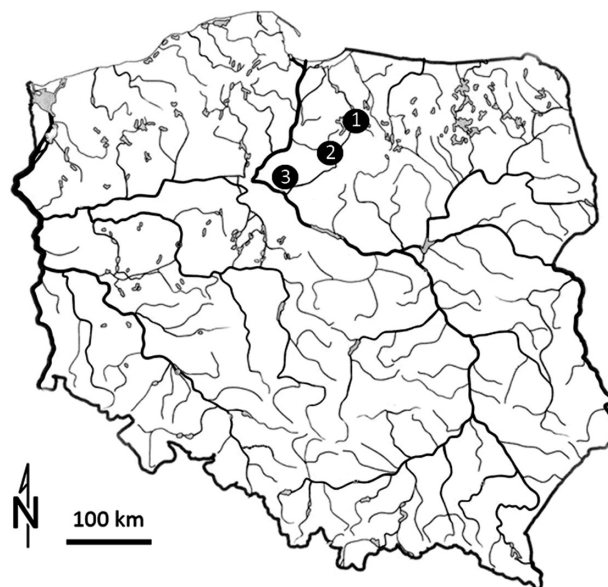


Figure 1 Study sites: Łabędź Lake (1), Sosno Lake (2), Kozielec Lake (3).

53°20'16.2", E 19°21'03.6"), which is characterized by the very low prevalence of trematodes in populations of this snail species (Cichy et al. 2017). The New Zealand mud snails were sampled from the lake's bottom (depth of ca. 0.5 m) using a benthic sieve KC-Denmark with a mesh size of 1,000 µm.

We obtained the cercariae of *E. aconiatum* from naturally infected *L. stagnalis* snails from Lake Koźielec (N 53°07'40.8", E 18°29'50.1") (Figure 1). The great pond snails were collected in the middle of summer (July and August 2019) as the peak of the prevalence of this trematode species is usually observed in snails during this period. In the laboratory, each individual of *L. stagnalis* was placed in a 50-mL glass beaker filled with tap water and exposed to a light source (lamps) for 2 h to stimulate cercariae shedding. The cercariae of *E. aconiatum* were recognized based on their morphological features (Faltýnková et al. 2007; Cichy and Żbikowska 2016a). Each snail species was kept separately in aquariums with a capacity of 15 L and a water temperature between 18 and 20 °C. Macerated lettuce and chlorella were food for snails, and the water was changed once a week. Two days before experiments, *Radix* spp. and *P. antipodarum* individuals were divided into 4 groups. Each group was placed at the appropriate temperature of 4, 10, 20, or 30 °C in Sanyo MIR 253 incubators to acclimate them to cold or warm temperatures.

Experimental design

Laboratory research consisted of a series of 3 experiments. All experiments were carried out in Petri dishes with a diameter of 50 mm and a height of 12 mm filled with 5 mL of dechlorinated tap water previously kept at the appropriate temperature for 2 h, depending on the thermal variant designated for experiments (4, 10, 20, and 30 °C). These thermal variants correspond to water temperatures recorded in the littoral zone of lakes of the temperate zone (Choiński 2006).

The first experiment aimed to study the lifespan of *E. aconiatum* cercariae in selected thermal variants (4, 10, 20, and 30 °C). We prepared 4 groups of cercariae. Each group of 6 cercariae of *E. aconiatum* freshly emerged from *L. stagnalis* (<1 h old) was placed in a Petri dish and then in Sanyo MIR 253 incubators at the set temperature. The number of living cercariae was determined at intervals of 0.5, 1, 2, 3, 6, 12, and 24 h. Further readings were carried out every 24 h until the death of the cercariae. We determined cercarial survival at a given time point based on their movement observed under a stereoscopic microscope Motic K-700. The experiment was performed in 10 repetitions for each thermal variant.

