Trematode Parasite Infection Affects Temperature Selection in Aquatic Host Snails*

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ABSTRACT

Animals infected by parasites or pathogens can exhibit altered behaviors that may reduce the costs of infection to the host or represent manipulations that benefit the parasite. Given that temperature affects many critical physiological processes, changes in thermoregulatory behaviors are an important consideration for infected hosts, especially ectotherms. Here we examined the temperature choices of freshwater snails (*Helisoma trivolvis*) that were or were not infected by a trematode (flatworm) parasite (*Echinostoma trivolvis*). Active snails that explored the experimental temperature gradient differed in their thermal preference based on their infection status, as parasitized snails chose to position themselves at a significantly higher temperature (mean: 25.4°C) compared to those that were uninfected (mean: 23.3°C). Given that snails rarely eliminate established trematode infections, we suggest that this altered thermal preference shown by infected hosts likely benefits the parasite by increasing the odds of successful transmission, either through enhanced production and emergence of infectious stages or by increasing spatial overlap with the next hosts of the complex life cycle. Further studies that employ experimental infections to examine temperature selection at different time points will be needed to understand the extent of altered host thermal preferences, as well as the possible benefits to both host and parasite.

Keywords: trematode parasite, aquatic snail host, fever, thermoregulation, null model.

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Introduction

Parasites can have many effects at various biological levels, from altering individual host fecundity (Forbes 1993) to acting as ecosystem engineers (Thomas et al. 1999). Among the best-known effects are alterations in the appearance or behavior of infected hosts (Poulin 1995, 2010; Moore 2013). Altered behaviors are typically attributed to four possible explanations: (1) an adaptive response by an infected host to aid in minimizing negative impacts or to rid the parasite, (2) a side effect of pathology or infection that coincidentally benefits the host or parasite, (3) no benefit to either party, or (4) a case of parasite manipulation for its own benefit, especially to increase the chances of successful transmission to the next host for those with complex life cycles (Poulin 1995, 2010; Moore 2013). Understanding the extent of host behavioral changes and the possible mechanisms driving these is important, as this may help to explain ways in which parasites can improve their transmission success.

While infection by parasites can change many aspects of host behavior, those relating to the thermal preferences of infected hosts are particularly significant, as this has many implications for pathology and transmission (Poulin 2006; Paull et al. 2012). Therefore, changes in thermoregulatory behavior may also have four possible outcomes as outlined above. Considering that trematode (flatworm) infections can have enormous fitness consequences for snails serving as their first intermediate hosts in their complex life cycle owing to the common occurrence of "parasitic castration" (Lafferty and Kuris 2009), we may expect parasitized snails to select warmer temperatures to combat trematode infection, especially if this provides an opportunity for compensatory reproductive effort (e.g., Minchella and Loverde 1981). This is in line with "behavioral fever" research, where ectothermic hosts select warmer temperatures to create conditions that are inhospitable for parasites or pathogens, thus improving host survival (e.g., Bernheim and Kluger 1976*a*, 1976*b*; Sherman et al. 1991; Kluger et al. 1998; Elliot et al. 2002; Woodhams et al. 2003). While some studies have shown that snails infected with lipopolysaccharide, a bacterial wall component meant to elicit an immune response, select a warmer temperature (Żbikowska et al. 2013), snails with patent trematode infections (i.e., actively producing infective motile stages—cercariae) selected cooler temperatures (Żbikowska 2004) and support the few documented cases of behavioral anapyrexia (reverse fever; see Müller and Schmid-Hempel 1993; Moore and Freehling 2002). Despite these inconsistencies, specific trematode infections may have different effects on the thermal behavior of

their snail hosts (Żbikowska and Cichy 2012), and the phase of trematode infection should also be considered, particularly whether cercariae are actively emerging from the snail (Żbikowska 2004; Żbikowska and Żbikowski 2015).

If infected hosts do not benefit from the selection of warmer temperatures, their parasites may instead. Indeed, the parasites may have the most to gain from host selection of warmer waters because snails cannot clear trematode infections (but see Goater et al. 1989) once they are established (Minchella et al. 1985). Furthermore, increased temperatures (by 10°C) can promote an 11-fold increase in cercarial production and emergence from snails (Mouritsen 2002), as well as successful penetration of cercariae into their second intermediate hosts (Paull et al. 2012). On the other hand, cold temperatures may benefit trematodes by prolonging the life of their snail hosts, which correspondingly increases the period for cercarial development (Żbikowska and Cichy 2012). It is also possible that neither party benefits from increased temperatures and host behavioral fever is merely a by-product of pathology. For example, warm temperatures can reduce freshwater snail survival regardless of trematode infection (Paull and Johnson 2011). In addition, most cercariae have decreased survival in warmer temperatures, likely due to their higher metabolic and locomotor activity, causing them to use their limited energy stores more rapidly (Pechenik and Fried 1995; Mouritsen 2002; Paull et al. 2012).

