

RESEARCH ARTICLE

Altered thermoregulation as a driver of host behaviour in glochidia-parasitised fish

Pavel Horký*, Ondřej Slavík and Karel Douda

ABSTRACT

Parasites alter their host behaviour and vice versa as a result of mutual adaptations in the evolutionary arms race. One of these adaptations involves changes in host thermoregulation, which has the potential to harm the parasite and thereby act as a defence mechanism. We used a model of the brown trout (*Salmo trutta*) experimentally parasitised with glochidia ectoparasitic larvae from the endangered freshwater pearl mussel (*Margaritifera margaritifera*) to reveal whether parasitisation alters fish behavioural thermoregulation. A study using radiotelemetry temperature sensors was performed during almost one year of the *M. margaritifera* parasitic stage. Glochidia-infested *S. trutta* altered their thermoregulation through active searching for habitats with different thermal regimes. The general preference for temperatures in infested fish varied and was either above or below the temperature preferred by uninfested individuals. Infested fish also preferred different temperatures across localities, whereas uninfested fish maintained their thermal preference no matter which stream they inhabited. Glochidia further induced the expression of a behavioural syndrome among *S. trutta* personality traits, suggesting that it might increase the probability that the fish host would occur in the glochidia temperature optimum. Our findings present the first evidence that thermoregulation plays a fundamental role in the relationship of affiliated mussels and their fish hosts. Incorporating thermoregulation as a factor in the study of this relationship can help to interpret results from previous behavioural studies, as well as to optimise management measures related to endangered mussels.

KEY WORDS: Host–parasite interaction, Thermoregulation, Behavioural fever, Freshwater pearl mussel, Telemetry

INTRODUCTION

Parasite–host interactions can be described as a never-ending evolutionary arms race of mutual adaptations and behavioural manipulations aimed at maximising the fitness of the parasite on the one hand and minimising the fitness costs of the host on the other (Moore, 2002). One of the important defensive mechanisms invoked by the host is fever, which can be defined as a deviation from a favourable temperature (Heinrich, 1993). Fever is widely recognised to occur in animals other than those with metabolically based thermal regulation, as parasitised ectotherms can regulate body temperature through changes in their behaviour (Vaughn et al., 1974; Bronstein and Conner, 1984; McClain et al., 1988).

This specific thermoregulation, called behavioural fever, is displayed by a wide range of taxa, ranging from insects (Boorstein and Ewald, 1987) to fish (Smith and Kramer, 1987), which seek warmer temperatures in response to parasite infection. The timing of behavioural fever is usually not random and has a clear relationship to the observed therapeutic effects that result in higher survival rates of parasitised individuals (e.g. houseflies *Musca domestica* infected with *Entomophthora muscae*; Watson et al., 1993). Nevertheless, the hosts might also respond to parasite infection through a temperature decrease. This type of adaptation, called behavioural chill, can equally harm a parasite (Müller and Schmid-Hempel, 1993; Hunt et al., 2016), suggesting that the defensive effect of temperature might be case specific. Conversely, host thermal preference could be also manipulated by parasites to enhance their own development (Horton and Moore, 1993), which can be strongly affected by temperature (DeGiusti, 1949; Novak et al., 1986; Müller and Schmid-Hempel, 1993; MacNab and Barber, 2012).

From the anthropogenic point of view, parasites are often suggested to be harmful and disgusting (Thomas, 2010). However, a parasitic stage might be a part of the lifecycle of endangered species that are generally favoured and protected by law. This is the case of freshwater mussels of the superfamily Unionoidea, which develop into juveniles via ectoparasitic larvae called glochidia (Kat, 1984). Several recent studies have shown that glochidia can alter the energy expenditure as well as physiology and behaviour of fish hosts (Slavík et al., 2017; Filipsson et al., 2017; Douda et al., 2017a). The development of the glochidia is also highly dependent on temperature, which can accelerate or decelerate its metamorphosis into the juvenile stage (Roberts and Barnhart, 1999; Watters and O'Dee, 1999). This raises the question as to whether glochidia parasitisation might change the thermal preferences of its host, and if so, how.

In the present study, the freshwater pearl mussel *Margaritifera margaritifera* and the brown trout *Salmo trutta* its primary host in Europe (Bauer, 1987), were selected as the parasite–host system to study this phenomenon. *M. margaritifera* numbers are declining worldwide, with the species nearly extinct in many areas and protected in all European countries (Lopes-Lima et al., 2017). *M. margaritifera* is one of the longest-living invertebrates, with the highest reported age of almost two hundred years in the northern part of their distribution (Helema and Valovirta, 2008). Most of the current populations have a prevalence of old individuals, suggesting that natural reproduction and parasitic/larval stage success is key for its conservation (Cosgrove et al., 2000; Hastie et al., 2000; Modesto et al., 2017).

Here, the hypothesis that glochidia would alter behaviourally determined thermoregulation of its host (e.g. Smith and Kramer, 1987) was tested in a field experiment using fish equipped with radiotelemetry sensor tags for measurement of body temperature. Diel movements, home range and dispersal of experimentally parasitised *S. trutta* were additionally analysed and predicted to

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differ from those of the control (e.g. Horký et al., 2014; Terui and Miyazaki, 2015). The relationship between observed behavioural characteristics and mean individual temperature preferences was also assessed to verify the prediction that glochidia induce the expression of a behavioural syndrome among *S. trutta* personality traits (e.g. Poulin, 2013).

MATERIALS AND METHODS

Study area

The study was carried out on the headwaters of the Vltava River above the Lipno Dam, located in the Šumava National Park, Czech Republic (Fig. 1). The study area included the main channel of the Vltava River, as well as its surrounding tributaries, e.g. Studená Vltava, Jezerní potok, Kobyli potok, Řasnice or Olšinka, depending on individual fish dispersal. The Vltava River headwaters studied here consist of mountainous oligotrophic streams with a predominantly pristine morphology.