The second experiment was performed to check whether (1) *E. aconiatum* cercariae can infect both the native host and alien host, and (2) whether the rise in ambient temperature affects the success of the invasion (e.g., the infectivity of cercariae is defined as the ability to penetrate the host and transform into living metacercariae). For this purpose, 1 individual of *Radix* spp. was placed in the Petri dish with 5 cercariae of *E. aconiatum* freshly emerging from *L. stagnalis*. We stained experimental cercariae for 10 min, using 5 drops of 0.5% Neutral Red Chempur dye per 50 mL of water in which *E. aconiatum* cercariae were present. It allowed us to distinguish metacercariae that could potentially be present in hosts due to natural infection from lakes and experimental metacercariae. Petri dishes with snails and parasites were placed for 48 h in Sanyo MIR 253 incubators at 4, 10, 20, and 30

°C, respectively. The same procedure was used for *P. antipodarum*. The experiment was performed in 25 repetitions for each thermal variant and each snail species. Petri dishes were replenished with water at the appropriate temperature depending on its loss during the experiment, especially at the highest temperatures. After the experiment, we counted metacercariae by squashing whole snails (*P. antipodarum*) or soft tissues of *Radix* spp. between 2 slides.

In the last experiment, *Radix* spp. individuals were infected with stained cercariae of *E. aconiatum* in the presence of *P. antipodarum* snails. Due to the different body sizes of both species of snails, we selected the biomass of snails so that the number of *P. antipodarum* specimens corresponded in terms of biomass to 1 specimen of *Radix* spp. Therefore, (1) 5 echinostome cercariae, (2) 1 individual of *Radix* spp., which was previously wiped with a paper towel and weighed using an analytical balance AS 110/X, and (3) the number of *P. antipodarum* individuals corresponding to 3 variants of the lymnaeid wet weight, were placed in the Petri dish. The total wet weight (g) of *P. antipodarum* individuals was (1) half of the wet weight of 1 *Radix* spp. individual (biomass variant 1, alien host 0.033 ± 0.003 , native host 0.058 ± 0.005), (2) was equal to the wet weight of 1 *Radix* spp. individual (biomass variant 2, alien host 0.049 ± 0.005 , native host 0.051 ± 0.005), and finally, (3) was 1.5 times the wet weight of 1 *Radix* spp. individual (biomass variant 3, alien host 0.058 ± 0.006 , native host 0.037 ± 0.004). We used 347 individuals of *P. antipodarum* (mean number of *P. antipodarum* per 1 Petri dish for all thermal variants was 6 ± 0.1 individuals) in the "biomass variant 1", 504 individuals of *P. antipodarum* (8 ± 0.1 individuals) in the "biomass variant 2", and 629 individuals of *P. antipodarum* (10 ± 0.2 individuals) in the "biomass variant 3". The experiment was performed in 3 repetitions for each thermal and biomass variant; each repetition consisted of the 5 experimental sets described above. The control group was *Radix* spp. (native host 0.061 ± 0.004) placed in the Petri dish with 5 cercariae of *E. aconiatum* without *P. antipodarum*. Necropsy to check for the presence of parasites was performed only for *Radix* spp.

Statistical analysis

The mean lifespan of echinostome cercariae was calculated for each Petri dish per day. Therefore, 3, 6, 12, and 24 h were treated as 0.125, 0.25, 0.5 part of the day, and 1.0 day, respectively. A 1-way ANOVA was used to check the differences in larvae lifespan in particular thermal variants. The lifespan of the larvae was considered to be the last time variant in which the larva was still alive. A Student's *t*-test was conducted to compare the ability of *E. aconiatum* cercariae to penetrate both the native host and alien host, taking into account the sums of recorded metacercariae in particular thermal variants in *Radix* spp. and *P. antipodarum*. A 1-way ANOVA was used to study the effect of temperature on the ability of *E. aconiatum* cercariae to settle in both the native host and alien host. A 2-way ANOVA was used to investigate echinostome cercariae's ability to infect *Radix* spp. snails in the presence of different biomass variants of *P. antipodarum* (factor 1) and different temperature variants (factor 2). Here, the sums of each 5 experimental sets were used for the analyses. We used Tukey's post hoc test for all ANOVAs. We used the term "prevalence" as the proportion of snails infected over the total number of snails, and the term "intensity" as the number of metacercariae per snail.