While there are possible beneficial and detrimental impacts of warm temperatures for both snails and trematodes, it is unclear what temperatures are preferred by infected and uninfected snails when given a choice. Given that trematodes vary in how they affect snails, and effects on host thermal responses from related parasite species cannot be generalized (Bates et al. 2011), we examined the thermal preference of *Helisoma trivolvis* snails that were or were not infected with the trematode *Echinostoma trivolvis*. As echinostomatid trematodes such as *E. trivolvis* have been shown to induce severe pathology and death in larval amphibians (Koprivnikar et al. 2012), thermal preferences of parasitized snails that may affect tadpole infection risk are of relevance. We investigated whether snails were able to respond to temperature in an experimental thermal gradient (TG), particularly if they would spatially orient toward a preferred temperature beyond that expected from a null mathematical model simulating random movement patterns, and compared the thermal preferences of trematode-infected or uninfected snails. If infected snails chose cooler temperatures, this could suggest a host adaptation to prolong their survival. In contrast, a choice by infected snails for warmer temperatures might indicate parasite manipulation to increase fitness through the enhanced production and emergence of cercariae or might indicate greater spatial overlap with larval amphibian second intermediate hosts.

Methods and Material

Trematode Life Cycle and Infected Host Acquisition

Echinostoma trivolvis has a complex life cycle requiring three different hosts (Johnson and McKenzie 2009). After eggs are released by adult worms dwelling within a vertebrate definitive host, free-swimming miracidia hatch and burrow into a snail host, such as *Helisoma trivolvis*, where they develop into sporocysts or rediae. The rediae asexually produce cercariae, the free-swimming form that leaves the snail to infect a second intermediate host, such as a larval amphibian or fish, encysting as metacercariae after penetration. These metacercariae remain in the second intermediate host until it is eaten by a definitive vertebrate host (a bird or mammal).

Helisoma trivolvis (ramshorn snails) were collected from late May to early August 2015 by hand from aquatic vegetation or by dipnetting from two ponds in Ontario (43.51°N, 80.09°W; 43.47°N, 80.24°W) and housed for at least 4 wk before behavioral trials commenced. This snail species is widely distributed across North America (Johnson et al. 2013), experiencing a correspondingly wide range of local temperatures. Snails ranging from 1.05 to 2.35 cm (shell diameter) were housed in Rubbermaid dishpans (40 cm × 31.8 cm × 15.2 cm) containing dechlorinated water at Brock University (St. Catharines, Ontario) and kept at room temperature (~22°C) on a 12L:12D photocycle (i.e., reflective of the diurnal cycle for the behavioral measurements). They were fed boiled spinach ad lib., with half water changes conducted every 1 or 2 d and full water changes weekly. We screened each snail for infection with *E. trivolvis* by inducing the emergence of free-swimming cercariae. To do so, snails were individually placed in a Petri dish with dechlorinated water approximately 30 cm under a 60-W light bulb such that the combination of heat and light induced cercarial emergence (Szuroczki and Richardson 2009). Cercariae emerged roughly 10–20 min after placement under the lamp, and their identity was verified using a compound microscope and published keys (Schell 1970). Snails were returned to their separate housing containers and allowed 1 wk to recover before behavioral experiments began.

Temperature Preference Experiments

To test for aquatic animal thermal preference, two apparatuses (53 cm × 27 cm) were constructed with plexiglass walls and a thermally conductive surface covered with white contact paper (Tattersall et al. 2012). Three plastic dividers created four individual lanes (53 cm × 6.75 cm) such that each animal could turn and move without constraint. We added 1.1 L of fully aerated water (i.e., water aerated overnight with air stones) to keep water levels low and avoid a vertical stratification of temperatures (Lefcort and Eiger 1993; Tattersall et al. 2012). In pilot trials, a vertical stratification of temperatures was unavoidable with a water level of 4 cm (to fully immerse large snails), and thus we filled each apparatus to a depth of ~1.2 cm, which was adequate to avoid a vertical cline of temperatures but deep enough to allow the foot and a portion of the shell of the snail to be submerged. A TG was established (within 30 min) by circulating fluid beneath each apparatus at both short ends. One water bath pumped a cold ethanol-water mixture through copper tubing on one end of the apparatus while hot water was pumped through copper tubing on the opposite end of the apparatus. Neither fluid was in contact with snails or the water within which they were placed. To achieve a gradient that spanned 15°–35°C, the water baths were set to 5° and 48°C, respectively.