Experimental design

Altogether, 52 individual *Salmo trutta* Linnaeus 1758 designated for radiotelemetry tag implantation were caught by electrofishing (650 V, 4 A, pulsed D.C.) at two selected streams (26 specimens in Jezerní potok and 26 in Studená Vltava) within the studied catchment. These streams have no existing *Margaritifera margaritifera* (Linnaeus 1758) populations, suggesting that the control fish remained uninfested; this was further confirmed through visual gill inspection. The fish were anaesthetised with 2-phenoxy-ethanol (0.2 ml l⁻¹), measured (standard length, L_S : mean 235 mm, range 206–350 mm) and weighed (body mass, M_b : mean 181 g, range 105–550 g). Radiotransmitters were implanted into the fish body cavities through a midventral incision that was closed with three separate stitches using sterile braided absorbable sutures (Ethicon-coated vicryl). The mass of the transmitter never exceeded 2% of the

body mass of the fish (Winter, 1983). Two different types of transmitters (Lotek Engineering, Inc., Canada) were used in our study. Twenty six *S. trutta* were tagged using the MST-820-T transmitters with temperature sensors in the Jezerní potok. The transmitter operational life was extended to 310 days by programming the tags to work continuously for 3 days and subsequently sleep for the following 11 days. The other twenty six *S. trutta* were tagged using the NTC-6-1 transmitters in the Studená Vltava. These transmitters worked continuously and their operational life was 357 days. The fish were held until they had recovered their body balance and showed spontaneous swimming activity. Prior to release close to the site of capture, half of randomly selected *S. trutta* (i.e. 50%) were infested with glochidia at each locality.

The *M. margaritifera* glochidia used for infestation were obtained from naturally reproducing specimens included in the national restoration programme. Glochidia obtained from 12 female mussels were pooled and used for infestation within 48 h of release. The viability of the glochidia was verified by evaluating their snapping action in an NaCl solution. The density of the glochidia suspension used for inoculation was 8527±2970 (mean±s.d.) viable glochidia l⁻¹, which was assessed by counting ten 10 ml sub-samples collected during the infestation procedure. The infestation procedure lasted 15 min, at a density of 1 fish l⁻¹ of glochidia suspension (individuals from each site were infested simultaneously). After the inoculation, the fish were checked for the presence of glochidia by visual inspection of the gill arches to ensure that the larvae had successfully attached. The control (uninfested) fish were treated with the same handling procedures (i.e. transfer among baths) to ensure similar handling stress to the infested fish.

The fish were monitored from 24 September 2014 until 20 August 2015. A group of randomly selected individuals equipped with temperature transmitters was tracked over a 24 h cycle once every 14 days. Two to twenty-three specimens were tracked during every 24 h cycle, with infested and uninfested fish represented as equally as possible, depending on the tracking conditions. The tracking equipment included two radio receivers (Lotek SRX 600; Lotek Engineering Inc., Ontario, Canada) and a three-element Yagi antenna equipped with a compass. Compass bearings were taken on the transmitter direction from locations positioned with the help of a GPS (GPS map 76S, Garmin LTD, USA). A computer program was developed to obtain fish position coordinates and to plot them on a map using the triangulation method proposed by White and Garrot (1990). The positions of the fish were determined during 8 subsequent 3 h intervals (06:00–08:59, 09:00–11:59, 12:00–14:59, 15:00–17:59, 18:00–20:59, 21:00–23:59, 24:00–02:59 and 03:00–05:59 h). After completion of a 24 h cycle, all the remaining specimens (equipped with temperature as well as standard transmitters) were positioned in the river network using a boat or by walking along the river banks; i.e. their position was also determined once every 14 days. In cases of relocation and related need to search for a fish position in a new unknown locality, its position was recorded less often. Temperature data from the sensor transmitters were stored automatically and subsequently downloaded from the receiver.

All of the experimental procedures were carried out in accordance with valid legislative regulations (Law no. 246/1992, §19, art. 1, letter c). Institutional Animal Care and Use Committee approved this research (no. 58176/2013-MZE-17214).

Temperature in the river network

Water temperature (°C) was measured at seven representative sites in the river network (Fig. 1) using automatic data loggers (Onset

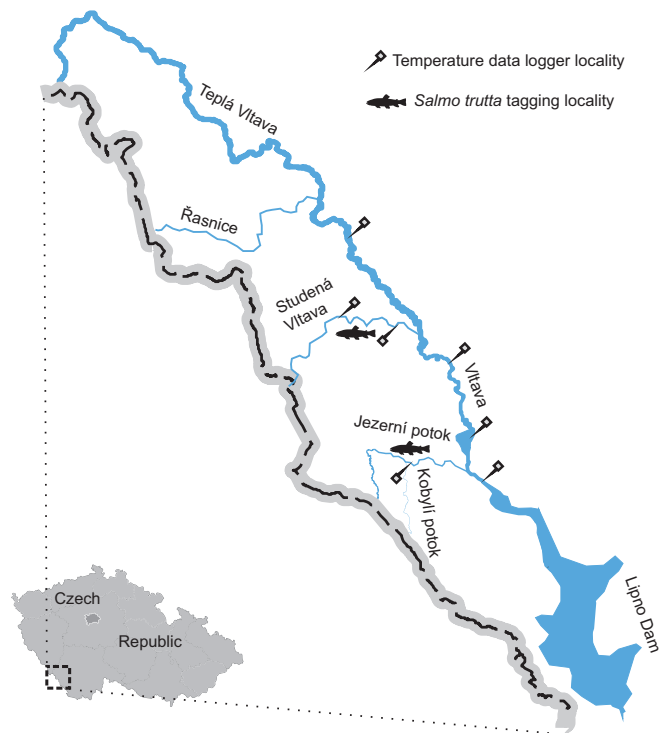


Fig. 1. Map of the Vltava river network studied. Temperature data logger and tagging localities are indicated.

Computer Corporation, Bourne, USA). The temperature was stored every 15 min throughout the duration of the study.

