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Thinking "outside the box": The effect of nontarget snails in the aquatic community on mollusc-borne diseases

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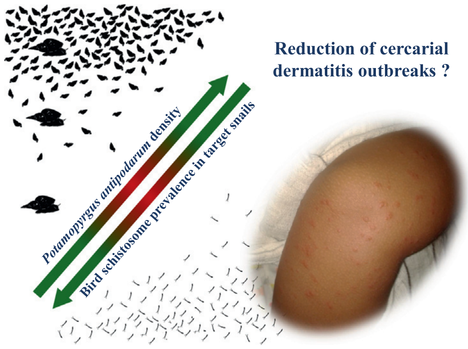
HIGHLIGHTS

- The presence of nontarget snails interferes with some digenean life cycles.
- Nontarget snails are likely to be a tool in "outside the box" strategies for parasite control.
- A comprehensive approach to overall ecosystem complexity is needed from a research perspective.

GRAPHICAL ABSTRACT



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ABSTRACT

There is a great need to understand the impact of complex communities on the free-living parasite stages that are part of them. This task becomes more complex as nonnative species emerge, changing existing relationships and shaping new interactions in the community. A relevant question would be: Can the coexistence of nontarget snails with the target hosts contribute to trematodasis control? We used field and experimental approaches to investigate nonnative competitor-induced parasite dilution. During a three-year field study, we investigated digenean infection in *Lymnaea stagnalis* from eight Polish lakes inhabited or uninhabited by *Potamopyrgus antipodarum*. Additionally, we verified the presence of digenean infections in the populations of *P. antipodarum*. Moreover, we conducted an experimental infection of *L. stagnalis* with miracidia of *Trichobilharzia szidati* under increasing densities of *P. antipodarum* and aimed to infect *P. antipodarum* with them separately. The prevalence of avian schistosomes in lymnaeid snails was significantly higher in uninhabited lakes than in lakes inhabited by *P. antipodarum*. Our study indicates that waters with a higher density of invaders have a lower prevalence of avian schistosomes in lymnaeid hosts. The results of experimental studies confirmed that the presence of high densities of *P. antipodarum* reduces the probability of target host infection. Both field and experimental studies rule out the role of *P. antipodarum* as a source of avian schistosome cercariae. Here, a nonnative species was tested as a diluter, which in practice may be harmful to the local environment. This work is not a call for the introduction of nonnative species; it is intended to be a stimulus for researchers to continue searching for natural enemies of parasites because, as our results show, they exist. Finding natural enemies to the most dangerous species of human and animal parasites that will pose no threat to the local environment could be groundbreaking.

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1. Introduction

Changes in biodiversity are one of the most pressing environmental problems of our time (Pereira et al., 2012; Rohr et al., 2020). The introduction of invasive species is considered among the drivers of declines and alterations in biodiversity (McGeoch et al., 2010). The possible impact of these processes on human and wildlife infectious diseases has generated intense debate (Rohr et al., 2020; Keesing and Ostfeld, 2021). Positive associations between nonnative host invasions and the incidence of zoonotic diseases have long been of concern (Zhang et al., 2022). For example, introduced species may modify host-parasite relationships in various ways (Dunn et al., 2012; Telfer and Bown, 2012). First, investigations focus on parasite spillover and cointroducing parasites with a nonnative host, which can spread to native species (Gozlan et al., 2005; Ortega et al., 2021; Santicchia et al., 2020). Second, there are many reports of parasite spillback and the possibility of using an introduced species as an alternative host for native and/or previously established parasites (Carolus et al., 2019; Schols et al., 2021). Nevertheless, nonnative species can also lead to a reduction in the spread of parasitic diseases (McIntire et al., 2021; Stanicka et al., 2021; Westby et al., 2019). They may reduce the abundance and richness of native species, e.g., through competition, predation or hybridization (Blackburn et al., 2004; Casas et al., 2004; Johnson et al., 2009; Kindinger and Albins, 2017). Nevertheless, such biotic homogenization could eliminate the transmission of some local infectious diseases (Pointier et al., 2011). Moreover, if nonnative species are not suitable hosts (parasites are unable to establish themselves in it or cannot infect it), they can also disrupt the transmission of parasite free-living stages by reducing their encounters with the target host (preferred by the parasite) in different ways, creating the so-called dilution effect (Johnson and Thieltges, 2010). For instance, nonhost species may prey on free-living larvae of parasites and consequently reduce the density of these infectious stages in the environment (Rohr et al., 2015; Born-Torrijos et al., 2021; Hobart et al., 2021; Stanicka et al., 2021). Additionally, nonhost species may constitute a physical barrier in the search for a host (Christensen, 1979). If they become infected, parasite larvae may be damaged in nonhost organisms (losing infectivity) (Gendron and Marcogliese, 2017) or killed by their immune system (Gendron and Marcogliese, 2016). However, Hopper et al. (2008) proved that not all nonhost species have the same effect on dilution.

Mollusc-borne diseases pose important public health issues worldwide. Molluscs serve as transmitting vectors of parasitic diseases that lead to cancers, infertility, organ failure, and even death. Millions of people struggle with these problems, especially in developing countries in Africa, Asia and Latin America. However, due to the global spread of molluscs, it can be expected that some endemic parasitic diseases may develop into worldwide epidemics (Lu et al., 2018). Among mollusc-borne human diseases, schistosomiasis is considered the most important. It is estimated that over 200 million people are infected in tropical and subtropical countries, and 800 million are at risk of being exposed (Hotez et al., 2014). In addition to human schistosomes, people can be attacked by avian schistosomes causing cercarial dermatitis worldwide. Avian schistosomes have two free-living larval stages in the life cycle, i.e., miracidia hatching from eggs and then penetrating intermediate hosts (gastropods), and cercariae actively emerging from intermediate hosts in search of definitive hosts. The similarity of skin-surface lipids of waterfowl (target host) and mammals can contribute to the fact that their cercariae penetrate human skin (Haas and van de Roemer, 1998). As a consequence, an inflammatory skin reaction, commonly known as cercarial dermatitis or swimmer's itch, occurs. Cercarial dermatitis is recognized as a neglected emerging disease that requires immediate attention, including the development of protective measures, due to its direct and indirect impacts on humans (Horák et al., 2015). Every year, there are new reports of cercarial dermatitis outbreaks from bathing areas after the summer season (Loker et al., 2022).

