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Effects of temperature and viscosity on miracidial and cercarial movement of *Schistosoma mansoni*: ramifications for disease transmission

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ABSTRACT

Parasites with complex life cycles can be susceptible to temperature shifts associated with seasonal changes, especially as free-living larvae that depend on a fixed energy reserve to survive outside the host. The life cycle of *Schistosoma*, a trematode genus containing some species that cause human schistosomiasis, has free-living, aquatic miracidial and cercarial larval stages that swim using cilia or a forked tail, respectively. The small size of these swimmers (150–350 μm) dictates that their propulsion is dominated by viscous forces. Given that viscosity inhibits the swimming ability of small organisms and is inversely correlated with temperature, changes in temperature should affect the ability of free-living larval stages to swim and locate a host. By recording miracidial and cercarial movement of *Schistosoma mansoni* using a high-speed camera and manipulating temperature and viscosity independently, we assessed the role each factor plays in the swimming mechanics of the parasite. We found a positive effect of temperature and a negative effect of viscosity on miracidial and cercarial speed. Reynolds numbers, which describe the ratio of inertial to viscous forces exerted on an aquatic organism, were <1 across treatments. Q_{10} values were <2 when comparing viscosity treatments at 20 °C and 30 °C, further supporting the influence of viscosity on miracidial and cercarial speed. Given that both larval stages have limited energy reserves and infection takes considerable energy, successful transmission depends on both speed and lifespan. We coupled our speed data with mortality measurements across temperatures and discovered that the theoretical maximum distance travelled increased with temperature and decreased with viscosity for both larval stages. Thus, our results suggest that *S. mansoni* transmission is high during warm times of the year, partly due to improved swimming performance of the free-living larval stages, and that increases in temperature variation associated with climate change might further increase transmission.

1. Introduction

Seasonal variation in temperature can strongly affect parasite and host abundance, behavior, development, and survival, and thus the spread of emerging and re-emerging infectious diseases (Altizer et al., 2006; Lafferty, 2009). However, the direction of these effects can be additive, antagonistic, or synergistic (Daszak et al., 2001; McMichael et al., 2006; Rohr et al., 2011; Raffel et al., 2012; Cohen et al., 2018), making it difficult to predict net outcomes on disease transmission. For example, high temperatures can accelerate parasite reproduction and development but can also increase parasite mortality and reduce parasite fitness (Lafferty, 2009; Amarasekare and Savage, 2012). Examining the life history traits of parasites at all stages in response to temperature is therefore crucial to accurately predict host-parasite dynamics under seasonal conditions, which have been shown to influence transmission via rates of host exposure to parasites and parasite infectivity (Shocket et al., 2018).

Temperature has non-linear effects on reproduction, survival, and infectivity of many parasites and hosts (Studer et al., 2010; Dell et al., 2011; Johnson et al., 2015; Mordecai et al., 2013; Cohen et al., 2017; Rohr et al., 2018). Free-living stages of parasites in aquatic systems, which can be larval or intermediate stages, are especially sensitive to changes in temperature because they have finite energy reserves that deplete faster at higher temperatures (Pietrock and Marcogliese, 2003). Q_{10}, a measure of the rate of change of a reaction for every increase in 10 °C, is often used to describe how strongly temperature affects a physiological process.

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Most biological processes have a Q_{10} between two and three, with values less than two indicating that a physiological process is not very temperature-dependent and values greater than two indicating temperature dependency. Q_{10} has been used to measure the sensitivity of life history traits of parasites and hosts to seasonally relevant temperature ranges (Morley, 2011, 2012; Paull and Johnson, 2011); however, the effects of temperature and viscosity are confounded for small aquatic organisms (Podolsky and Emlet, 1993; Fuiman and Batty, 1997; Gemmell et al., 2013), so obtaining accurate Q_{10} measurements for free-living larvae requires parsing out the effects of viscosity.

Viscosity is inversely correlated with temperature and Reynolds numbers are used to describe the ratio of inertial to viscous forces exerted on an organism in a fluid. Small aquatic organisms typically have a Reynolds number less than one, which indicates that viscous forces dominate their locomotive ability and they must overcome resistance (i.e. viscous drag) to move through the water (Purcell, 1977). Conversely, large aquatic organisms with a Reynolds number greater than one are dominated by inertial forces and changes in the viscosity of a fluid will have less of an impact on locomotion (DeMont and Hokkanen, 1992). That is, larger aquatic organisms can generate enough force to resist viscous drag. Reynolds numbers are commonly reported in fluid mechanics literature and less commonly in parasite literature, but may offer additional insights into parasite locomotion and swimming performance across temperatures.