Data analyses

Radiotelemetry data from 49 *S. trutta* were included in our analyses. Three specimens were caught by otters shortly after tagging and therefore were not considered further. Another 12 specimens were lost throughout the duration of the study. These specimens were presumably also caught by otters, herons or fisherman, and their data were included until we were no longer certain they were still live. Four localities with data from infested and uninfested individuals (i.e. Jezerní potok, Kobylí potok, Studená Vltava and Vltava) were used for locality-dependent analyses.

The temperature sensor transmitters (range from -6 to 34°C ; 50 stepwise temperature values; i.e. accuracy to 0.8°C) allowed automatic saving of individual *S. trutta* body temperature data every 5 s. To avoid the dependence of consecutive points in the raw continuous data (a type of M-dependence structure; Moon and Velasco, 2013), a regular interval grid approach was applied, resulting in 1213 lines in the final dataset. Thus, every fish was assigned one mean temperature value in every interval of diel observation (8 subsequent 3 h intervals). The temperature data for fish that were positioned in the longitudinal profile only were treated in the same way and assigned to one appropriate interval. Mean interval temperature data were further referred to as 'body temperature' (T_b).

Movement during a 24 h cycle was determined as the distance (m) between fish positions at two subsequent 3 h intervals, and is henceforth referred to as 'diel movement'. Three 'light intervals' (day, twilight and night) were used to describe the diel activity patterns. These were determined based on the absolute values of illumination (EV), according to Slavík et al. (2007); i.e. twilight ranged between 2 and 6 EV, day was higher than 6 EV and night was lower than 2 EV. The distance (m) between the locations of a fish in two successive 14 day intervals was referred to as a 'longitudinal movement'. The 'longitudinal range' of a specimen was computed as the distance between the two positions of the fish that were the furthest apart during the entire study period. The 'home range' (HR) size was determined using the minimum convex polygon (MCP) method (Aebischer et al., 1993). Despite the known limitations of the MCP (Nilsen et al., 2008), it remains a frequently used HR calculation method, for various reasons (Powell, 2000; Row and Blouin-Demers, 2006). For example, MCP is more accurate than kernel estimators when the sample size is small (Boyle et al., 2009); therefore, it was used in the present study, which had eight points per HR. Samples were grouped into 'seasons': spring (21 March to 20 June), summer (21 June to 20 September), autumn (21 September to 20 December) and winter (21 December to 20 March). While size usually influences fish behaviour, this was not our target variable and we were interested in comparing infested versus uninfested fish, independent of size. Thus, to ensure the independence of diel and longitudinal movements, longitudinal range and home range on fish size, these variables were corrected by dividing by the individual fish mass, according to previous *S. trutta* behavioural studies (Aarestrup et al., 2005; Slavík et al., 2012). In further analyses, we used values corrected for fish mass only. Mean values for body temperature, diel movements, longitudinal movements, longitudinal range and home range were computed as means of all values gathered for particular individual fish across the whole study period.

Statistical analyses

Statistical analyses of body temperature, mean body temperature, water temperature, diel movements, longitudinal movements,

longitudinal range and home range were performed using the SAS software package (SAS Institute Inc., version 9.4; www.sas.com) and a linear mixed model (LMM) with random factors (PROC MIXED). The data were transformed for normality prior to the LMM analyses when necessary. The random factors were used to account for the repeated measures collected for the same experimental units (individual fish) across the duration of the experiment. The significance of each explanatory variable (and its possible two-way interactions) was assessed using *F*-tests. Fixed effects and their interactions that were not statistically significant were not discussed further. The differences between classes were tested with *t*-tests, and a Tukey–Kramer adjustment (Adj. *P*) was used for multiple comparisons. The degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger, 1997).

A GENMOD procedure with binomial distributions was designed to estimate the probability that *S. trutta* relocated to a new stream (i.e. probability equal to 1) rather than remaining in the same stream (i.e. probability equal to 0). We applied an analysis of repeated measurements based on the generalised estimating equation (GEE) approach (Liang and Zeger, 1986), which is an extension of a generalised linear model and provides a semi-parametric approach to longitudinal data analysis. We used a REPEATED statement to account for the repeated measures collected for the same experimental units (individual fish) across the duration of the experiment.

RESULTS

Temperature in the river network

Temperature in the river network showed general seasonal trends, being highest in summer (mean 15.38°C , range 8.18 – 25.03°C) and lowest in winter (mean 3.71°C , range 0.23 – 5.45°C ; $F_{3,19\text{E}4}=105\,831$, $P<0.0001$). This trend was also observed in particular localities ($F_{18,19\text{E}4}=446.55$, $P<0.0001$). Temperatures showed longitudinal variability, increasing from the sources to the lower river network sections ($F_{6,19\text{E}4}=681.46$, $P<0.0001$; Fig. 1). Significant temperature differences between localities were observed (Adj. $P<0.05$), reaching an average difference of 2.3°C between the lower (726 m a.s.l.) and upper (773 m a.s.l.) locality, allowing fish to find a temperature optimum by their movements in the river network.

S. trutta body temperature

A simple general model showed that glochidia-infested fish had lower body temperature than uninfested ones ($F_{1,1213}=5.80$, $P<0.0161$; Fig. 2A). As a second step, *S. trutta* body temperature was analysed using several separate models and data subsets (Table 1) in order to follow seasonal and locality-dependent trends and to provide a more detailed answer to the thermal preference question. These models revealed that the general preference for lower temperatures shown by infested fish could vary across localities and seasons. Thus, in the Jezerní potok during the spring, body temperature was lower in infested *S. trutta* than in uninfested fish (Adj. $P<0.05$; Fig. 2B), while in the Kobylí potok in winter, body temperature was higher in infested *S. trutta* than in uninfested fish (Adj. $P<0.05$; Fig. 2C). The results from Vltava and Studená Vltava indicated potential differences that followed the trends observed at previous localities (lower body temperature of infested *S. trutta* in Studená Vltava during spring and higher body temperature of infested *S. trutta* in Vltava during winter), but these differences did not reach statistical significance (Table 1).