The elimination of the first intermediate host (the source of cercariae) from water bodies is considered to be the basal method of the control for most digenean infections (Bullard and Overstreet, 2008; Sokolow et al., 2018). This solution may be implemented in various ways, for instance, using molluscicides, modification of the habitat of molluscs, manual or mechanical removal of molluscs from bodies of water and “outside the box” strategies such as repellents, traps and natural enemies (Sokolow et al., 2018). However, there are concerns that the above treatments may be temporary and insufficient (Bullard and Overstreet, 2008; Sokolow et al., 2018). As a result, the potential correlation between alterations in biodiversity and disruption of parasite transmission to the host is under debate (Rohr et al., 2020; Keesing and Ostfeld, 2021).

The New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae), which has been recognized as the world's most widespread aquatic invader, occurs on five continents (Geist et al., 2022) and may constitute an interesting research model for the analysis of host-parasite interactions (Marszewska et al., 2020). Flexible feeding abilities provide *P. antipodarum* with access to multiple food resources, which gives them a competitive advantage over native species (Geist et al., 2022). However, native molluscs coexist with them in invaded areas (Żbikowska et al., 2021).

An interesting question is whether the coexistence of nontarget snails with target hosts contributes to trematodasis control. In this report, we aimed to address this question by conducting research on a system employing avian schistosomes, *L. stagnalis* and *P. antipodarum* as research models of parasite larvae, suitable hosts, and nonnative species. The following four tasks were carried out: i) a three-year field study on avian schistosome prevalence (against the background of other digenean infections) in *L. stagnalis* from water bodies coinhabited and uninhabited by *P. antipodarum*, ii) a search for avian schistosome larvae in *P. antipodarum* originating from Polish lakes, iii) experimental infection of *L. stagnalis* with miracidia of *T. szidati* in the presence of increasing densities of *P. antipodarum*, and iv) infection of *P. antipodarum* with miracidia of *T. szidati* under laboratory conditions. We predicted that the prevalence of avian schistosomes in the target intermediate hosts (*L. stagnalis*) would be lower in lakes with *P. antipodarum*. We also expected that the experimental transmission of miracidia of *T. szidati* to the target host would be disturbed by *P. antipodarum* and that *P. antipodarum* would not be a suitable host of avian schistosomes belonging to the genus *Trichobilharzia*. Consequently, we hypothesized that invaders can be considered a relevant player in “outside the box” strategies to control possible digenean epidemics and that monitoring the composition and abundance of aquatic ecosystem inhabitants is an important element in predicting epidemic size and spread.

2. Materials and methods

2.1. Research area and snail sampling

Based on preliminary observations conducted in 2017, four lakes inhabited by *L. stagnalis* together with *P. antipodarum* and four lakes without *P. antipodarum* in two parts of Poland were selected for the presented field research (Fig. 1, Table A.1).

To exclude the influence of basic abiotic factors, the values of the physical and chemical parameters of water (conductivity, pH) were measured in July each year with the core sampler and a MultiLine P4 (WTW) Universal Pocket Sized Metre (Weilheim, Germany).

To obtain the highest possible representativeness and repeatability of the results, field studies were conducted for three growing seasons in 2018, 2019 and 2020. Mollusc samples were collected once a month from May to September from each lake.

For parasitological diagnostics, a single sample of *L. stagnalis* was counted from 30 to 40 specimens (shell length > 4.0 cm), while a single sample of *P. antipodarum* consisted of 200 specimens (shell length > 0.3 cm). Lymnaeid gastropods were gathered manually from the macrophytes, lake bottom, stones, bridges and sunken wood along the accessible lake shoreline to a depth of approximately 1.0 m. *Potamopyrgus antipodarum* was always collected from the same sampling site as *L. stagnalis*; for this purpose, sand was removed from the bottom of the bodies of water with a spatula and then sieved through a hydrobiological sieve with a mesh diameter of 3 mm. Additionally, specimens of *P. antipodarum* were also manually gathered from macrophytes.

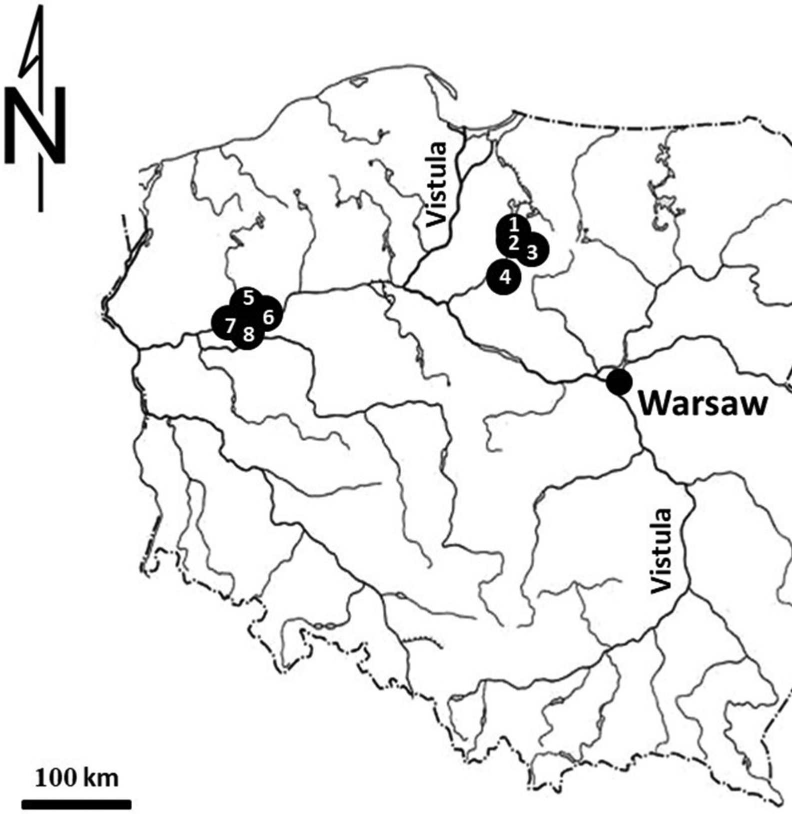

Fig. 1. Study sites in Poland, Central Europe: 1 – Lake Szymbarskie, 2 – Lake Slim, 3 – Lake Iławskie, 4 – Lake Sosno, 5 – Lake Drawsko, 6 – Lake Czaplino, 7 – Lake Siecino, and 8 – Lake Kaleńskie.