Schistosoma mansoni is a trematode species that causes schistosomiasis, one of the most prevalent neglected tropical diseases of humans (Hotez, 2013). Approximately 200 million people are infected and more than 800 million individuals are at risk of contracting the disease in endemic areas, particularly in sub-Saharan Africa (WHO, 2019). *Schistosoma mansoni* eggs enter freshwater through human feces and hatch into the first larval stage, called miracidia. These ciliated, 150 μm long organisms swim and mostly infect *Biomphalaria* spp. but can also infect other aquatic snails as an intermediate host. Once inside, miracidia develop into cercariae, which are slightly larger than miracidia (250–350 μm) and swim with a forked tail. Cercariae are released diurnally by snails back into freshwater and infect humans via skin penetration.

Few studies have examined the role of temperature and viscosity in parasite motility, even though free-living larval stages of parasites are small, likely operate under a Reynolds number less than one, do not feed, and thus have limited energy for propulsion. The swimming speed of larval stages has been found to increase the likelihood of infection due to increased contact rates with hosts (Fingerut et al., 2003; Pasternak, 2004), but this will not occur if mortality occurs before infection. Thus, there is a need to quantify the swimming mechanics of miracidia and cercariae in response to changes in temperature and viscosity, and the estimated distance that these larval stages can travel based on swimming speed and survival. The greater the distance these larval stages can travel, the presumed higher probability of transmission given that finding and penetrating a host takes considerable energy.

The main objectives of this study were to i) decouple and quantify the constitutive effects of temperature and viscosity on miracidial and cercarial speed, miracidial power during swimming, and cercarial tail-beat frequency and ii) calculate the theoretical maximum distance miracidia and cercariae can travel across their natural temperature range. To provide additional measures of how strongly temperature and viscosity affect movement, we calculated Q_{10} values and Reynolds numbers for miracidial and cercarial speed. We expected that higher temperatures would result in higher metabolic rates and thus faster swimming speeds, more power expended for miracidia, and quicker tail-beat frequencies for cercariae. However, we also expected that miracidial and cercarial speed, miracidial power, and cercarial tail-beat frequency would decrease under viscous forces because miracidia and cercariae are small and likely operate at Reynolds numbers less than one.

2. Materials and methods

2.1. Obtaining Schistosoma mansoni miracidia and cercariae

We harvested livers from Swiss Webster mice exposed to the NMRI strain of *S. mansoni*. A liver homogenate was passed through a 106 μm sieve (Fisherbrand, ASTM E-11 Standard, USA) and the solution that passed through the sieve was caught in another container. This solution, which contained *S. mansoni* eggs, was subsequently passed through a 45 μm sieve (Fisher Scientific Company, ASTM E-11 Standard, USA). We rinsed the eggs from this sieve with 1.2% saline into a beaker with an artificial freshwater solution modified from Baer et al. (1999) to induce hatching.

To obtain cercariae, *Biomphalaria glabrata* (NMRI strain) were exposed to 10–20 *S. mansoni* miracidia. Individuals were held at room temperature (22 °C), fed ad libitum (6 g of fish flakes and 6 g of spirulina in 2.5 g of agar), and tested for infection 8 weeks post-exposure. On the day of the experiment, infected snails were placed under artificial light for 1 h at room temperature (22 °C) to induce cercarial emergence. All cercariae were homogenized into one beaker with the same artificial freshwater solution described above.

2.2. Manipulation of temperature and viscosity

Water was either heated using a 200 W submersible aquarium heater or cooled using a 1/10 hp aquarium chiller unit. The water temperatures tested were 10 °C, 20 °C, and 30 °C. All data recordings (filming) were performed in a recirculating water bath to maintain the temperature within ± 1 °C and reduce the presence of small-scale convective currents within the filming vessel. We manipulated viscosity independently of temperature at 20 °C and 30 °C by dissolving methylcellulose polymer (25cP) (MC). A Cannon-Fenske routine viscometer was used to determine the concentration of methylcellulose solution that matched the kinematic viscosity of water at 10 °C ($\nu = 1.31 \times 10^{-6}$ $m^2 s^{-1}$). We had a total of five treatments: 10 °C, 20 °C, 30 °C, 20 °C with MC, and 30 °C with MC. Previous studies have shown no effect of methylcellulose on the metabolic rates of organisms (Luckinbill, 1973; Fuiman and Batty, 1997).