Another batch of models was fitted for comparison of body temperatures of infested and uninfested fish separately in different

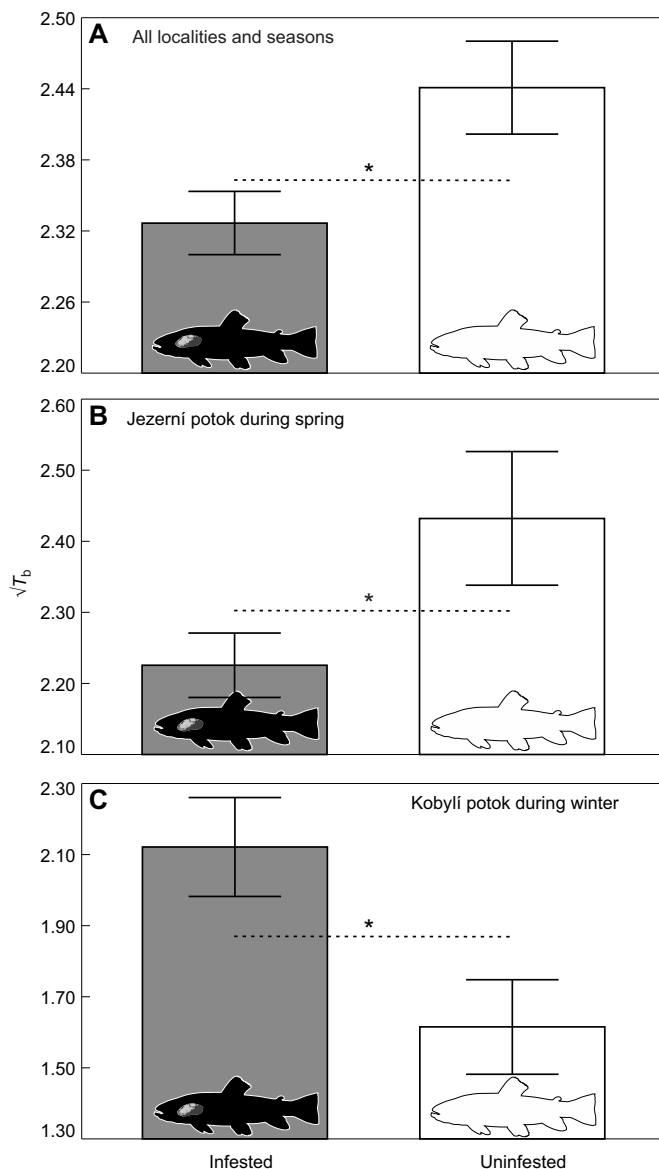


Fig. 2. Body temperature in infested and uninfested *Salmo trutta* at different localities. (A) Fish infested with *M. glochidia* (black) showed generally lower body temperature (T_b) when compared with uninfested specimens (white) ($n=1213$). This trend was also followed in Jezerní potok during spring (B; $n=292$), whereas in Kobyli potok during winter (C; $n=52$), infested fish showed higher T_b . Values are adjusted means (\pm s.e.m.) predicted by a mixed model from square root transformed T_b data. Significant differences between classes (Adj. $P < 0.05$) are indicated with asterisks.

localities across seasons (Table 2). These models showed that infested *S. trutta* had higher body temperature in Kobyli potok in winter (Adj. $P < 0.05$; Fig. 3A), whilst in summer the body temperature was higher in Studená Vltava (Adj. $P < 0.05$; Fig. 3B). Nevertheless, uninfested fish showed no body temperature differences among localities across seasons (Table 2), suggesting that they maintained their thermal preferences no matter which stream they inhabited.

***S. trutta* body temperature and behavioural syndromes**

The mean body temperature variability of individual infested fish could be explained by their altered mean diel movements ($F_{1,14}=6.36$, $P < 0.0244$; Fig. 4A) and mean home range size

Table 1. Type 3 tests of fixed effects for *Salmo trutta* body temperature in relation to glochidia treatment

Locality	Season	Num DF	Den DF	<i>F</i>	<i>P</i>
Jezerní potok	Spring	1	292	3.92	0.0486
Studená Vltava	Spring	1	43	1.34	0.2541
Jezerní potok	Summer	1	68.3	0.00	0.9532
Studená Vltava	Summer	1	59	2.58	0.1134
Jezerní potok	Autumn	1	347	0.07	0.7988
Kobyli potok	Autumn	1	9	0.59	0.4637
Jezerní potok	Winter	1	281	0.32	0.5749
Kobyli potok	Winter	1	29.2	6.91	0.0135
Vltava	Winter	1	13.9	0.52	0.4840

Results are from particular LMM models including different data subsets across localities and seasons. Num DF, numerator degrees of freedom; Den DF, denominator degrees of freedom. LMM models indicating a significant influence of glochidia are in bold. Combinations with non-available data for a particular locality and season due to fish relocation were excluded.

($F_{1,14}=21.18$, $P < 0.0004$; Fig. 4B). In other words, the more an individual parasitised fish moved and the larger the home range it occupied, the higher temperature it preferred. No similar relationship was observed in the uninfested fish, suggesting that glochidia induced the expression of a behavioural syndrome among *S. trutta* personality traits. The relationship between mean individual temperature and movements in the river network (i.e. longitudinal range and movements) was non-significant for either infested or uninfested fish.

Movements in the river network

The infested *S. trutta* showed a higher probability of remaining in their original stream ($\chi^2=16.76$, d.f.=1; $P < 0.0001$; Fig. 5A), where they showed a higher intensity of movements in the longitudinal profile when compared with uninfested fish ($F_{1,332}=5.06$, $P < 0.0251$; Fig. 5B). Nevertheless, the presence of glochidia did not influence the dispersal distance, as the longitudinal range did not differ between infested and uninfested fish, and infested fish generally relocated to the same streams as the uninfested ones did. Thus, *S. trutta* has the potential to disseminate *M. margaritifera* across the whole studied river network at proven distances up to 25 km. Movements in the longitudinal profile also showed general seasonal trends ($F_{3,319}=9.03$, $P < 0.0001$), with the lowest activity observed in summer. The probability of stream change was lowest in autumn and highest in winter ($\chi^2=9.17$, d.f.=3; $P < 0.0271$). Longitudinal movements also showed a locality dependence ($F_{7,374}=9.74$, $P < 0.0001$).