2.2. Parasitological examination of snails

The collected snails were identified as *L. stagnalis* or *P. antipodarum* based on morphological descriptions (Piechocki and Wawrzyniak-Wydrowska, 2016). All collected gastropods were subjected to autopsy. To search for the developmental stages of digenean trematodes (sporocysts, rediae and cercariae), a microscopic investigation of the hepatopancreas and gonads of lymnaeid snails and the whole body of *P. antipodarum* was performed. Morphological identification of digenean trematodes was based on fully developed cercariae (Cichy and Żbikowska, 2016) using a light microscope (Axio Lab. A1, Carl Zeiss). Due to the cryptic species diversity of digenean larvae (Kudlai et al., 2021), cercariae were identified at the genus level. Only the larvae of the genus *Echinoparyphium* have been distinguished as *E. recurvatum* and *E. aconiatum*. In our study, the term “prevalence” means the proportion of *L. stagnalis* individuals infected with one genus/species of digenean trematodes (e.g., *Trichobilharzia*) in relation to all individuals in a sample, while the term “overall prevalence” means the proportion of snails infected with any of the recorded parasite genera.

2.3. Density of snail populations

The presence of *P. antipodarum* was investigated in all lakes during each sampling of snails. Three bottom samples were always collected, each consisting of the contents of four core samplers described by Poznańska-Kakareko et al. (2017) with a diameter of 40 mm. In the laboratory, specimens of *P. antipodarum* were counted, and the obtained values were recalculated to 1 m^2 (Fig. A.1).

Each year, the density of *L. stagnalis* was examined in July, the period when their populations reach one of their highest densities (Cichy, 2013). The study of lymnaeid density was carried out using the “frame method” presented by Cichy (2013). More precisely, the test consisted of setting up 16 frames (square-shaped with a side length of 25 cm) next to each other and arranging them in a square. The frames used were composed of heavy material to prevent them from floating in the water column. The frames were placed in the littoral zone (both nonovergrown and overgrown with macrophytes) to a water depth of 1 m. Then, the specimens of *L. stagnalis* on the bottom, stones and macrophytes within the set frames were counted. In each lake, the frames were placed 5 times along the shoreline where the molluscs were collected for parasitological research. Then, the obtained values were recalculated to 1 m^2 (Fig. A.2).

2.4. Obtaining miracidia

Two specimens of *Anas platyrhynchos* f. *dom.* (definitive hosts) were experimentally infected with cercariae of *T. szidati* (Digenea: Schistosomatidae) at the Department of Parasitology (Charles University, Prague, Czechia) (Kolářová et al., 2010). The droppings of the ducks with parasite eggs were collected 18 days after the experimental infection, and avian schistosome miracidia (first larval stage, infectious for the intermediate host) were hatched and collected based on the methodology presented by Marszewska et al. (2020). Care and maintenance of used birds were performed following European Directive 2010/63/EU and Czech law (246/1992 and 359/2012) for a biomedical study involving animals. Experiments were carried out with the legal consent of the Expert Committee of the Section of Biology, Faculty of Science, Charles University, Prague, Czechia, and the Ministry of Education, Youth and Sports of the Czech Republic, under ref. no. MSMT-33740/2017-2.

2.5. Experimental snails

Adult parthenogenetic females of *P. antipodarum* with a shell height of approximately 3.0 mm from a natural source, Sosno Lake, Poland (Fig. 1, Table A.1), were used in the experiments. The snails were collected over a month before the experiment and then maintained under laboratory conditions to ensure that the miracidia or early sporocysts recorded by histology were not of environmental origin. Before setting up the tests, the specimens of *P. antipodarum* were investigated for the presence of patent trematode infection according to Żbikowska et al. (2006).

For the suitable host for *T. szidati*, we used young specimens of *L. stagnalis* with a shell height of approximately 9.0 mm; the age/size of the snails used was selected based on previous research (Marszewska et al., 2018; Marszewska et al., 2020). The lymnaeid snails were obtained from laboratory breeding in the Department of Parasitology, Charles University, Prague, Czechia.

2.6. Experimental infection of *P. antipodarum* with miracidia of *T. szidati*

The experiment was performed in a 96-deep-well plate with round wells (with a diameter of 6.5 mm and a height of 10.8 mm). We placed 200 μl of dechlorinated tap water (20 °C), one *P. antipodarum* snail, and three freshly hatched miracidia per well into 41 wells in total. Then, the plate was covered and kept at a constant temperature of 20 °C and artificial photoperiod of 12/12 h. The time of snail exposure to miracidia was 24 h, which was comparable to the maximum lifespan of free-living miracidia in the external environment (Horák et al., 2015).

Next, 30 specimens of *P. antipodarum* experimentally exposed to miracidia were immediately transported in an aerated container from Charles University (Czechia) to Nicolaus Copernicus University in Torun (Poland), where they were placed in aerated 10-litre aquaria in a room with a constant temperature of 20 °C and a natural photoperiod. Once a week, one-third of the aquarium water volume was changed, and the molluscs were fed multicomponent food in the form of a falling tablet (Shrimps tablet, Glopex, Poland). Every day, the activity of *P. antipodarum* was observed. Dead specimens were immediately removed and autopsied. After two months, all surviving individuals of *P. antipodarum* were checked for digenean infection by autopsy.