Results

Experiment 1

Cercarial survival was temperature dependent (1-way ANOVA, $F_{3,36} = 204.2$, $P < 0.001$) (Figure 2) and decreased with the increasing temperature. The differences were observed between all the thermal variants used ($P < 0.02$). Microscopic observations demonstrated that the extremely low motility of *E. aconiatum* cercariae in the lowest thermal variant was strongly limited to weak tail movement and infrequent stretching of the front of the body at the bottom of the Petri dish.

Experiment 2

There were no significant differences between the ability of *E. aconiatum* cercariae to enter both the native host and alien host, and transform into metacercariae ($t = 0.46$, $df = 6$, $P = 0.66$). One-way ANOVA showed that there was a difference in the number of metacercariae formed in these hosts in different thermal variants ($F_{3,96} = 30.21$, $P < 0.001$ and $F_{3,96} = 31.96$, $P < 0.001$, for the native host and alien host, respectively) (Figure 3). The number of metacercariae increased with temperature. Significant differences ($P < 0.05$) occurred between all tested thermal variants, except for the following: 10×20 °C for the native host ($P = 0.2$) and 20×30 °C for the alien host ($P = 0.2$). The necropsy after the experiments showed no natural infection with trematode species in experimental snails.

Experiment 3

We found significant differences in the ability of *E. aconiatum* cercariae to penetrate the native host at different biomass variants of alien host (2-way ANOVA, $F_{3,32} = 18.76$, $P < 0.001$). The number of echinostome metacercariae in *Radix* spp. decreased with an increase in the biomass variants of *P. antipodarum* (Figure 4). Statistically significant differences occurred between biomass variants 1 and 3 and between the control group and all biomass variants ($P < 0.05$). Such

differences were not recorded between the biomass variants 1 and 2 ($P = 0.4$) as well as 2 and 3 ($P = 0.4$). The analysis also confirmed a statistically significant influence of temperature on the incidence of *E. aconiatum* cercariae in penetrating hosts ($F_{3,32} = 13.34$, $P < 0.001$). The post hoc test showed no significant differences between the thermal variant 20 versus 10 °C ($P = 0.4$) and 20 versus 30 °C ($P = 0.3$). It also showed no interaction between the tested factors, that is, temperature and biomass of the alien host ($F_{9,47} = 1.126$, $P = 0.37$). The necropsy after the experiments showed no natural infection with trematode species in experimental *Radix* spp.

Discussion

Our research demonstrated that a dilution effect occurs between the native host and alien host in experimental conditions, as *P. antipodarum* reduce the number of echinostome metacercariae in native *Radix* spp. The dilution effect observed between the second intermediate hosts may occur by several mechanisms. According to Civitello et al. (2015), these include interspecies interactions between hosts and factors affecting parasite transmission. In our experiments, the impact of *P. antipodarum* on the *Radix* spp. was quite challenging to observe due to the small number of specimens used. However, in nature, the relative competence of those hosts might be a possible mechanism underlying the dilution effect (Huang et al. 2013). A high abundance of the alien host might lead to a reduction in the native host that would counteract “dilution” caused by the alien host. Although *Radix* spp. and *P. antipodarum* co-occur in nature (Lewin et al. 2015), they are not very strong competitors. For instance, they have different food requirements and respiration methods (Dillon, 2000). Therefore, we assume that the dilution effect between the native host and alien host was most likely associated with the echinostome cercariae transmission. The relatively low specificity of these larval stages toward the second intermediate hosts (McCarthy and Kanev 1990) could affect this phenomenon. We found no differences in the ability of *E.*