Table 2. Type 3 tests of fixed effects for *S. trutta* body temperature in relation to locality

Glochidia treatment	Season	Num DF	Den DF	<i>F</i>	<i>P</i>
Infested	Spring	1	262	1.27	0.2617
Uninfested	Spring	3	76	1.10	0.3533
Infested	Summer	1	103	9.98	0.0021
Uninfested	Summer	1	50	1.70	0.1979
Infested	Autumn	1	208	1.06	0.3034
Uninfested	Autumn	1	148	2.48	0.1176
Infested	Winter	3	181	4.19	0.0067
Uninfested	Winter	2	74.7	0.60	0.5517

Results are from particular LMM models including different data subsets across glochidia treatments and seasons. Num DF, numerator degrees of freedom; Den DF, denominator degrees of freedom. LMM models indicating significant locality influence are in bold.

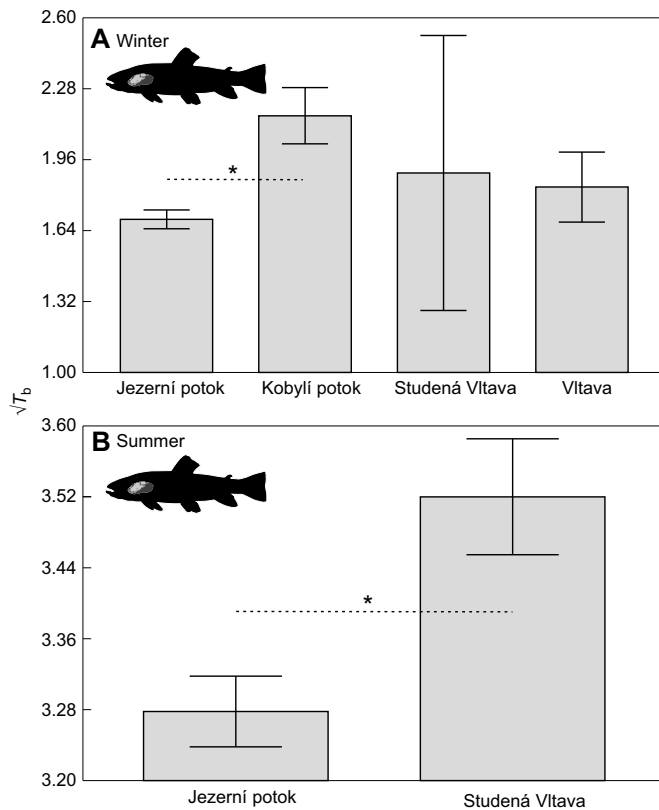


Fig. 3. Body temperature of glochidia-infested *S. trutta* across localities and seasons. T_b of fish infested with *M. margaritifera* glochidia differed across localities in winter (A; $n=255$) and summer (B; $n=103$). Values are adjusted means (\pm s.e.m.) predicted by a mixed model from square root transformed T_b data. Significant differences among classes (Adj. $P<0.05$) are indicated with asterisks.

Diel movements and home range

Glochidia-infested fish showed larger diel movements ($F_{1,203}=5.46$, $P<0.0205$; Fig. 6A) and had smaller home ranges when compared with uninfested fish ($F_{1,673}=5.76$, $P<0.0167$; Fig. 6B). In other words, infested fish occupied a smaller area, where they moved more intensively, when compared with uninfested fish. Glochidia-infested fish showed increased diel movements at night ($F_{4,386}=3.94$, $P<0.0038$; Fig. 7A), while uninfested fish showed no differences across light intervals. Diel movements of glochidia-infested fish also increased during the summer ($F_{6,998}=5.26$, $P<0.0001$; Fig. 7B). Glochidia-infested fish had significantly smaller home ranges during spring and autumn ($F_{3,955}=19.51$, $P<0.0001$), when the home range size generally peaked ($F_{3,955}=36.75$, $P<0.0001$). The home range also varied among localities ($F_{3,977}=156.92$, $P<0.0001$).

DISCUSSION

Behavioural fever has a reported therapeutic effect against various fish pathogens, ranging from viruses (Boltaña et al., 2013) to parasites (Mohammed et al., 2016), confirming that a behavioural defence can be an effective mechanism of immunity (Thomas and Blanford, 2003; Parker et al., 2011; De Roode and Lefevre, 2012). In this study, we experimentally showed that *S. trutta* generally decreased its body temperature as a response to *M. margaritifera* infestation. Furthermore, detailed analyses revealed temporal and spatial dependence in this relationship, varying from behavioural chill to behavioural fever. A similar variability in thermal preference

