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Thermal preferences of bird schistosome snail hosts increase the risk of swimmer's itch

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ABSTRACT

Ambient temperature strongly affects host parasite interactions, especially when both are ectothermic. Bird schistosomes, which cercariae are known as agents of swimmer's itch and their snail hosts can be a good example of this phenomenon. The snails of these parasites play the key role, as the source of harmful larvae. Cercarial dermatitis is noted even in areas when prevalence of parasites in snail populations is very low. The main question is what adaptation in snail-fluke association can lead to a sufficient number of cercariae causing swimmer's itch in lake water? The influence of ambient temperature on snail survival and cercarial production as well as the thermal preferences of two host species naturally infected with bird schistosomes were studied. The 24-h preferences of *Lymnaea stagnalis* infected with *Trichobilharzia szidati*, and *Planorbarius corneus* infected with *Bilharziella polonica* were recorded using an oblong thermal gradient set (OTGS). Both cercariae releasing hosts of bird schistosomes preferred a significantly lower temperature than non-infected snails. Additionally, at a higher temperature, the survival of snail hosts was shortened as a result of the increase in daily cercarial expulsion. An especially interesting result concerns the release of a significantly larger total cercariae number by *L. stagnalis* at lower than at higher temperatures. These data indicate that preferences of infected snails to low temperature microhabitats can increase host survival and parasite success, as well as affecting the increase in the number of invasive larvae in the environment increasing the risk of swimmer's itch.

1. Introduction

The long-term monitoring studies show the impact of recent climate trends on species physiology, distribution and phenology (Hughes, 2000). Ambient temperature strongly affects the outcome of interactions between parasite and host (Poulin, 2006); therefore, there is a need to broaden knowledge about this topic. The parasites that constitute a global problem are bird schistosomes. The ubiquitous range of these parasites, and the often accompanying swimmer's itch caused by them, is a field for cooperation between researchers and owners of water recreation areas. Cercariae – the pathogenic larvae emerging from host snails – adhere to human skin due to a chemokinetic reaction, followed by penetration (Brant and Loker, 2009). Despite the fact that humans are accidental hosts and the parasites can not complete their life cycle in the human body, the manner of skin penetration is consistent with the course of this process in birds (Horák et al., 2008; Haas and Haeberlein, 2009). The symptoms of swimmer's itch appear after two hours in bathers (Żbikowska, 2003), and are accompanied by allergic changes in the skin and even the respiratory system (Bayssade–Dufour et al., 2001). These symptoms are observed with particular intensity in children and can last up to several weeks (Marszewska et al., 2016).

According to numerous reports on dermatitis of cercarial etiology, symptoms of swimmer's itch occurred in people bathing in waters where the prevalence of these parasites in the snail host populations did not exceed several percent (Chamot et al., 1998; Lévesque et al., 2002; Farahnak and Essalat, 2003; Skírnisson and Kolářová, 2005; Jouet et al., 2008). For instance, the prevalence of *Trichobilharzia* spp. infection in European snails ranges from 0.05% to 5% (Soldánová et al., 2013). The question arises what factor has an impact on the high prevalence of cercarial dermatitis when the observed infection of snails appears to be low? The observation of seasonal changes in the prevalence of bird schistosomes in snails shows that in various types of lakes, patent infection of these parasites is present from May to September (Żbikowska, 2004a). Given the at least two-month intramolluscan development period, the release of cercariae in early spring concerns only those snails that were able to overwinter with the parasite inside the body (Dvořák et al., 1999). In the summer months and early autumn, cercariae emerging from host snails may be the result of a new transfer from final hosts – birds – to intermediate hosts –

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molluscs (Soldánová et al., 2016). The persistence of larvae of bird schistosomes in the snails' bodies throughout the year requires adaptation, among other things, to changing thermal conditions in the environment. The question arises whether larvae of bird schistosomes developing in the snails' bodies react only to fluctuations in the ambient temperature, or whether their presence may be the cause of changes in the thermal preferences of the infected host? Our previous research on the changes in host thermal preferences induced by parasites presented that it is not only a physiological defence response of the host but also the result of digenean larvae development manifestation (Żbikowska, 2004b, 2005, 2011). The present study was aimed at investigating (i) the prevalence of bird schistosomes in snail hosts inhabiting Polish lakes, (ii) the thermal behaviour of intermediate hosts of bird schistosomes (*Trichobilharzia szidati* or *Bilharziella polonica*), (iii) snail host survival at two different temperatures, and (iv) total cercarial production within snail hosts, as well as the daily rate of cercarial expulsion. We assume that the obtained results will present new arguments to explain the phenomenon of the persistence of the high risk of swimmer's itch even in the case of the low prevalence of infection.