The remaining snails (11 specimens) were fixed in Bouin's fluid for histological examination 24 h after exposure to miracidia. In addition, 12 noninfected snails (control) were also fixed for histological examination (for comparison purposes). Following fixation, the samples were dehydrated in a graded ethanol series (Chempur, Piekary Śląskie, Poland), cleared with xylene (POCH, Gliwice, Poland), and embedded in paraffin wax (Chempur, Piekary Śląskie, Poland). Serial cross-sections (4–6 μm thick) were cut with a rotary microtome (Hyrax M55, Zeiss, Oberkochen, Germany). Histological slides were deparaffinized, rehydrated, and stained with Ehrlich hematoxylin (Carl Roth, Karlsruhe, Germany) for 5 min and a 1 % ethanol solution of eosin Y (Analab, Warszawa, Poland) for 5 min. Then, the slides were dehydrated in 96 % ethanol and twice in isopropyl alcohol (Leica, Wetzlar, Germany), cleared in Clearene (Leica), and embedded in CV Ultra (Leica). Snail tissues on the glass slides were analysed under a light microscope (Eclipse 80i, Nicon, Tokyo, Japan) in a bright field using 4, 10, 20, 100 × objective magnification and photographed using a digital camera (Axio Cam 305 colour, Zeiss) and image acquisition software (ZEN 3.3 blue edition, Zeiss).

2.7. Exposure of *L. stagnalis* to the miracidia of *T. szidati* in the presence of *P. antipodarum*

The experimental molluscs *P. antipodarum* (nonnative species) and *L. stagnalis* (susceptible species) were exposed to the miracidia of *T. szidati* in 6 deep-well plates with round wells (diameter of 35 mm and height of 17.5 mm) filled with 2.5 ml of conditioned tap water at 20 °C. We tested three densities of *P. antipodarum*: I: 50, II: 100 and III: 200 specimens per well. The fourth (control; 0) variant was without the presence of *P. antipodarum*. All variants were analysed through 10 replications. One individual of *L. stagnalis* was placed in the central part of each well. For each well with the molluscs, three newly hatched *T. szidati* miracidia were added. The plates were covered and kept in a breeding room at 20 °C and an artificial photoperiod of 12/12 h. The time of exposure of the snails to miracidia was 24 h. After exposure, the specimens of *L. stagnalis* were transported in containers lined with gauze, lettuce and a small volume of water from Charles University (Czechia) to Nicolaus Copernicus University in Torun (Poland), where they were placed in aerated 10-litre aquaria in a room with a constant temperature of 20 °C and a natural photoperiod. Twice a week, one-third of the aquarium water volume was changed, and the snails were fed lettuce (*Lactuca sativa* L.) or a multicomponent food in the form of falling tablets (shrimp tablet, Glopex, Poland). Every day, the activity of the snails was observed. Dead specimens of *L. stagnalis* were removed and immediately autopsied. After two months, all surviving individuals of *L. stagnalis* were checked for digenean infection by autopsy.

2.8. Statistical analysis

The differences in water conductivity and pH between lakes inhabited and uninhabited by *P. antipodarum* were assessed using one-way ANOVA.

To investigate the differences in the digenean infection prevalence between lakes inhabited and uninhabited by *P. antipodarum* according to year, a generalized linear mixed model (GLMM, multinomial, logit) was constructed, with nominal categorical response indicating the infection of each *L. stagnalis* individual (0 – not infected, 1 – *Diplostomum* infection, 2 – *Echinoparyphium* (*E. aconiatum*) infection, 3 – *Plagiorchis* infection, 4 – *Trichobilharzia* infection, 5 – *Opisthioglyphe* infection, 6 – *Hypoderaeum* infection, 7 – *Paryphostomum* infection and 8 – *Sanguinicola* infection); two categorical factors (year and *P. antipodarum* presence); and site (lake) as a random effect. Digenean trematode species with an overall prevalence of <0.5 % were excluded from the analysis.

The relationship between the density of *P. antipodarum* individuals and the prevalence of *Trichobilharzia* in *L. stagnalis* was assessed using Spearman rank correlation.

The relationship between the density of *L. stagnalis* and *P. antipodarum* individuals in lakes where they co-occur was assessed using a mixed general linear model (dependent variable: density of *L. stagnalis* individuals; continuous predictor: density of *P. antipodarum* individuals) with site (lake) as a random effect.

To assess the differences in infection in *L. stagnalis* experimentally exposed to the miracidia of *T. szidati* at different densities of *P. antipodarum* between variants (groups I, II and III – with 50, 100 and 200 individuals of *P. antipodarum*, respectively), a generalized linear model (GLZ; binomial, logit) was constructed (dependent binomial variable: infected/not infected, categorical factor: experimental group). It was not possible to include the control group “0” (without *P. antipodarum*) in the model because all *L. stagnalis* individuals that survived to the end of the experiment were infected (no variance in the control group). Thus, the differences in prevalence between the control group “0” and groups I, II and III were tested separately using tests between proportions, with the Benjamini–Hochberg procedure that decreases the false discovery rate (FDR) when conducting multiple comparisons (FDR parameter was set to 0.1) (Benjamini and Hochberg, 1995).

3. Results

3.1. Field surveys

We collected a total of 3833 specimens of *L. stagnalis*. In the first year of research (2018), *L. stagnalis* originated from four lakes coinhabited (Czaplinek, Iławskie, Siecino and Sosno) and four lakes uninhabited (Drawko, Kaleńskie, Kamionka and Szymbarskie) by *P. antipodarum*, from which 650 and 632 of the lymnaeid specimens were collected, respectively. In the following two years, no presence of *P. antipodarum* was recorded in Lake Iławskie; *L. stagnalis* snails were collected from three coinhabited and five uninhabited study areas by *P. antipodarum*. In 2019 and 2020, 494 and 485 specimens of *L. stagnalis* originated from the inhabited area as well as 795 and 777 from the area uninhabited by *P. antipodarum*, respectively.