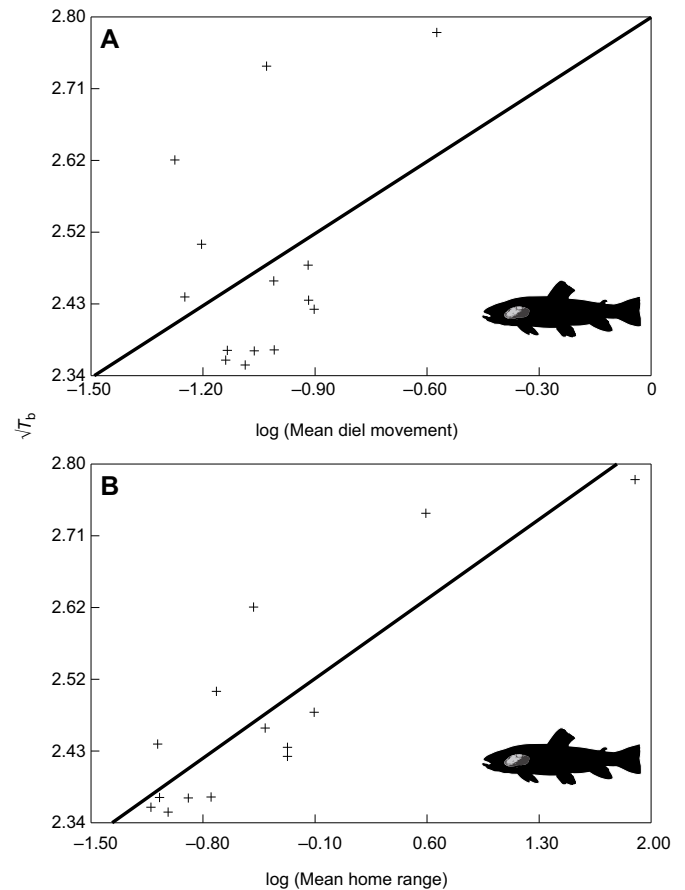


Fig. 4. Mean individual body temperature of glochidia-infested *S. trutta* versus mean diel movements and home range. Mean individual body temperature of fish infested with *M. margaritifera* glochidia increased with mean individual diel movements in metres (A; $n=14$) and mean individual home range in m^2 (B; $n=14$). Values are predicted by a mixed model from square root transformed body temperature data. The lines were fitted by: $y=0.308x+2.7993$ ($r^2=0.16$) in A and $y=0.1458x+2.5394$ ($r^2=0.75$) in B.

was observed in the three-spined stickleback *Gasterosteus aculeatus* infected with the bird tapeworm *Schistocephalus solidus*; these fish changed their thermal preferences according to the developmental stage of the parasite (MacNab and Barber, 2012). Higher temperatures were favoured during the first phase of infection, and when parasites increased in mass, their fish hosts switched to a cooler temperature preference. Similarly, *S. trutta* displayed behavioural fever in winter and behavioural chill in the subsequent spring. If we adopt the arguments of MacNab and Barber (2012), then an increase in temperature preference could be interpreted as an attempt to initiate an immune response (Scharsack et al., 2007), whilst the subsequent spring decrease in temperature preference could reflect a response to the increasing energy demands of the growing parasites at higher temperatures (Davies and Walkey, 1966). Nevertheless, this relationship could also be interpreted from the parasite point of view. In this case, the tendency to prefer higher temperatures in winter and subsequently to maintain lower temperatures in spring could indicate the need to stabilise environmental variability and remain closer to the preferred temperature optimum of the glochidia. The ‘life–dinner’ principle (Dawkins and Krebs, 1979) would suggest that a stronger selection pressure should exist for *M. margaritifera* to adjust the temperature preference of its host than vice versa. Temperature is a key driver of glochidia developmental timing and success (Roberts and Barnhart,

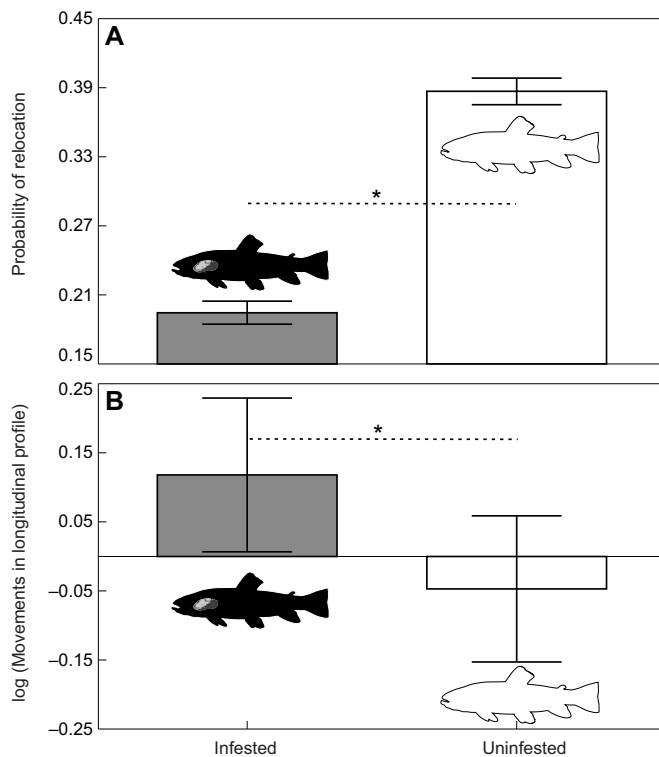


Fig. 5. Comparison of *S. trutta* movements in the river network across treatment. Fish infested with *M. margaritifera* glochidia showed a higher probability of remaining in their original streams (A; $n=627$) and a higher intensity of longitudinal movements (B; $n=585$) when compared to uninfested specimens. Data in A are estimated using a GENMOD procedure with binomial distributions. Data in B are adjusted means (\pm s.e.m.) predicted by a mixed model from log-transformed longitudinal movement data. Significant differences among classes (Adj. $P<0.05$) are indicated with asterisks.

1999; Watters and O'Dee, 1999), whereas altered temperatures within physiological limits are not fatal for *S. trutta*. Furthermore, the general tendency of infested fish to display behavioural chill with its emphasis during spring when temperatures in rivers generally increase could be viewed in the context of *M. margaritifera* juvenile fitness and survival. Marwaha et al. (2017) found a strong positive relationship between the length of the *M. margaritifera* parasitic phase and the post parasitic growth rate, size at excystment and post parasitic survival. Thus, the tendency to remain in colder temperatures could be linked to the slowdown of glochidia development, resulting in parasitic phase extension and an increase in the probability of survival. In addition, juvenile and adult *M. margaritifera* have a limited possibility of moving larger distances, especially upstream (Schwalb et al., 2011). Thus, the thermal regime of the habitat where they leave the host could play a fundamental role in their future growth (Negus, 1966) and metabolic processes (Doucet-Beaupré et al., 2010), which would further reinforce the importance of host temperature selection for *M. margaritifera* performance. The observed increase in diel activity of *S. trutta* during summer, when the glochidia leave their hosts, could also be attributed to this possibility. However, behavioural changes within parasite–host interactions should not be considered separately from the host or parasite point of view only (Moore, 2013). As argued by Ezenwa et al. (2016), the behavioural traits within parasite–host interactions may be frequent subjects of coevolution, suggesting that the changes in behaviour are reciprocally influenced by parasite as well as by its host, thereby generating a