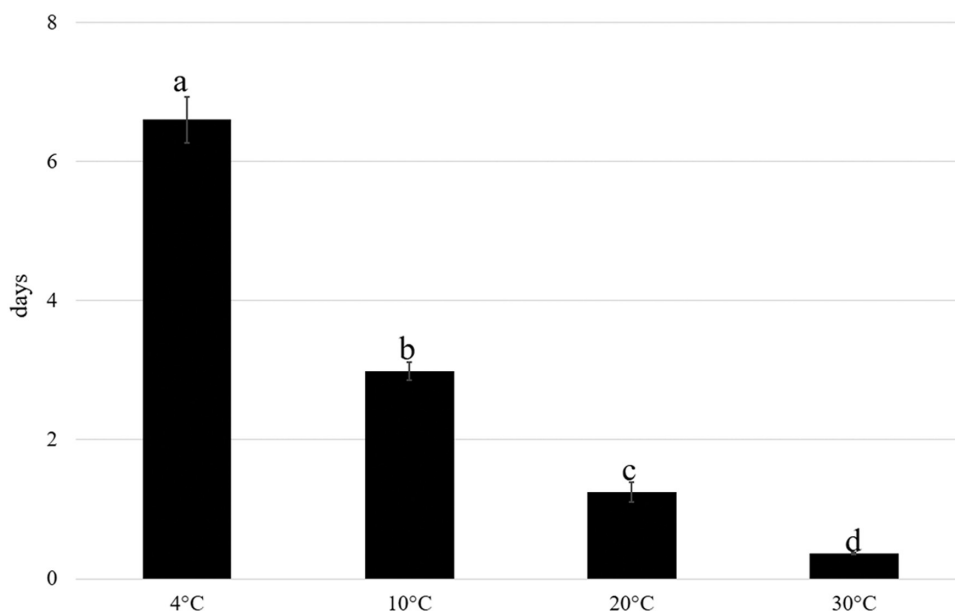


Figure 2 The mean survival ($\pm SE$) of *Echinoparyphium aconiatum* cercariae in different thermal variants (repetitions: $n = 10$, cercariae per repetition: $n = 6$). The different letters (a–d) label statistically significant differences (Tukey test, $P < 0.02$).

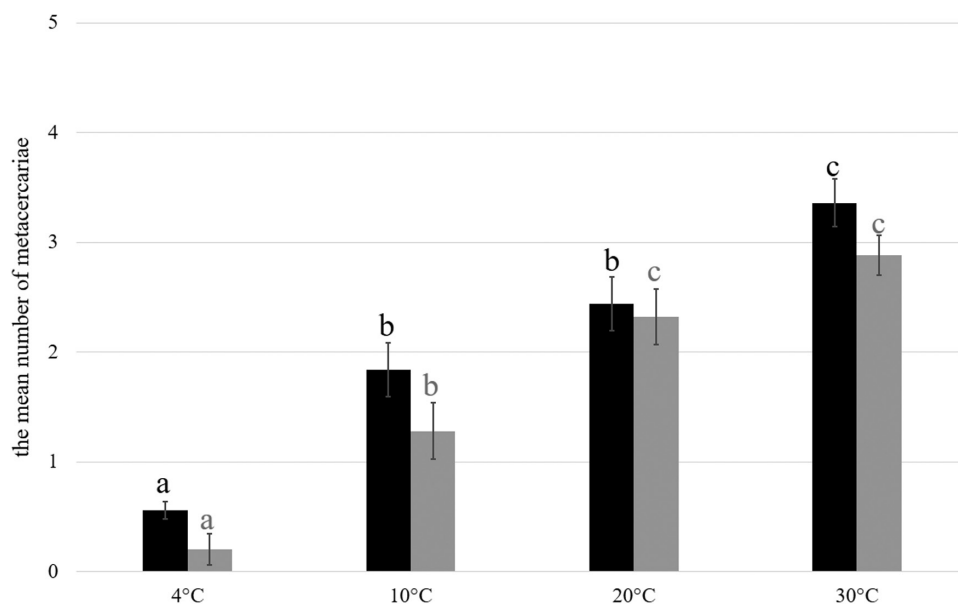


Figure 3 The mean number (\pm SE) of *Echinoparyphium aconiatum* metacercariae in the native host (black bars) and alien host (gray bars) in different thermal variants (repetitions: $n = 25$, cercariae per repetition: $n = 5$). The different black and gray letters (a–c) label statistically significant differences (Tukey test, $P < 0.05$) between different thermal variants for native host and alien host, respectively.