Measurements taken with a thermocouple meter (Sable Systems TC1000) every 1–2 cm along each individual lane showed a linear TG, which was confirmed at the beginning and end of every trial and for every lane. When the water baths were off, the water in the apparatus remained at room temperature (~22°C) and created a constant temperature (CT) environment.

Temperature measurements of each lane were taken before each trial along six points to calculate the water temperatures experienced by each snail because these could differ up to two degrees (at the extreme hot and cold ends) between trials within the same lane and between apparatuses. Each trial consisted of two groups of snails (four infected and four uninfected snails, later confirmed by dissection). Individual snails were randomly assigned to each apparatus and lane and were placed in the middle of their apparatus lane randomly facing the left or right side. All trials started at 9 a.m., were conducted under lab ambient light conditions (15-W compact fluorescent lamp lighting), and lasted for 8 h, giving the snail ample time to explore the apparatus and select a position or temperature. Two webcams, one above each apparatus, captured time-lapsed images of the snails every 30 s. Pilot studies indicated that shorter intervals did not change the results. After each trial, all water was removed from each apparatus, followed by a wipe down with ethanol and then a water rinse before leaving the gradient to stand overnight.

In total, 124 snails were tested: six uninfected snails and one infected snail in the CT environment, with 92 and 25 uninfected and infected snails, respectively, in a TG. After testing, the snails were patted dry to accurately measure their weight (g) and shell diameter (cm) and then individually frozen in labeled bags for later dissections to confirm their infection status. Thawed snails were dissected to confirm infection (or not) by *E. trivolvis* by gently cracking the shell and carefully teasing apart the body tissue under a dissecting microscope to check for presence of rediae (Koprivnikar and Walker 2011).

Behavior Analysis

All images were processed in FIJI/ImageJ (Schindelin et al. 2012) using the manual tracking plug-in to locate the position of each snail within the apparatus every 30 s of the 8-h recording period. Positional data (x- and y-coordinates) were saved for each image to locate the spatial position of the snail within its lane (i.e., position along the gradient in centimeters) and to interpret position preference in the absence (CT) or presence (TG) of temperature gradients. For the snails placed in a TG, their spatial position along the longitudinal axis (i.e., the x-position data) was used to infer temperature preference given that the corresponding range of temperatures within the TG was measured for each trial. Thermal preference was calculated using mean temperature, and thermoregulatory precision was calculated using the intraindividual standard deviation of thermal preference. These values were assessed every hour and for every snail. Snail activity (movement in centimeters) was calculated using total distance moved over the entire experimental period.

Null Model

To assess null models for thermoregulatory behavior, a correlated random walk simulation approach was taken (adapted from Turchin 1998), incorporating displacement data (cm/s), turning angles (α), and concentration factors (ρ_α) empirically determined from our study. The model started in the center of the TG and was allowed to move in a random direction (0°–360°) away from the origin, at a velocity drawn at random from the observed displacements (0.0128 $\pm$ 0.009 and 0.00147 $\pm$ 0.0006 cm/s for active and inactive models, respectively), and at subsequent turning angles drawn from a wrapped normal distribution with $\mu_\alpha = 0$ and $\rho_\alpha = 0.995$ (for information on circular statistics, see Lund and Agostinelli 2001). Each step was 1 s, and new x- and y-coordinates were calculated from the new trajectory angle and displacement, with temperature estimated from the x-position using conversions from the thermal preference experiments. We included boundaries to keep the model within the dimensional constraints of the TG (x: 0–53 cm; y: 0–7.5 cm). On arriving at a boundary, the new trajectory was randomly drawn from a bimodal wrapped distribution with angles that were parallel to the boundary (0° and 180° if exceeding the Y-axis boundary and 90° and 270° if exceeding the X-axis boundary), but with relaxed concentration factors ($0.5\,\rho_\alpha$) to allow for both reflection off the boundary and parallel wall movements. Since many snails naturally moved parallel to the walls when in contact, these turning angles allowed the models to achieve similar behavior if encountering the boundaries while retaining the desired random quantities.