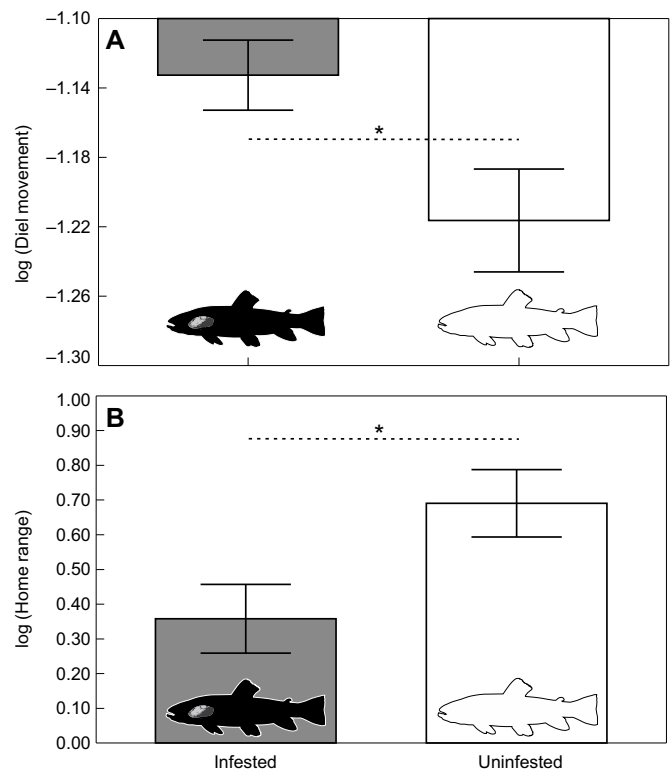


Fig. 6. *S. trutta* diel movements and home range across treatments. Fish infested with *M. margaritifera* glochidia showed a higher intensity of diel movements (A; $n=1024$) and a smaller home range (B; $n=1024$) when compared with uninfested specimens. Values are adjusted means (\pm s.e.m.) predicted by a mixed model from log-transformed diel movements (A) and log-transformed home range (B) data. Significant differences between classes (Adj. $P<0.05$) are indicated with asterisks.

feedback loop concept. We can speculate that multiple feedback loops (Ezenwa et al., 2016), along with adaptive phenotypic plasticity (Kochin et al., 2010) and individual tolerance to parasites (Råberg, 2014), can occur within our studied system and contribute to variations in host and parasite thermoregulatory behavioural traits.

Metabolic costs and internal energetic requirements are considered to be stable on an individual level and are linked to a set of behavioural features attributed to animal personalities (Biro and Stamps, 2008; Careau et al., 2008; Herrera et al., 2014). Basically, proactive and reactive personalities with different behavioural/energetic strategies are distinguishable (Koolhaas et al., 1999; Sih et al., 2004; Réale et al., 2007; Castanheira et al., 2015). Recent findings suggest that fish with proactive and reactive personalities show different thermal preferences (Rey et al., 2015). Furthermore, Cerqueira et al. (2016) validated thermal preferences as a proxy to assess personality traits in fish, suggesting thermal preference as a key to revealing the adaptive meaning of personalities within populations. Our data demonstrate that parasitised fish express a relationship between individual thermal preference, home range size and movement activity, whereas uninfested fish do not. In other words, glochidia induced the expression of a behavioural syndrome in *S. trutta* personality traits. Parasites may manipulate their hosts in various ways (Barber and Dingemanse, 2010; Poulin, 2013), suggesting that the modification of more than one trait at the same time could be more beneficial (Thomas et al., 2010). Only a few studies have documented the influence of parasites on behavioural syndromes (Poulin, 2013), despite the fact that this influence is expected to be common, as

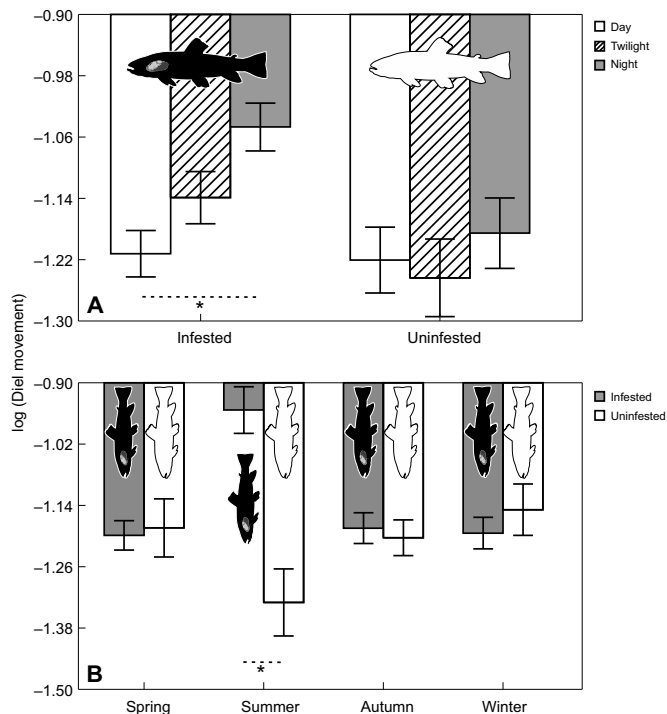


Fig. 7. Comparison of diel movements of infested and uninfested *S. trutta* during particular light intervals and seasons. Fish infested with *M. margaritifera* glochidia showed increased diel movements at night, while uninfested fish did not show any differences across light intervals (A; $n=1024$). Diel movements of glochidia-infested fish also increased during summer (B; $n=1024$). Values are adjusted means (\pm s.e.m.) predicted by a mixed model from log-transformed diel movement data. Significant differences between classes (Adj. $P < 0.05$) are indicated with asterisks.

many behavioural traits are influenced by the same chain of endocrine or physiological phenomena (Benesh et al., 2008). For example, Coats et al. (2010) found a relationship between phototaxis, swimming activity and vertical distribution, but only among parasitised amphipods, suggesting that these relationships might increase the probability of transmission to the final host. Accordingly, the relationship observed in the present study between the individual thermal preferences, home range size and movement activity of parasitised *S. trutta* might increase the probability that the fish host would occur in the glochidia temperature optimum.