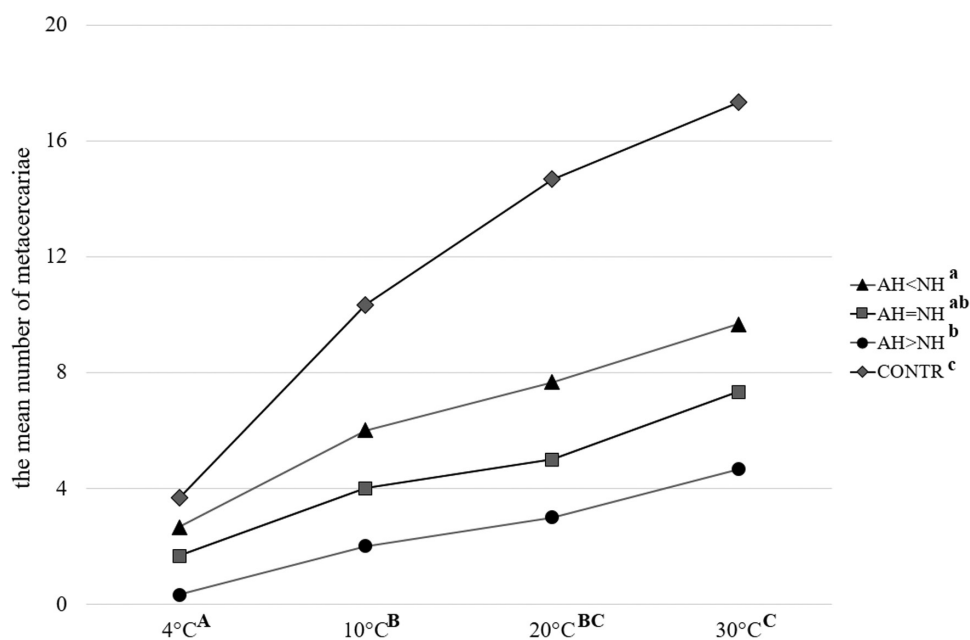


Figure 4 The mean number of *Echinoparyphium aconiatum* metacercariae in the native host in relation to different alien host biomass and thermal variants (repetitions: $n = 3$, cercariae per repetition: $n = 25$). The different lowercase (a–c) and uppercase (A–C) label statistically significant differences (Tukey test, $P < 0.05$) between different biomass and thermal variants, respectively.

aconiatum cercariae to penetrate the native host and alien host, suggesting that transmission of parasites to both host species and formation of metacercarial stages occur with similar probabilities. It is consistent with the observations of Körner and Haas (1998) that echinostome cercariae are generalists with low specificity toward the second intermediate hosts. Contrary, the host specificity of miracidia is very high. These larval stages are specialists searching for the specific molluscan host (Kalbe et al. 2000). Therefore, the dilution effect in the case of parasites with complex life cycles should be considered for specific developmental stages that may react

differently to host stimuli (Zimmermann et al. 2016). For instance, Larson et al. (2020) found no evidence for the dilution effect in experiments using *P. antipodarum* and native snails as the first intermediate hosts for highly specific miracidia. Contrary, we observed dilution when using this alien snail and native *Radix* lymnaeids as the second intermediate hosts for less-specific echinostome cercariae. A similar observation was made in our field study using other host–parasite systems involving *Dreissena polymorpha* (Pallas, 1771), an alien zebra mussel fouling the shell of native unionids (Marszewska and Cichy 2015). Although the total infection

prevalence of both alien and native hosts was similar, *D. polymorpha* had significantly more echinostome metacercariae. Consequently, the presence of this alien host on the shell of the unionids reduces the chance of transmission of echinostome cercariae to native host, promoting the dilution effect.