The water conductivity differed significantly between lakes (Fig. 2a; one-way ANOVA, $F = 30.2$, $p < 0.0001$), while it did not differ significantly between lakes inhabited and uninhabited by *P. antipodarum* (one-way

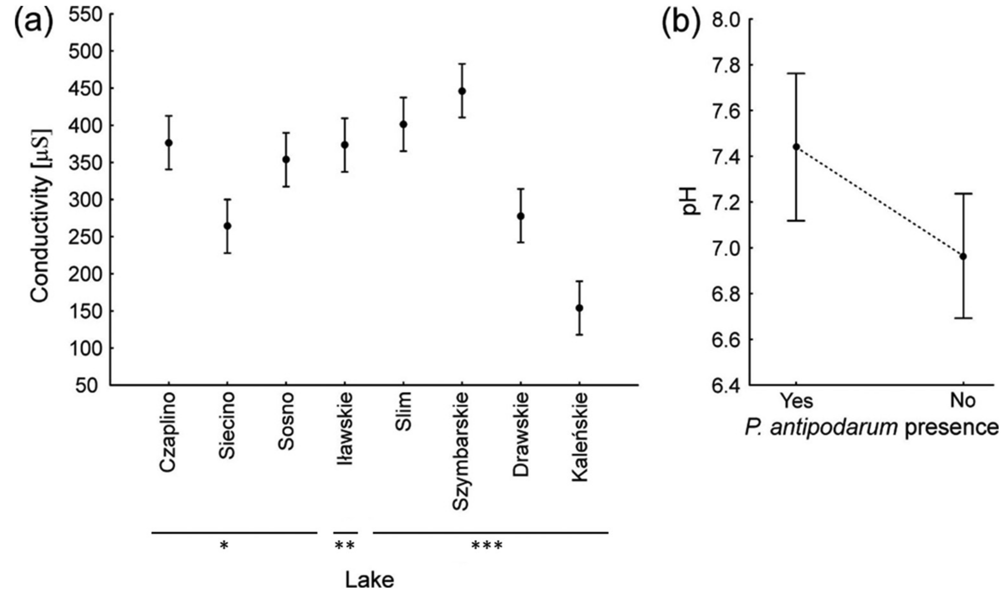

Fig. 2. The differences in the (a) mean water conductivity between surveyed lakes and (b) the differences in mean pH between lakes inhabited and uninhabited by *Potamopyrgus antipodarum* (* – lakes inhabited by *P. antipodarum*, ** – inhabited only in the first year of the study, *** – uninhabited).

ANOVA; $F = 0.05$, $p = 0.826$). The highest conductivity was recorded in Lake Szymbarskie, while the lowest was in Lake Kaleńskie (Fig. 2a). The water pH did not differ significantly between lakes (one-way ANOVA; $F = 0.9$, $p = 0.5089$) but differed significantly between lakes inhabited and uninhabited by *P. antipodarum* (Fig. 2b, one-way ANOVA; $F = 5.5$, $p = 0.0287$).

The overall prevalence of infection with larval flukes in *L. stagnalis* for all of the investigated bodies of water was 25.7 % (19.0 %, 29.2 %, and 27.6 % for subsequent years). The detected digenean larvae in the lymnaeid snails belonged to 11 genera. The dominant cercariae belonged to the genera *Diplostomum*, *Echinoparyphium*, *Plagiorchis*, *Opisthioglyphe*, *Trichobilharzia*, and *Paryphostomum* (Table A.2). The other genera of cercariae (*Hypoderaeum*, *Sanguinicola*, *Tylodelphys*, *Notocotylus*, and *Asymphylodora*) were less frequent (Table A.2). The digenean larvae were recorded in 25.3 % (21.2 % in 2018; 29.5 % in 2019; 26.4 % in 2020) and 25.9 % (16.8 % in 2018; 29.1 % in 2019; 30.2 % in 2020) of all examined *L. stagnalis* originating from the inhabited and uninhabited lakes by *P. antipodarum*, respectively.

Avian schistosomes (the genus *Trichobilharzia*) were recorded in 1.6 % of all collected specimens of *L. stagnalis*. The prevalence for the sites coinhabited by *P. antipodarum* was 0.2 % (0.3 % in 2018; 0.0 % in 2019; 0.4 % in 2020), while for the sites uninhabited by *P. antipodarum,* it was 2.5 % (1.7 % in 2018; 2.4 % in 2019; 3.3 % in 2020).

The results of the GLMM model showed that differences in the prevalence of infection of *L. stagnalis* individuals between lakes inhabited and uninhabited by *P. antipodarum* were significant in *Diplostomum*, *Echinoparyphium* (*E. aconiatum*), *Plagiorchis* and *Trichobilharzia* infections, whereas differences in infestation prevalence between years were significant in *Diplostomum*, *Echinoparyphium* (*E. aconiatum*), *Trichobilharzia*, *Opisthioglyphe*, *Hypoderaeum* and *Paryphostomum* infections (Table 1). The dilution effect (lower infestation prevalence in lakes inhabited by *P. antipodarum* compared with uninhabited lakes) was shown in *Trichobilharzia*, *Opisthioglyphe* and *Hypoderaeum* infections; however, the difference was significant only in *Trichobilharzia* infections (Table 1).

A total of 4000, 3000 and 3000 specimens of *P. antipodarum* were collected from the study area in 2018, 2019 and 2020, respectively. Autopsy of the snail specimens revealed no infection with sporocysts, rediae or cercariae of digenean trematodes.

The highest densities of the individuals of *P. antipodarum* in 2018 were recorded in Lake Czaplino and Lake Siecino, followed by Lake Iławskie and Lake Sosno (Fig. 3). As mentioned above, the population of *P. antipodarum* completely collapsed in Lake Iławskie in 2019, and it did not recover the following year. A gradual decline in the population density of this species can be observed for Lake Sosno in the following years (2019 and 2020), while the density of individuals of *P. antipodarum* for Lake Czaplino and Lake Siecino ranged from approximately 4000 to approximately 7000 individuals per m^2 along the investigated lake shorelines (Table A.3).

Table 1
The results of the generalized linear mixed model (GLMM; multinomial, logit) showing the differences in the prevalence of infection of the digenean genus between lakes inhabited and uninhabited by *Potamopyrgus antipodarum* and years. Significant effects are shown in bold font. SE – standard error.