A temperature sensitivity component ($Q_{10} = 2$) was also included to account for temperature-sensitive locomotion (Crozier and Pilz 1924), which is essential to demonstrate the extent to which random motion within the gradient predicts a nonuniform distribution of animals within the gradient (Anderson et al. 2007). Each replication represented a single snail movement over 28,000 s. We assumed that the snail moved continuously but could vary velocity at random each second according to the empirical displacement observations. Each model was allowed to run for the same duration as the live experiments (8 h) and was replicated 10,000 times, providing a final output of selected temperature over time (sample outputs are provided in figs. A1 and A2, available online). The 10,000 replications allowed for an empirical distribution of temperature of the null model for comparison to the live animals, using the final hour of the model to compare to the live animal thermal preferences. Both active and inactive snails (see "Statistical Analyses" below) were modeled according to the displacements measured empirically, since inactive animals will not sample as much of the available space as active animals would.

Statistical Analyses

Statistical analyses were conducted in R v. 3.4 (R Core Team 2016). Activity data (total distance moved) in the CT snails were compared to the TG snails and infected snails were compared to uninfected snails using a general linear model, using a log transformation to assist with unequal variances. During this analysis we noticed that some snails moved very little over the

course of the experiment, which impacted the assumptions regarding behavioral thermoregulation. Thus, we elected to classify snails as "active" or "inactive" based on an apparent bimodal distribution of total distances moved. From the distribution, 58 cm was used as a cutoff; snails that moved more than 58 cm over the 8 h were classified as active, while those moving less than 58 cm were classified as inactive. These distances also closely corresponded to the longitudinal dimension of the choice chamber, and thus, an active snail that moves at least the minimum distance of the choice chamber objectively samples 50% of the available temperatures if it started in the middle and made only a simple decision to move to one end and back.

The influence of infection status on temperature preference and thermoregulatory precision (i.e., variation in selected temperature as indicated by intraindividual SD) was assessed with linear mixed effects modeling (LMM), accounting for the following fixed effects: infection status (infected or uninfected), elapsed time (hours in apparatus), movement, and the hour by infection interaction. Snail ID, apparatus number, lane number, and date were included as random effects, while a random slope with respect to hour was assessed for each snail. Mixed effects models were fit using the lme4 package in R (Bates et al. 2015). Model selection procedures from the MuMIn package (Barton 2016) were used to rank and select the model with the lowest Akaike information criterion. Residuals were visually examined for normality using quantile-quantile (Q-Q) plots. To obtain *P* values, likelihood ratio tests (type II Wald's χ^2 test) using the car package (Fox and Weisberg 2011) were performed on the top-ranked models. Finally, the mean values of the final hour of each snail's selected temperature were compared to the respective null model's final hour means, according to the snail's movement and infection status, using Anderson-Darling tests of distributional differences. To conservatively account for sample sizes of the different groups, and since distribution tests are sensitive to slight deviations in large-sample distributions, we used bootstrapping to draw similarly sized samples at random from the null model distributions and used the mean *P* values from these bootstrapped ($N = 10{,}000$) comparisons.

Results

Total distance traveled was significantly lower (Pearson's $\chi^2_1 = 4.28$, $P = 0.034$) in the snails within the TG (mean: 247 cm) compared to those in the CT trials (mean: 494 cm; fig. 1), although infected snails did not move less overall compared to uninfected snails (Pearson's $\chi^2_1 = 2.61$, $P = 0.106$). After grouping snails into active and inactive categories, 23 snails were scored as inactive, 14 of which were uninfected and nine of which were infected. The remaining 101 snails were scored as active, with 84 that were uninfected and 17 that were infected. This categorical measure of activity in snails was therefore associated with their infection status (Pearson's $\chi^2_1 = 4.4$, $P = 0.04$); more uninfected snails were active than inactive, and while it was evident that infected snails were still active, a higher proportion of infected snails were scored as inactive.

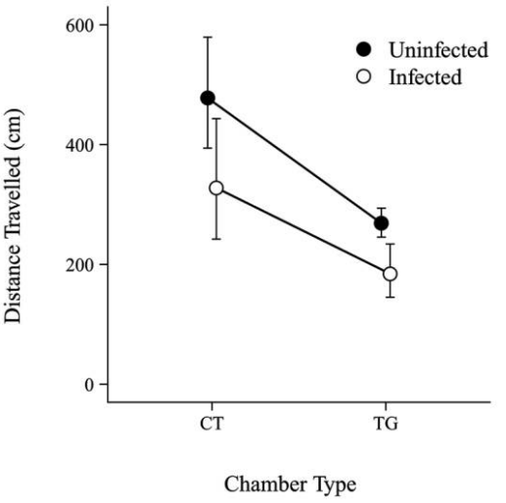

Figure 1. Behavior of freshwater snails (*Helisoma trivolvis*) during an 8-h period in a constant temperature (CT) and a thermal gradient (TG). The mean (± SE) total distance travelled in either a CT environment of ~22°C ($N = 7$) or a TG that spanned 15°–35°C ($N = 117$) is depicted, along with the distances moved by infected and uninfected snails. The test environment significantly influenced total distance moved ($P = 0.034$), although infection status did not ($P = 0.10$).