2. Material and methods

2.1. Animals

All *Lymnaea stagnalis* and *Planorbarius corneus* individuals which were studied were collected from May to October during three seasons, 2012–2014, in ten lakes situated in central Poland: Gopło (52°36′N, 18°21′E), Tarpno (53°29′N, 18°49′E), Tynwałdzkie (53°40′N, 19°37′E), Zbiczno (53°20′N, 19°23′E), Strażym (53°20′N, 19°26′E), Bachotek (53°17′N, 19°28′E), Wiecanowskie (52°41′N, 17°55′E), Jeziorak (53°42′N, 19°39′E), Śmiłowskie (53°20′N, 17°54′E), Rudnik (53°25′N, 18°44′E). Over 2000 specimens of *L. stagnalis*, shell height 35–40 mm, and 2000 individuals of *P. corneus*, shell diameter 25–35 mm, were collected. Snails were tested for cercarial shedding, by placing individuals separately in a small amount of tap water under a light source for 2–24 h. In such conditions, parasitic larvae left their host. Cercariae species were identified on the basis of morphological features, according to Našincová (1992), Faltýnková et al. (2007, 2008), and Cichy and Żbikowska (2016). Snails infected with bird schistosome larvae, and control non-infected individuals (from the same localities) were selected for observation in a thermal gradient. All of the individuals used in the thermobehavioural experiment were acclimated for at least 24 h preceding the test to a temperature of 19 °C. During this time they were kept separately in containers filled with spring water in natural light, and they were fed on lettuce. All infected snails before being placed in the thermal gradient were tested for cercarial shedding, and only those with patent infection were used in this thermal experiment.

2.2. Experiment in the thermal gradient

Four groups of 10 snails each were studied using a thermal gradient: (i) experimental *L. stagnalis* infected with *T. szidati,* (ii) control *L. stagnalis* non-infected, (iii) experimental *P. corneus* infected with *B. polonica,* and (iv) control *P. corneus* non-infected. In the early morning (at 7:00 a.m.) snails were individually placed in an oblong thermal gradient (length 1100 mm, width 60 mm, height 100 mm, filled with water at a volume of 0.4 L) (Żbikowska and Cichy, 2012) with temperatures at the opposite ends of + 8 °C and + 38 °C, respectively, which were generated by circulating fluids (Petrygo Q and water) controlled by Poly Science ultrathermostats. Individual snails were placed at the point at 19 °C, and could move freely along the gradient themselves. The experiment lasting 24 h was conducted in an air conditioned room at an ambient temperature of 19–20 °C. During the experiment the snails were not fed. After the experiment we performed an autopsy of all examined snails to exclude those with a pre-patent infection.

Data of the temperature selected by each individual during the 24 h experiment were automatically recorded and computed at 3-min intervals using a custom data acquisition computer program GRAD (Żbikowska and Żbikowski, 2015). For the data analysis, excel plotting, and presentation, the temperature recordings of the control and experimental snail groups of 10 snails each were pooled into 24-h averages.

2.3. Snails' survival and cercarial expulsion in a constant temperature experiment

A further 40 individuals were used in the experiment of snail survival and cercarial expulsion: control (10 individuals of each species), *L. stagnalis* infected with *T. szidati* (10 ind.), and *P. corneus* infected with *B. polonica* (10 ind.). All snails were kept individually in containers filled with spring water in natural light at a constant temperature, in a Sanyo incubator. Half of the individuals of each group were kept at 19 °C and the other half at 26 °C. Temperature values were selected according to the range of lake water temperature during the snail collection period. Monitoring of cercarial expulsion and snail host survival were carried out in accordance with the procedure presented by Marszewska et al. (2016).

2.4. Statistical analysis

We applied a two-way ANOVA for: (i) the average temperature selected by snails during the 24 h of the experiment (factors: snail species, parasitic infection), and (ii) the effects of the controlled temperature conditions on the number of cercariae released from infected individuals (until the death of snails), as well as on the number of larvae released per day (i.e. their shedding speed) (factors: snail species, temperature conditions). Moreover, we ran a three-way ANOVA with parasitic infection, controlled temperature conditions and snail species as factors to test the effects on snail lifespan. We used the Tukey test as a post-hoc procedure.

3. Results

3.1. Snails infected with bird schistosomes

From the total snail sample comprising approximately 4000 animals, 37 specimens of *L. stagnalis* and 28 of *P. corneus* were naturally infected with bird schistosomes – *Trichobilharzia szidati* and *Bilharziella polonica*, respectively. Hosts of bird schistosomes were observed from May to September in eight out of the ten studied water bodies, but the average prevalence of those parasites in snail populations was very low (Table 1).

3.2. Thermal behaviour of snails

Snail species and parasite infection influenced the thermal behaviour of snails during the 24 h experiment in a thermal gradient (Table S1) (two-way ANOVA, $F_{1,36} = 175.7$, $P < 0.001$). Moreover, although both species under study presented a similar algorithm of behaviour in a thermal gradient, *P. corneus* individuals infected with parasitic larvae showed stronger thermo-behavioural reaction than *L. stagnalis*, and chose the lowest temperature (Fig. 1, Table 2). The analysis of the average temperature over 24 h indicated that both the infected *P. corneus* and *L. stagnalis* specimens chose statistically significant cooler microhabitats than non-infected ones (Table 2).

3.3. Snail lifespan and cercarial production in controlled temperature conditions

The research revealed that snail species, presence of parasite infection and temperature had an effect on the lifespan of the studied