Digenean infection	Effect	Estimate	SE	t	*p*
Diplostomum	Intercept	−4.46	0.60	−7.38	**<0.001**
	Year (2020)	0.75	0.20	3.76	**<0.001**
	Year (2019)	0.97	0.19	5.02	**<0.001**
	P. antipodarum presence (yes)	1.74	0.61	2.86	**0.004**
Echinoparyphium (*E. aconiatum*)	Intercept	−5.62	1.13	−4.96	**<0.001**
	Year (2020)	0.62	0.25	2.46	**0.014**
	Year (2019)	0.95	0.24	3.95	**<0.001**
	P. antipodarum presence (yes)	1.14	0.38	2.99	**0.003**
Plagiochris	Intercept	−4.45	0.47	−9.53	**<0.001**
	Year (2020)	0.20	0.23	0.89	0.376
	Year (2019)	0.33	0.22	1.45	0.146
	P. antipodarum presence (yes)	1.78	0.50	3.55	**<0.001**
Trichobilharzia	Intercept	−4.18	0.44	−9.42	**<0.001**
	Year (2020)	0.90	0.34	2.36	**0.009**
	Year (2019)	0.49	0.37	1.33	0.184
	P. antipodarum presence (yes)	−1.89	0.73	−2.58	**0.010**
Opisthioglyphe	Intercept	−4.02	0.75	−5.33	**<0.001**
	Year (2020)	0.81	0.27	3.05	**0.002**
	Year (2019)	0.52	0.28	1.87	0.062
	P. antipodarum presence (yes)	−1.0	0.67	−1.48	0.139
Hypoderaeum	Intercept	−5.24	0.61	−8.59	**<0.001**
	Year (2020)	1.13	0.53	2.13	**0.033**
	Year (2019)	0.08	0.64	0.13	0.899
	P. antipodarum presence (yes)	−0.43	0.67	−0.65	0.518
Paryphostomum	Intercept	−5.29	0.67	−7.96	**<0.001**
	Year (2020)	0.65	0.41	1.58	0.114
	Year (2019)	1.08	0.39	2.80	**0.005**
	P. antipodarum presence (yes)	0.44	0.77	−0.65	0.574
Sanguinicola	Intercept	−6.48	1.09	−5.97	**<0.001**
	Year (2020)	−1.13	0.80	−0.140	0.160
	Year (2019)	−0.27	0.59	−0.46	0.647
	P. antipodarum presence (yes)	2.04	1.15	1.78	0.076

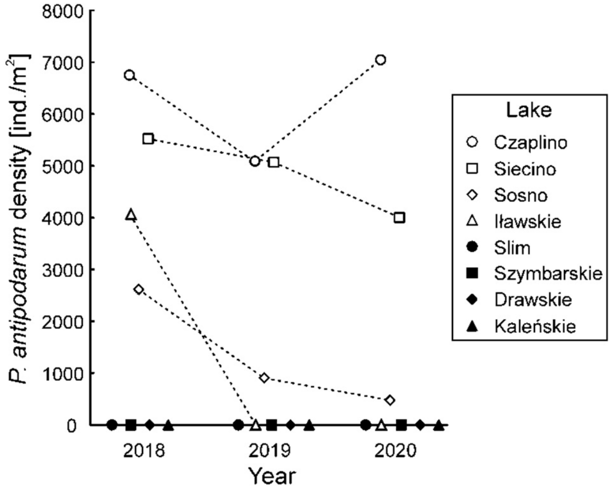

Fig. 3. Changes in *Potamopyrgus antipodarum* density between lakes surveyed in 2018, 2019 and 2020.

The relationship between the density of the individuals of *P. antipodarum* and the prevalence of *Trichobilharzia* infection in *L. stagnalis* was significant (Fig. 4, Spearman rank correlation, $N = 11$; $R = -0.78$, $p = 0.0044$). The higher the density of *P. antipodarum* was, the lower the prevalence of *Trichobilharzia* infection in *L. stagnalis* (Fig. 4).

The relationship between the densities of *P. antipodarum* and *L. stagnalis* in lakes where they co-occur was significant (mixed GLM; $\beta = 0.88$, $F = 28.7$, $p = 0.0007$; Fig. 5).

3.2. Laboratory experiment

By the end of the laboratory experiment (60 days), 70 % of *P. antipodarum* specimens exposed to the miracidia of *T. szidati* survived. In both the dead snails (nine specimens) and live snails (21 specimens), no digenean larvae were recorded during autopsies.

The analysis of histological slides revealed the presence of foreign bodies of unknown origin in both *P. antipodarum*-infected and noninfected individuals (Fig. A.3).

All specimens of *L. stagnalis* exposed to the miracidia of *T. szidati* in the presence of 100 and 200 specimens of *P. antipodarum* survived the entire study period, whereas those in the presence of 50 specimens of *P. antipodarum* or the control group (no *P. antipodarum* snails) died (in the second week) at rates of 10 % and 40 %, respectively (Table A.4); no larvae of *T. szidati* were recorded in these specimens. All control specimens surviving the study (100 %; variant 0) were infected with cercariae and/or sporocysts of *T. szidati*, whereas 66.7 %, 50 % and 30 % of *L. stagnalis* individuals were infected in variants I (in the presence of 50 individuals of *P. antipodarum* per specimen of target snail), II (100 individuals of *P. antipodarum*) and III (200 individuals of *P. antipodarum*), respectively (Fig. 6). The differences between variants I, II and III were not significant (GLZ, binomial, logit; $W = 2.45$, $p = 0.2945$; Fig. 6). The difference between variants 0 and I was not significant (test between proportions, $p = 0.1167$; Benjamini–Hochberg significance: not significant; Fig. 6), whereas significant differences were shown between variants 0 and II (test between proportions, $p = 0.0367$; Benjamini–Hochberg significance: significant; Fig. 6) and variants 0 and III (test between proportions, $p = 0.0063$; Benjamini–Hochberg significance: significant; Fig. 6).

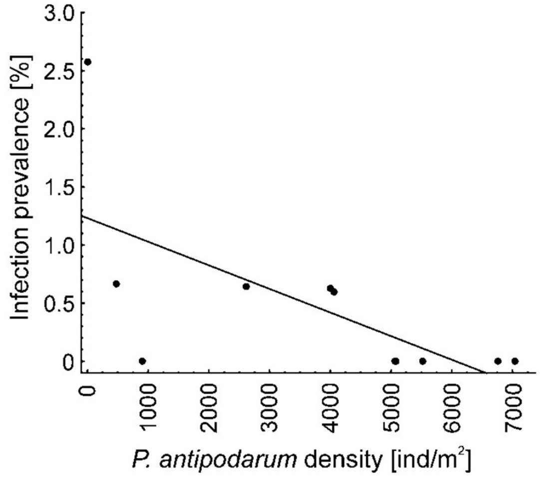

Fig. 4. The relationship between the density of *Potamopyrgus antipodarum* and the prevalence of *Trichobilharzia* in *Lymnaea stagnalis* populations.