The temperature and the increase in alien host biomass are the proposed mechanism for the dilution effect in the studied host–parasite system. Our result showed that cercarial survival is temperature dependent and decreases with the increasing temperature. It is consistent with the predictions assuming faster consumption of energy reserves by these developmental stages in conditions of elevated water temperature (Morley 2011; Morley and Lewis 2015). While high temperature reduces cercarial survival, thus limiting the transmission to the second intermediate host (both the native host and alien host), low temperature reduces cercarial infectivity and may restrict the dilution effect. In our research, echinostome cercariae in the lowest thermal variant were weakly active and lay at the bottom of the Petri dish. It is highly probable that in the autumn–winter period, during which a decline in the prevalence of mollusks is observed (Esch and Fernandez 1994), cercariae having low motility sink to the bottom of the water reservoir and, as a component of zooplankton, passively enters the food chain of benthic hosts (Mironova et al. 2019; Stanicka et al. 2021). It can lead to removing transmission stages from the environment and reducing the prevalence of native host. Although these assumptions are based solely on observations of the cercariae behavior and do not constitute direct evidence, they may indicate that additional hosts and/or consumers (greater biodiversity) favor the dilution effect. For instance, Cichy and Żbikowska (2016a, 2016b) found that metacercariae of *Diplodiscus subclavatus* (Pallas, 1760) entered the nonhost organism, the benthic snail *Viviparus contectus* (Millet, 1813), via a passive route. The highest prevalence of pre-adults in snails was recorded in the cooler months (early autumn). It was correlated with the preceding peak of cercariae shedding by planorbid snails—the first intermediate hosts in this life cycle. Although both examples refer to different trematode forms, metacercariae of *D. subclavatus* in the environment and pre-adults in the nonhost organism versus cercariae of *E. aconiatum* in the environment and metacercariae in nonhost organisms, the mechanisms are similar. Our study also supported the hypothesis that the dilution effect is biomass dependent (Johnson and Thielges 2010). We observed a decrease in the number of metacercariae in the native host with an increase in the biomass of the alien host. Therefore, we assume that the high density of *P. antipodarum* communities observed frequently in the environment (Kerans et al. 2005) may constitute a protective shield for native species of snails against the invasion of echinostome cercariae. Such a positive effect is rarely highlighted in the literature as most studies are based on 2, rather than multispecies systems.

Although our experimental studies showed that the alien host, *P. antipodarum*, reduces the number of echinostome metacercariae in native *Radix* spp., the magnitude of this phenomenon in nature and the further fate of *E. aconiatum* metacercariae may be more complex. We found living metacercariae in the alien host and native host, indicating that *P. antipodarum* is an “additional” rather than a “dead-end” host for these digenean stages. Some authors suggest that due to the relatively hard shell of *P. antipodarum*, it is passed through the digestive system of some vertebrates, for

example, fishes (Butkus and Rakauskas 2020), but presumably not through the digestive tract of birds, which are final hosts (van Leeuwen et al. 2012). The authors found only empty or broken pieces of *P. antipodarum* shells in the digestive tract of birds, suggesting that potentially infected snails were digested. Moreover, some species of trematodes, such as metacercariae of *Microphallus* spp., can develop into adults in ducks after the digestion of infected *P. antipodarum* snails (Levri and Lively 1996). Although *P. antipodarum* may cause lower prevalence in native, second intermediate hosts such as *Radix* snails, thus promoting the dilution effect, it may probably support the amplification effect at the level of final hosts. For instance, Evans et al. (1981) and McCarthy (1999) found that metacercariae of *Echinoparyphium recurvatum* originating from different species of the second intermediate hosts were able to establish and transform into adult forms inside ducks. If both species of mollusks from our study are equally competent as hosts, then the increased infection in *P. antipodarum* snails may lead to more infection in the definitive host. Consequently, greater inputs of miracidia might counteract any short-term benefit of reduced infection in native *Radix* spp. hosts. However, at the moment, research on the contribution of *P. antipodarum* to the birds’ diet is limited (Ponyi 1994), and further studies should answer the question about the fate of *E. aconiatum* derived from *P. antipodarum* snails.

The parasitological consequences of biological invasions are still poorly understood as parasites remain a neglected component of ecological research (Gómez and Nichols 2013). Considering the dilution effect in snail–trematode systems, ecological (biodiversity of potential hosts), biological (temperature-dependent life-history traits of both the hosts and parasites such as survival and infectivity), and evolutionary (specificity of transmission stages) features should be included. Understanding the generality of the dilution effect and checking whether it occurs in various host–parasite systems can shed new light on the risk and dynamics of emerging diseases during progressive climate change and biological invasions.

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Author Contributions

AC: Conceptualization, developing methods, conducting the research, data interpretation, and writing the original draft. AS, AC, and EŻ: Data analysis. AS, EŻ: Commenting and editing drafts of the manuscript

Data Availability

Data are available from the authors on reasonable request.

Conflict of Interest Statement

All authors declare no conflicts of interest.

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