The best model for temperature selection included elapsed time, infection status, activity, and the interaction between infection and activity, while only the interaction term was significant (LMM hour effect, type II Wald's $\chi^2_1 = 2.91$, $P = 0.086$; activity: $\chi^2_1 = 1.14$, $P = 0.286$; hour × activity: $\chi^2_1 = 6.06$, $P = 0.014$). Infection status alone was not significant ($\chi^2_1 = 2.42$, $P = 0.120$). The snails that selected the warmest temperatures were those scored as active and infected (fig. 2*a*). To assess thermoregulatory precision, we focused only on active snails that were objectively sampling the available temperatures. The best model for thermoregulatory precision included both infection status and elapsed time as fixed effects. Elapsed time and infection status were significant (LMM hour effect, type II Wald's $\chi^2_1 = 3.73$, $P = 0.0535$; infection: $\chi^2_1 = 8.34$, $P = 0.0039$), showing that while all snails selected temperatures more precisely over time, infected snails had much higher thermoregulatory precision compared to uninfected snails (fig. 2*b*).

Null models yielded distributions within the TGs that varied according to activity level, showing nonuniform, low temperature bias with high variabilities in those models with active rates of movement ($\mu = 20.64$°C, SD = 5.0°C) and centrally focused, nearly symmetrical distributions ($\mu = 22.9$°C, SD = 2.4°C) in models with low rates of movement (fig. 3). The models with active rates of movement showed adequate dispersal throughout the gradient, whereas the models with low rates of movement did not disperse far from the introduction point (see figs. A2, A3). Comparing both active infected and active uninfected snails (fig. 3) to the active null model yielded significant differences ($P = 0.018$ for

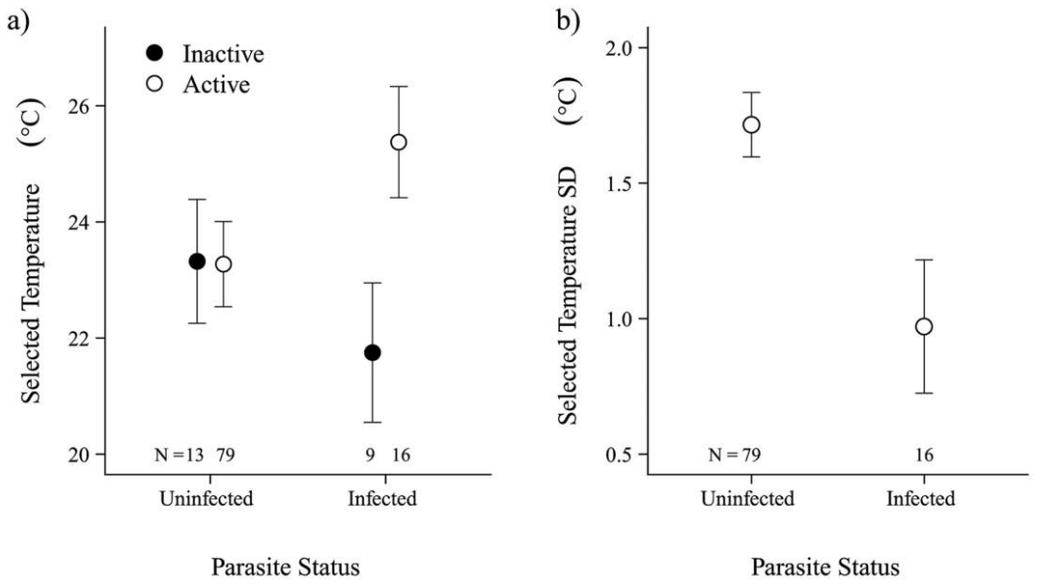

Figure 2. *a*, Temperature selection in freshwater snails (*Helisoma trivolvis*) during an 8-h period depicting mean selected temperature, separated into those that were uninfected or infected by the trematode parasite *Echinostoma trivolvis* as well as those that actively explored an experimental apparatus that spanned 15°–35°C (significant interaction between movement and infection status; $P = 0.014$). *b*, Temperature selection in freshwater snails (*H. trivolvis*) during an 8-h period depicting variability of the individual snails' selected temperature, that is, thermoregulatory precision, assessed from intraindividual standard deviations of temperature over the entire time period for the active infected versus active uninfected snails ($P = 0.0039$). Error bars represent the model standard errors. Sample sizes (N) for each category are depicted.

active infected; $P = 0.002$ for active uninfected), whereas inactive snails were not significantly different from the inactive null model ($P = 0.409$ for inactive infected; $P = 0.222$ for inactive uninfected). Active snail distributions were also significantly different from the inactive null model distributions ($P = 0.038$ for infected; $P = 0.0003$ for uninfected), further supporting the notion that behaviors of active snails differed from null, random conditions.