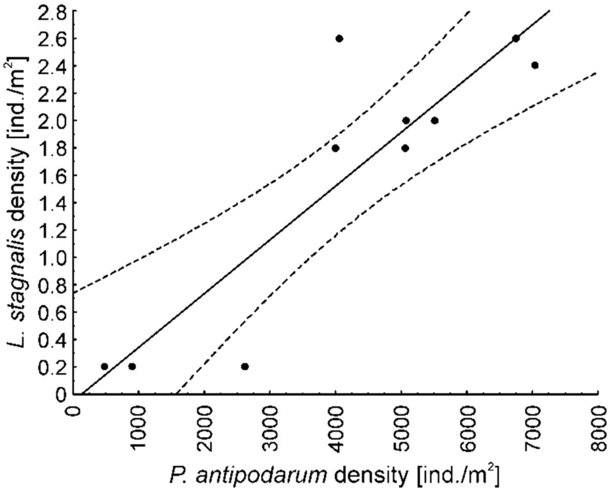

Fig. 5. The relationship between the densities of *Potamopyrgus antipodarum* and *Lymnaea stagnalis* with 95 % confidence bounds (dashed lines).

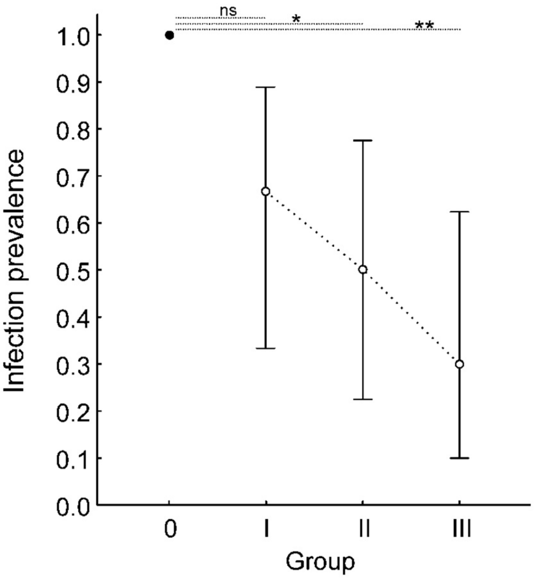

Fig. 6. The prevalence of infection in four experimental variants (groups) of *Potamopyrgus antipodarum*: 0 – 0, I – 50, II – 100 and III – 200 specimens per specimen of *Lymnaea stagnalis*. Differences between groups I, II, and III, tested using GLZ (binomial, logit), were not significant. Differences between groups 0 and I, 0 and II, and 0 and III were tested using tests between proportions. ns – not significant, * – $p < 0.05$, ** – $p < 0.01$.

4. Discussion

4.1. Field evidence

Many introduced species can imperil native taxa, although invaders may also have the opposite effect (Nelson et al., 2015). Here, we tested the scenario in which the nonnative competitor reduces the parasite prevalence in native target molluscs. Westby et al. (2019) indicated that invaders can reduce parasitism in native species without affecting the abundance of these hosts; therefore, the reduction in parasitism need not be the result of changes in host density. In nonnative areas, the populations of *P. antipodarum* reach large densities; therefore, ecologists speculate that this species may contribute to changes in the structure of dominance in aquatic ecosystems (Żbikowska et al., 2021). Our field observations did not show any major indications that the presence of the competitor's population would affect the density of the native population of *L. stagnalis*. The increase in the density of the nonnative species was accompanied by an increase in the density of the native target host, which likely indicates favourable environmental conditions for molluscs in the research area. However, we showed a population decline for the nonnative species. Overall, European populations of *P. antipodarum* show significant fluctuations in occurrence; their high densities and subsequent extinction as a result of the low genetic diversity of their individuals can be observed (Stanicka et al., 2020).

Our nonmanipulative field study confirms previous reports that the coexistence of alien species may reduce the prevalence of certain parasites in native species (Kelly et al., 2009; Żbikowska et al., 2021). Excluding the possibility that dilution occurs by nonselective filter feeders and free-living parasite stages are ingested as a byproduct (Stanicka et al., 2021), the detection of a mollusc that would dilute the miracidia of a wide range of digenean species appears doubtful due to species-specific differences in the behaviour of these larvae (Marszewska et al., 2020). Lootvoet et al. (2013) indicated that host specificity is a crucial property of parasites. We detected a dilution for three digeneans, but it was significant only for avian schistosomes, which we focused on in further analyses. Subsequently, we showed that target host populations occurring with higher densities of *P. antipodarum* have a lower prevalence of avian schistosomes. Kelly et al. (2009) indicated that an increase in the diluter abundance index, namely, the ratio of the number of diluters to the number of native target hosts, may weaken parasitism. Nevertheless, in our field studies, we did not consider the numerous biotic and abiotic environmental factors that may influence disease dynamics (Laidemitt et al., 2019; Westby et al., 2019). One of the important factors related to trematode-borne disease transmission is the presence/abundance of definitive hosts, which is extremely difficult to assess in the case of avian schistosomes because it appears likely that even minimal contact time between the definitive host and the lake ecosystem (e.g., by a nonresident, possibly migrating bird) may already be significant (Rudko et al., 2022). Therefore, it is necessary to compare the results of field studies with those obtained under controlled laboratory conditions.

Another argument in favour of *P. antipodarum* as a diluter of digenean miracidia is the lack of detection of intrasnail sporocysts, rediae and cercariae. Different authors emphasize the extremely sporadic role of *P. antipodarum* as a nonnative first intermediate host (Stanicka et al., 2020). Nevertheless, there is one molecular confirmation of a patent infection of *P. antipodarum* by a digenean from Europe, which indicates that the parasite belongs to the Aporocotylidae native to New Zealand (Gérard et al., 2017).