Fish behaviourally regulate their body temperature by selecting habitats with thermal regimes that optimise their physiological performance (Reynolds et al., 1976; Ward et al., 2010). Temperatures in the studied river network increased from the sources to the lower river sections, with significant differences between localities, which allowed the fish to find their temperature optima through movements in the river network. Nevertheless, our data support the possibility that the thermal regime was regulated on the habitat scale rather than on the stream selection principle. Uninfested fish remained in comparable temperatures across the whole river network, and they maintained their thermal preferences regardless of which stream they inhabited. Behavioural compensation through habitat selection was also observed in lizards that maintain preferred body temperature across a thermal environmental gradient in mountains (Adolph, 1990). By contrast, infested fish displayed different thermal preferences across localities, occupying smaller home ranges and moving more intensively during the diel cycle when compared with uninfested

specimens. They also had a higher probability of remaining in the original stream, where they showed higher intensities of movement in the longitudinal profile. Therefore, the behavioural thermal selection observed within our studied system could be assumed to occur through active searching for habitats with different thermal regimes within the same stream. In support of this, the habitat shift of glochidia-infested fish hosts observed in a previous study (Horký et al., 2014) could also be attributed to thermoregulatory behaviour, suggesting its importance across various mussel–fish host systems. Furthermore, the fish thermal regime is closely related to energy expenditure (Tanaka et al., 2000). A difference of 1°C can change the rate of many physiological processes by 6–10% (Johnston and Bennett, 1996). This suggests that an increased energy consumption by fish infested with glochidia (Slavik et al., 2017) could also be related to changes in the host thermal regimes observed in the present study.

If we consider that there is an arms race between *M. margaritifera* and *S. trutta*, we might conclude that this particular race has no clear winner. Both species have rather reached their mutual local optimum (Dawkins and Krebs, 1979). This raises the question of how this mutual optimum would evolve in the near future, as the thermal parasite–host adaptations described in our study might be influenced by climate change and increasing temperatures. Climate change is expected to have a large impact on the functioning of whole ecosystems (Hoegh-Guldberg et al., 2007), including parasite–host interactions (Harvell et al., 2002; Dobson, 2009; Lafferty, 2009; Cramp et al., 2014), and freshwater mussels are already living close to their upper thermal tolerances (Pandolfo, 2010). As shown by Catenazzi (2016), for the fire salamander *Salamandra salamandra*, climate change has the potential to alter the metabolism of ectotherms, with severe implications for energetic demands and related behavioural strategies. Riverine fishes, including *S. trutta*, are expected to shift their preferred habitats in response to climate and land use changes until 2050 (Radinger et al., 2017). Whether and how the stationary *M. margaritifera* will be able to follow the habitat shifts of its host is doubtful. This is especially true in small populations with specimen distribution restricted to several river kilometres, as in the system studied here, where future success is closely related to host-dependent dispersal (Schwalb et al., 2011). Our results are promising in this sense, as they suggest that *M. margaritifera* glochidia do not limit the dispersal distance of its host, allowing fish to migrate for distances of over 25 km across the river network. Host-related dispersal over a distance of several kilometres was also reported for the related species *Margaritifera laevis* (Terui and Miyazaki, 2015). However, utilisation of this dispersal potential is determined by related management issues. Above all, the river networks are fragmented worldwide with various types of obstacles, ranging from weirs to dams (Nilsson et al., 2005). Migration obstacles, even when equipped with fish passes, limit the probability that glochidia-infested fish would negotiate them (Horký et al., 2014). Thus, obstacle removal should be preferred as an overall solution to fragmentation (O’Hanley et al., 2013) in river networks with populations of endangered mussels in order to facilitate their natural dispersal. Considerable problems could also arise in fisheries management; for example, the legal catch and consumption of fish hosts by fisherman could stop the natural recruitment and potential dispersal of affiliated mussels from the very start. Considering the fact that recreational fishermen have the potential to seriously impact and overfish whole populations (Arlinghaus et al., 2013), fishing in networks with endangered mussels should perhaps be prohibited. Other fisheries management

practices, such as stocking, should also be avoided due to the altered behavioural patterns of hatchery-reared fishes (Huntingford, 2004) and host compatibility, which is specific at the population level in the mussel–fish relationship (Douda et al., 2014, 2017b). Our results suggest that the management unit for these recommended measures is the whole catchment, as even small streams surrounding the mussel population in the main channel could include infested hosts that have the potential to disperse the affiliated mussels.

In conclusion, the present study successfully incorporates the thermoregulation issue into research on the host–affiliate relationships between freshwater fish and mussels. Despite the fact that radiotelemetry with physiological sensors could be used to study parasite load in fish (Broell et al., 2016), measurement of temperature data with the radiotelemetry equipment is rarely conducted in the wild (Adelman et al., 2014). However, laboratory results do not necessarily mirror the true behavioural features of animals in their native habitats (Calisi and Bentley, 2009). This fact is important to consider, especially in studies like ours, due to specificities of the observed parasite–host system. Freshwater pearl mussels develop on their host from late summer to late spring, which makes it impossible to simulate natural temperature variability, a crucial aspect related to the goal of our study. The findings presented here indicate that parasitised *S. trutta* change their thermal preferences as a specific response, suggesting that the observed parasite–host system is more complex and complicated than expected. Further research is needed to answer the questions that were revealed by our study in more detail. Precise identification of these relationships will obviously depend on high-quality longitudinal data (Ezenwa et al., 2016). This research should be conducted as soon as possible because *M. margaritifera* numbers are decreasing and ongoing changes in natural thermal regimes through climate change can violate the basis of this relationship.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

All co-authors contributed to collection of data, design of the experiments and other work related to manuscript preparation. Writing - original draft: P.H.; Writing - review & editing: O.S., K.D.

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