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Symptoms of behavioural anapyrexia – Reverse fever as a defence response of snails to fluke invasion

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ABSTRACT

The subject of the research was the thermal preferences of *Planorbarius corneus* individuals infected by larvae of digenetic trematodes. Snails were obtained over two consecutive years, 2009 and 2010, from 10 water bodies located in central Poland. The relationship between the seasons and the occurrence of patent invasions in hosts found in the shore-zone of lakes was observed. Behavioural experiments conducted on *P. corneus* individuals placed in a thermal gradient demonstrated that parasite infection had an impact on the thermal preferences of the snails. Individuals that shed cercariae of *Bilharziella polonica*, *Cotylurus sp.*, *Notocotylus ephemera*, *Rubenstrema exasperatum/Neoglyphe locellus*, *Rubenstrema opisthovitellinum*, or *Tylodelphys excavata* displayed symptoms of behavioural anapyrexia, similarly to experimentally injured snails. This response increased the survival of infected individuals while simultaneously prolonging the period of shedding of dispersive forms of parasites. This point of view was upheld by the observation that infected snails bred at 19 °C lived longer than at 26 °C and the shedding rate of cercariae at a lower temperature was lower than at a higher one.

1. Introduction

Planorbidae are of interest to both parasitologists and immunologists because they include snails of the *Biomphalaria* genus, which are intermediate hosts of schistosomes. An exhaustive review of the research on the immunobiology of gastropods, with particular focus on schistosome hosts, is presented in Loker (2010). From the data collected by the author there emerges a picture of a considerable similarity between the defence processes described in snails and the responses observed in vertebrates. A barrier for many pathogens is, as for vertebrates, mucus-producing epithelium (Lo, 1995; Sapp and Loker, 2000). Garcia et al. (2010) identified and characterised the involvement of a snail homologue of the cytokine Macrophage Migration Inhibitory Factor (MIF) in *Biomphalaria glabrata* immune responses to infection by *Schistosoma mansoni*. Ottaviani (2006) hypothesises on the universality of the mechanisms of defence processes in animals, and even uses the term immunocytes for snail hemocytes and fixed phagocytes (Ottaviani, 2001).

Research on Gastropods' defence reactions is almost entirely devoted to interactions in the snail-trematode system (Lie et al., 1981; Bayne and Yoshino, 1989; Loker et al., 1989; Fryer and Bayne 1990; Amen et al., 1991; Yoshino and Vasta, 1996; Adema and Loker, 1997; Horak and Van der Knaap, 1997; Sapp and Loker, 2000; see review: Loker, 2010), but the concentration of researchers on the cellular and humoral processes in snails means that relatively little is known of the ability of snails to generate non-specific reactions, analogous to fever, which may follow a similar course in all animals. Independent of the systematic position, many ectotherms developed a behavioural mechanism for changing body temperature, involving moving to warmer or cooler microhabitats (Portner 2002; Zippay et al., 2004).

The condition for thermo-behavioural response is the ability to perceive thermal stimuli. Snails possess this ability, as the observations of various authors can testify (Kavaliers et al., 1983; Kavaliers and Hirst, 1986; Marshall et al., 2010). Depending on the intensity and direction of the thermal stimulus these animals react behaviourally, increasing or decreasing exposure to specific temperature conditions (Muñoz et al., 2005; Achaval et al., 2005). Lefcort and Bayne (1991) observed changes in the thermal behaviour of *B. glabrata* exposed to miracidia of *S. mansoni*, and earlier Cabanac and Rossetti (1987) investigated the thermal preferences of *Lymnaea auricularia* (=*Radix auricularia*) after the injection of bacterial pyrogenic factors. However, due to the lack of clear results in the form of behavioural fever generated by molluscs this work was not continued. The absence of behavioural fever in snail hosts during trematode invasion postulated by Lefcort and Bayne (1991) is also confirmed by the results of research conducted in our laboratory. However, the absence of behavioural fever does not mean the absence of a thermo-behavioural response in snail hosts. Similarly to *B. glabrata* exposed to *S. mansoni* miracidia, individuals of *L. stagnalis* shedding cercariae

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of *Plagiorchis elegans*, *Diplostomum pseudospathaceum*, *Trichobilharzia szidati* chose microhabitats with a statistically significant lower temperature compared to the control (Żbikowska, 2004, 2005, 2011). The question appears whether the phenomenon of behavioural anapyrexia (reverse fever – according to Glossary of Terms for Thermal Physiology, 1987) observed in *L. stagnalis* and in *B. glabrata* can be considered to be a typical defence response of snail hosts to digenetic trematode invasion. Moreover, it is interesting to know whether the thermal preferences of infected hosts are reflected in the seasonal occurrence of patent invasions of trematodes in snails found in the shore-zone of lakes. This second aspect may be of significance for the transmission of parasites causing "swimmers'itch" in humans or affecting farm animals in natural watering holes.