4.2. Laboratory evidence

To confirm the hypothesis that *P. antipodarum* causes the dilution effect toward avian schistosomes attacking intermediate hosts, we also performed an experimental infection of *L. stagnalis* with *T. szidati*. The obtained results confirm that the presence of *P. antipodarum* reduces the probability of target host infection, which is consistent with previous experimental studies involving *R. labiata* and *T. regenti*. Moreover, both here and in an earlier experiment, we showed that the risk of infecting the target host decreases with the increasing density of the nonhost coinhabitant (Marszewska et al., 2018). The mechanism causing this phenomenon is still unknown. In the natural environment, *P. antipodarum* may not constitute a physical barrier against miracidia (which may have been the case under the experimental conditions used here, as high densities of nontarget snails were used in a relatively small experimental arena) because it mainly inhabits the bottom of bodies of water, while *L. stagnalis* most often dwells on macrophytes. Additionally, it belongs to pulmonate snails, which are often exposed to the water surface (Piechocki and Wawrzyniak-Wydrowska, 2016).

The eggs of *T. szidati* in the definitive host's faeces sink to the bottom of the bodies of water, and the miracidia hatch there and begin their search for the snail host (Skála et al., 2020). Therefore, it is likely that chemical signals from *P. antipodarum* preferring the bottom sites interfere with those emitted by *L. stagnalis*. Our previous research indicates that the chemokinetic reaction of avian schistosome miracidia is effectively disrupted in the presence of excretory-secretory products derived from *P. antipodarum* (Marszewska et al., 2020). Additionally, *T. szidati* larvae, during experiments in the choicechambers, left the base arm and moved to the arm filled with water conditioned by *P. antipodarum* (Marszewska et al., 2020). As a result, the question was raised regarding whether the miracidia of the avian schistosome could penetrate *P. antipodarum*. Combes and Moné (1987) indicated that digenean miracidia can, in some cases, penetrate nonhost species of molluscs. It is well known that the snail immune system effectively kills unspecific species of digenean larvae (Bayne et al., 2001; Sapp and Loker, 2000; Skála et al., 2020). In unsuitable hosts, early sporocysts are surrounded by haemocytes and destroyed (Bayne et al., 2001). Our experiment did not prove the presence of *T. szidati* in *P. antipodarum* exposed to the parasite; the "foreign bodies" were not recognized as trematode larvae. Interestingly, Combes and Moné (1987), in histological studies, showed that *Schistosoma mansoni* miracidia can penetrate different species of pulmonate snails and not prosobranchs (including the genus *Potamopyrgus*). The above phenomenon may contribute to the interruption of the transmission of schistosome miracidia to target hosts by unsuitable snails.

Digenean miracidia in the external environment have a limited lifespan, and they only survive by finding a host snail. Avian schistosome miracidia failure may be due to an ineffective search for a host as a result of attraction to the substances emitted by an unsuitable host and/or unsuccessful attempts to adhere to an unsuitable host. Interaction with the nonhost organism can cause parasite exhaustion or damage to parasite invasion tools. As a result, miracidia may be less successful when attempting to infect a target host (Chernin and Perlstein, 1969; Combes and Moné, 1987).

5. Conclusions

Digenean trematodes, because they have two free-living larval stages, are an excellent research model for investigating the dilution effect in parasite transmission control. Fewer studies have been carried out on digenean miracidia than on cercariae, which is certainly associated with the difficulty of obtaining miracidia for the experiments. There is no doubt that diluting miracidia leads to a significant reduction in the number of cercariae released into the environment; this may be a better way to combat outbreaks of mollusc-borne diseases. Although there are numerous species-dependent characteristics of parasites (Marszewska et al., 2020), schistosomes serve as a unique model for interdisciplinary study, including those focused on disease ecology and the One Health paradigm (Loker et al., 2022).

Here, based on a combination of field surveys and laboratory investigations, we confirm that the presence of nonhost snails interferes with some digenean life cycles (snail host finding and penetration). This investigation is important, as it provides insight into parasite-host-diluter interactions that may serve as the basis for the development of possible mollusc-borne disease control biological measures, including the areas of most serious infectious diseases in the tropics and subtropics. The parasitological perspective should include a comprehensive approach to the overall ecosystem complexity; researchers should also look past the target host and parasite, at least as far as possible.

Notably, the net result of disease dilution and resource competition (between hosts and diluters) is challenging to predict (Hall et al., 2009). In addition, the detected parasite dilution in the naturally presented densities of the nonnative competitor in their nonnative areas generally did not result in a complete decline of the local parasites in the intermediate host. While molluscs infected with digenean trematodes may release large amounts of infectious larvae, cercariae (Marszewska et al., 2016), even the presence of a single infected first intermediate host may pose a risk of disease outbreaks. It is likely that the dilution effect caused by nonnative competitors may be a tool in “outside the box” strategies for mollusc-borne disease control, but each parasite-host-diluter system requires individual research, interpretation and careful cost–benefit analysis.

Changes in the species composition of the host community may influence the risk of disease transmission (Marsot et al., 2013; Rohr et al., 2020). Understanding the ecological interactions in complex communities will increase disease knowledge (Shaw and Civitello, 2021). There is a wide need to investigate how infectious agents interact with ecosystems and to understand the dilution effect in the context of infectious disease dynamics and infection risk (Roberts and Heesterbeek, 2018). Determining the size of possible epidemics is a significant challenge in disease ecology (Strauss et al., 2018). Despite the number of questions still arising regarding the influence of the dilution effect on possible parasitic epidemics and nonnative species on residents, getting to know the composition and abundance of aquatic ecosystem inhabitants inhabited by a host is certainly a key element in predicting the size and spread of mollusc-borne diseases.

This work is not a call for the introduction of nonnative species; it is intended to be a stimulus for parasitologists and ecologists to continue searching for natural enemies of parasites because, as our results show, they exist. As we have previously postulated in our other research, the introduction of nonnative species to new areas may have adverse consequences (Stanicka et al., 2022). It is important to continuously draw the public's attention to this issue (Stanicka et al., 2022). Finding natural enemies to the most dangerous species of human and animal parasites that will pose no threat to the local environment could be a groundbreaking finding.

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CRediT authorship contribution statement

A.S., A.C., J.B., P.H. and E.Ż. conceived the ideas and designed methods. A.S. conducted field research, the breeding of experimentally infected molluscs and the autopsy of gastropods. A.S., A.C. and J.B. conducted the experimental infection. A.M.L. and J.T. conducted the histological study. A.M.Ć. performed the statistical analysis. A.M.Ć. and A.S. prepared the figures and tables. A.S. wrote the first draft of the manuscript, with contributions from A.M.Ć. All authors contributed critically to the drafts and provided final approval for publication.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157264.

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