Discussion

Infection by parasites or pathogens has been shown to affect thermoregulation in ectotherms, and we found this for freshwater snails (*Helisoma trivolvis*) harboring the trematode *Echinostoma trivolvis*. As the distribution of actively moving snails in the TG differed significantly compared with the null model, this indicates that snails that are more active have a preferred temperature and do not simply move randomly with respect to water temperature. The fact that snails exposed to the TG traveled less than those in the CT environment further supports the conclusion that thermal cues shape *H. trivolvis* behavior. While there was no overall significant difference in temperature preference between infected and uninfected snails, analyses that incorporated activity showed that active infected snails selected warmer temperatures compared to active uninfected snails. In other words, of the snails that actively explored the TG, those with an infection ultimately chose a position that corresponded to a higher temperature. Because temperature can have a large effect on both digenean flukes (i.e., trematode worms) and their snail hosts, this can accrue possible benefits to either party as well as have implications for transmission to the next host in their complex life cycle.

Although our results give some support for behavioral fever, it is not clear why infected snails may prefer warmer ambient temperatures. For example, Paull and Johnson (2011) demonstrated that survival of *H. trivolvis* was lower at higher temperatures (26°C) compared with snails kept at lower temperatures (13°C), regardless of infection status. Although warmer temperatures have been shown to increase snail growth, snails are rarely able to clear a trematode infection once established (Minchella et al. 1985), so there are no clear benefits to attaining a larger size in this context (Paull and Johnson 2011). In addition, immune function may actually decline in extremely warm temperatures for freshwater snails (Seppäla and Jokela 2011).

Notably, because many snail hosts are castrated by trematodes, any changes to improve host survival should benefit only the parasite (Hechinger et al. 2009). This suggests that a preference for warmer temperatures by infected snails here may be a case of parasite manipulation to improve the chances of successful transmission of cercariae to second intermediate hosts, such as larval amphibians. Many studies have shown that warmer temperatures increase cercariae production and emergence, as well as the odds of successful host penetration, all of which should improve transmission success (Jensen and Mouritsen 1992; Mouritsen and Jensen 1997; Mouritsen 2002; Poulin 2006; Bates et al. 2011; Paull and Johnson 2011).

There are likely various reasons why some studies have reported that trematode-infected snails show a preference for