2. Materials and methods

2.1. Animals

The native range of *Planorbarius corneus* is from Europe to central Asia. Snails of this species, very common in European lakes, were collected from May to October during two seasons, 2009–2010, in ten lakes situated in central Poland: Goplo (52°36'N 18°21E), Tarpno (53°29'N 18°49E), Nowodworskie (53°18'N 17°31E), Zbiczno (53°20'N 19°23E), Strazym (53°20'N 19°26'E), Bachotek (53°17'N 19°28'E), Wiecanowskie (52°41'N 17°55'E), Wolickie (52°51'N 17°54'E), Smilowskie (53°20'N, 17°54'E), Rudnik (53°25'N 18°44'E). Over 3000 specimens, shell diameter 25–35 mm, were collected. Snails were tested for cercariae shedding, and were provisionally divided into experimental groups. The presence of cercariae was checked by placing a snail in small amount of tap water under a light source for 2–24 h. In such conditions, larvae left their host. Cercariae species were identified on the basis of morphological features, according to Nasincova (1992) and Faltynkova et al. (2007, 2008). Individuals that did not shed cercariae were provisionally classified as not infected, and this was ultimately verified after the end of the thermal experiments by means of autopsy. Snails infected with the most commonly found species of trematodes were selected for observation in a thermal gradient. All of the individuals used in the thermobehavioural experiment were acclimated for at least 3 weeks preceding the test to a temperature of 19 °C. During this time they were kept in containers filled with spring water in natural light. They were fed on lettuce and the water in the containers was changed weekly. All infected snails before being placed in the thermal gradient were tested for cercariae shedding, and two additional groups were created: snails with patent (cercariae shedding) and pre-patent invasion. Non-infected, control animals were also divided into two groups – snails without treatment, and individuals wounded with a needle (dimension: 0.1 mm). The treatment was repeated four times, and anatomical location of the wounding was foot. Wounded snails were immediately placed into the thermal gradient.

2.2. Observations in the thermal gradient

Snails (shell diameter 30 ± 3 mm) were individually placed in an oblong thermal gradient (length 1100 mm, width 60 mm, height 100 mm, filled with water at a volume of 0.4L) (Żbikowska, 2006) 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 PolyScience ultrathermostats. Individual snails placed in the trough could move freely along the gradient itself; snails were left undisturbed in the trough for 24 h. The experiment was conducted in an air conditioned room at a temperature of 19–20 °C. Data for each distinct 24 h experimental period were collected every 3 min by the computer program GRAD.

2.3. Snails' survival in a constant temperature experiment

After the experiment in the thermal gradient eight groups of snails – control (60 individuals) and individuals infected with *Notocotylus ephemera* (60 ind.), *Bilharziella polonica* (40 ind.), *Tylodelphys excavata* (60 ind.), *Cotylurus* sp. (60 ind.), *E. spiniferum* (30 ind.), *Rubenstrema exasperatum/Neoglyphe locellus* (60 ind) or *R. opisthovitellinum* (60 ind.) – were kept at a constant temperature. Half of the number of individuals of each group were kept at 19 °C and the other half at 26 °C. Survival, and cercariae shedding were recorded.

2.4. Statistical analysis

The average daily temperatures chosen by the studied groups of individuals were calculated. All data were analysed by ANOVAs, followed by Tukey multiple comparisons of means if significant.

3. Results

3.1. Parasites' prevalence in snail populations

During the two-year study in the shore-zone of lakes 3089 individuals of *P. corneus* were collected. Around 34% of such snails were infected with trematode larvae, and in 13% of the specimens *Chaetogaster limnaei* was found (Table 1). Among the individuals infected by trematodes were the first intermediate hosts, metacercariae hosts and snails with mixed trematode/trematode or trematode/*Ch. limnei* invasions. In studied snails 9–10 species of cercariae were determined (Table 1).

Seasonal changes in the prevalence of trematodes in studied populations of *P. corneus* are presented in Fig. 1. In the studied gastropods, patent invasions of dominant trematode species occurred with a certain regularity. A relatively large number of snails shedding cercariae was noted in the cooler periods of the research season (in May and/or September). In the heat of the summer months (July–August) only snails with patent invasion of *E. spiniferum* and *T. excavata* occurred in greater numbers compared to the cooler period.

3.2. Thermal preferences of snails

On the basis of the thermal preference studies of snails it was established that control individuals uninfected and undamaged by a needle which were studied in the period from June to September displayed a tendency to move in the direction of the warm end of the thermal gradient. The daily average of the preferred temperatures was 27.1 °C (SD = 3.2). The experimental damage to the body surface and the natural invasion by trematodes had an impact on the thermal preferences of *P. corneus* individuals (ANOVA: $F_{9,426} = 9.823$; $P \ll 0.0001$). Tukey's test for unequal N revealed that differences between thermal preferences of some experimental groups of snails and control animals were statistically significant (Table 2).

As follows from Table 2, snails with pre-patent invasion of the majority of the studied parasites were thermophilically similar to control individuals, while the majority of the snails with a patent invasion of parasites chose significantly lower temperatures than the control. Only in two cases were no statistically significant differences noted in the thermal preferences between control snails and those shedding cercariae. These were hosts shedding larvae of *E. spiniferum* or *O. ranae*.