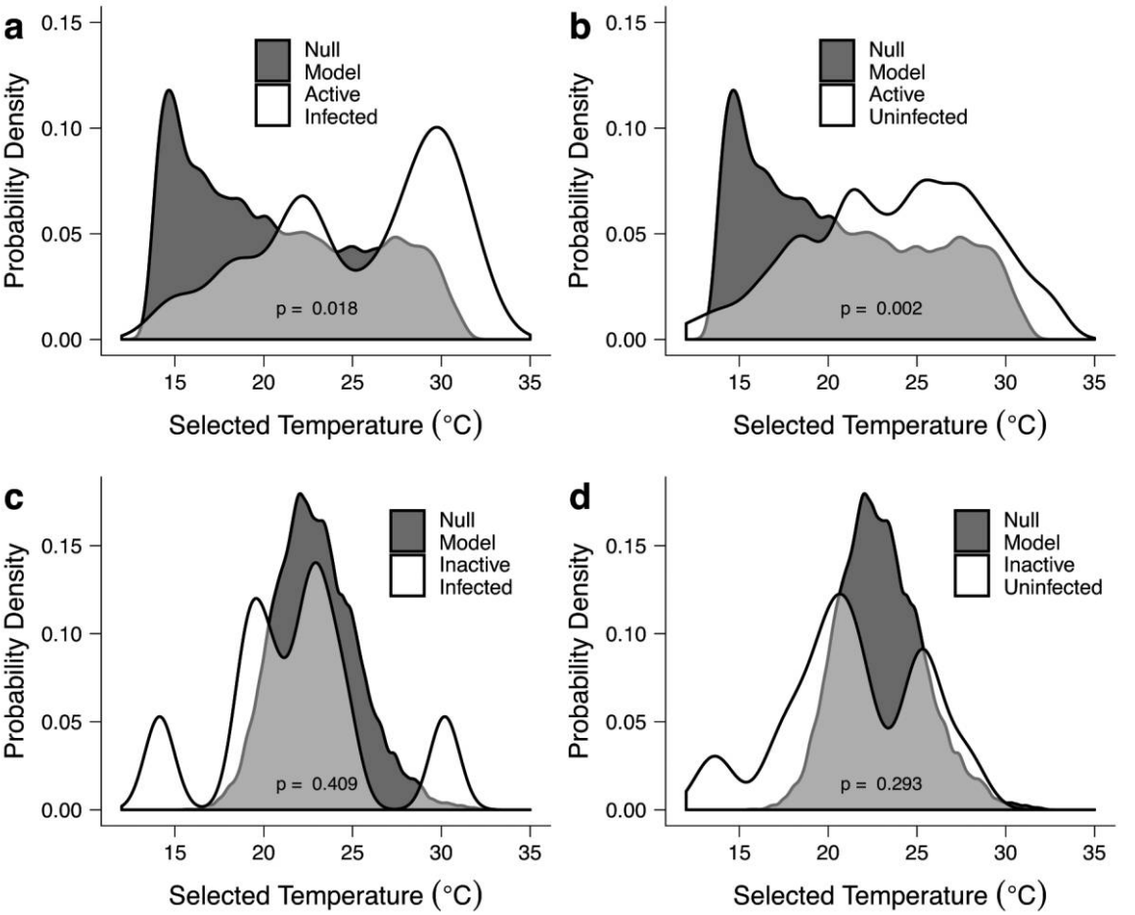

Figure 3. Probability density distributions of observed selected temperatures of snails that were active infected (*a*), active uninfected (*b*), inactive infected (*c*), or inactive uninfected (*d*) compared to their respective null model distributions derived from correlated random walk simulations ($N = 10{,}000$). Distributions depict the mean selected temperature for a snail in the final hour of the experiment or simulation. Randomly moving, active simulations show cold-weighted probability densities, whereas active snails are shifted to significantly higher selected temperatures. Inactive animals overlap substantially with their null model distributions. *P* values represent Anderson-Darling tests of differences in the distributions between observed values and the randomly drawn samples from the distributions (*P* values are the mean of bootstrapped values).

warmer temperatures and others have not. Changes in thermal behavior of snails stemming from trematode infection may be species specific, as seen in freshwater and marine snails (Żbikowska 2004, 2005; Bates et al. 2011). For instance, marine snails (*Zeacumantus subcarinatus*) infected with the trematode *Philophthalmus* spp. had a reduction in host thermal tolerance compared with those infected with *Maritrema* spp., even though both trematodes infect the same host tissues (Bates et al. 2011). On heating, *Philophthalmus*-infected snails did not display altered thermal preferences and fell into a coma, whereas *Maritrema*-infected snails were more active and selected higher temperatures than uninfected snails (Bates et al. 2011). Snails may also differ in their thermal preference depending on the stage of their trematode infection, especially in whether they harbor prepatent (no cercariae shedding) or patent infections. Infected snails that select colder temperatures (i.e., show signs of behavioral anapyrexia) are believed to benefit from reduced epithelial damage due to a slowdown in cercariae development and emergence (Żbikowska 2004). Given the various possible influences on snail host thermal preference, more research is needed to understand how temperature affects the association between trematode parasites and their snail hosts and the possible benefits that might drive snails to choose different temperatures. Further studies are especially needed for instances of behavioral anapyrexia, as it is unclear why infected snails may choose cooler temperatures if they rarely eliminate trematodes and have no opportunity to reproduce, unless this represents some unknown benefit for parasite transmission or is instead an artifact of temperature-sensitive locomotion, as revealed through temperature-sensitive null model approaches.

It is also important that studies of thermal preference use appropriate controls and null models to test whether animals are specifically responding to temperature when given a TG. Particularly with small ectotherms, the distribution of animals in an apparatus could be biased by two important factors: (1) time, as small, slow-moving animals may take longer to reach certain areas of the apparatus, or (2) metabolism and locomotion, as cold

areas of a TG would quickly immobilize animals with low thermal inertia. These factors could lead to misleading findings regarding thermal preference; thus, a null model is useful to confirm temperature selection in ectotherms as it demonstrates expected distributions in the absence of choice or preferences (e.g., Anderson et al. 2007). By comparing snails' total distance moved in a CT environment versus that in a TG environment, we provide further evidence that snails responded differently in the presence of TGs. We also grouped our snails into active or inactive categories based on their overall propensity to move within the apparatus. While animals are expected to reduce activity as they settle to a preferred temperature, some snails were inactive throughout the duration of the experiment, did not fully explore the apparatus, and remained withdrawn in their shells. Inactive snails, regardless of infection status, remained near the initial introduction point at the center of the apparatus. For parasitized snails, such inactivity may be related to general "sickness behaviors" that have been reported for infected animals, including anorexia, depression, and fever (Hart 1988, 1990).

For inactive uninfected snails, the midpoint of the apparatus was also similar to the housing temperature to which they were acclimated; therefore, they may not have been motivated to move far from the introduction point. This is further supported by the fact that active uninfected snails chose temperatures similar to those of the inactive uninfected snails, showing that the former returned to the initial introduction point despite exploration. The only group of snails that selected warmer temperatures was thus the active and infected snails, suggesting that parasitic infection may alter host thermoregulatory behaviors, but whether this is host mediated or parasite mediated is still unknown. The fact that the active infected snails showed substantially lower individual variation in selected temperature emphasizes the greater importance of thermoregulatory precision to those snails selecting warmer temperatures; in other words, the shift to warmer temperatures is not a random occurrence but appears to reveal a particular thermal strategy. It is unclear, however, why some infected snails were more active than others; various factors such as host condition, duration of parasitism, and infection patency may contribute to host behavior. Future studies can attempt to address these factors by experimentally infecting snails, which may also help to determine whether thermoregulatory behavior is mediated by the host immune system, is a by-product of parasitic infection, or represents parasite manipulation.

In this study, naturally infected snails were collected from the field to investigate their temperature preference. However, by examining thermal preferences of snails with established infections, we may lose critical information regarding the importance of thermoregulatory behavior during key events of the parasite life cycle, including snail resistance to miracidia during the initial point of infection, as well as sporocyst and rediae development. Miracidia induce an immune response in snails (Sandland and Minchella 2003), which could affect snail temperature preference differently early on when it might still be possible to fight the infection. For instance, *Biomphalaria glabrata* snails reduced their preferred temperature when penetrated by *Schistosoma mansoni* miracidia (Lefcort and Bayne 1991), suggesting that this behavioral anapyrexia increased host chances of survival as well as the number of circulating hemocytes to fend off the parasite (Pflüger et al. 1984). Consequently, there may be more crucial temporal windows during which thermal preferences may be altered that should be examined.

The tendency of trematode-infected snails to prefer warmer temperatures in our study also has consequences for their spatial distribution via microhabitat selection in natural habitats. Warm, shallow waters may optimize parasite transmission in cases where ectothermic second intermediate hosts choose to bask. Notably, larval amphibians, the second intermediate host for *E. trivolvis*, are often found in warmer waters (Brattstrom 1962). Therefore, infected snails selecting warmer temperatures could reflect parasite manipulation to transport them closer to their next host, serving as an extended parasite phenotype (Hechinger et al. 2009). This is particularly beneficial since most cercariae have very short life spans, typically less than 24 h (Johnson et al. 2004). However, these "pulses" of cercariae released in response to warm temperatures may be detrimental to the next intermediate host (e.g., Mouritsen 2002), particularly if there is a high abundance of infected snails, as pathology is often intensity dependent. Further studies are thus needed to understand the mechanism(s) that prompt(s) active infected snails to choose warmer temperatures so as to determine whether this may be an example of parasite manipulation or simply a by-product of pathology.

Temperature greatly affects many physiological processes, including that of parasites within their ectothermic hosts, and therefore changes in host thermal preference can influence parasite development and other factors relevant to transmission. We found that active *H. trivolvis* snails that were infected with the trematode parasite *E. trivolvis* chose warmer temperatures over time compared to active but uninfected snails. This altered behavior may be an instance of host manipulation by a parasite, as a preference for warmer waters is likely to increase the odds of successful transmission for *E. trivolvis* by promoting the production of infectious stages and/or causing spatial overlap with the next host in the parasite life cycle, especially because snails rarely rid themselves of established trematode infections. However, further research is needed to determine whether this change in thermoregulatory behavior by infected hosts truly confers any fitness advantages to the parasite, and it is also necessary to identify the mechanism(s) involved in a host behavioral alteration before it can truly be labeled as manipulation (Lafferty and Shaw 2013). Future studies to understand how temperature selection in snails may vary at different stages of infection (prepatent, patent, and exposed but uninfected) will also help to elucidate the possible benefits to either the host or the parasite and ultimately determine how this may affect the transmission of digenean trematodes, as well as how this may be affected by global climate change.

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