2.3. High-speed videography of miracidia and cercariae

Once *S. mansoni* miracidia and cercariae were collected, individuals were transferred to a glass observation cuvette (1 cm × 5 cm × 5 cm, Model 3-I-40, Starna Cells, Inc., USA) with water at 10, 20, 30 °C, 20 °C with MC, or 30 °C with MC. We used a high-speed camera at 1280 × 1024 pixel resolution (Edgertronic SC1, USA) to film miracidia and cercariae at 100 frames per second (fps), 250 fps, or 500 fps, depending on the organism's swimming speed (Fig. 1). All videos were imported into ImageJ (Schneider et al., 2012) for kinematic analyses. We only analyzed individuals that swam in the camera's focal plane over the entire period of quantification to minimize error associated with out of plane movements, which resulted in unequal sample sizes across treatments. We calculated average miracidial speed at 10 °C (n = 17), 20 °C (n = 24), 30 °C (n = 36), 20 °C with MC (n = 11), and 30 °C with MC (n = 21) by tracking individuals frame by frame and measuring the distance individuals traveled within that time period. We employed the same method for cercarial speed at 10 °C (n = 8), 20 °C (n = 10), 30 °C (n = 16), 20 °C with MC (n = 10), and 30 °C with

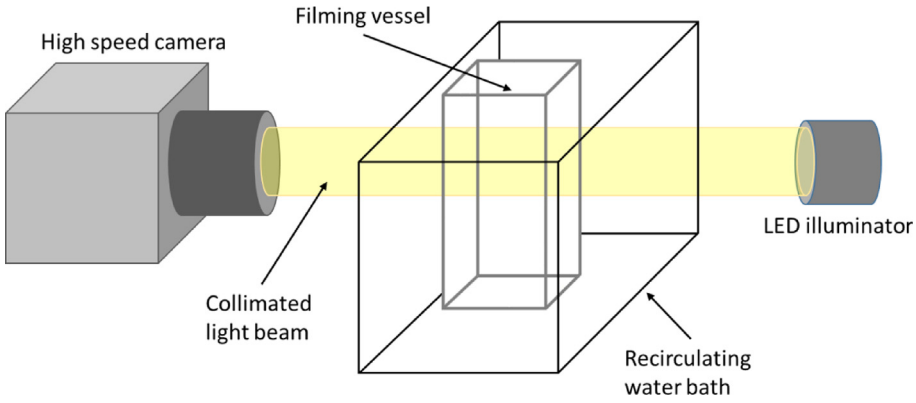

Fig. 1. Experimental set-up is shown here. Note, this diagram is not drawn to scale.

MC (n = 8). Water was seeded with neutrally buoyant particles (5–20 μm diameter) so for each swimming sequence, the speed of an in-focus nearby particle was also quantified to correct for any effects of convection currents present in the observation cuvette. We calculated net miracidial and cercarial swimming speeds and accounted for any convective currents present in the filming vessel by subtracting the speeds of nearby neutrally buoyant tracer particles. For cercarial tail-beat frequency, we manually counted five tail-beats per individual at five random intervals and calculated the average number of tail-beats per second per individual at 10 °C (n = 10), 20 °C (n = 10), 30 °C (n = 21), 20 °C with MC (n = 10), and 30 °C with MC (n = 10). An example of miracidia at 30 °C (15fps, Supplementary Movie S1) and cercariae at 30 °C (10fps, Supplementary Movie S2) without methylcellulose can be viewed online.

2.4. Statistical analyses

All statistical analyses were conducted in R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria, 2017). We applied a $\log_{10}$ (x + 1) transformation to miracidial and cercarial speed to adhere to assumptions of normality. To determine the effects of temperature independent of viscosity, we analyzed the miracidial and cercarial data separately and used two-sided t-tests to compare mean miracidial speed, cercarial speed, miracidial power, and cercarial tail-beat frequency between 10 °C and 20 °C with MC, 10 °C and 30 °C with MC, and 20 °C with MC and 30 °C with MC. Due to unequal sample sizes, the variance between some groups were unequal and a Welch's t-test was conducted instead. A sequential Bonferroni correction was then employed to account for multiple comparisons (Benjamini and Hochberg, 1995), with sequential P values of less than 0.05, 0.025, and 0.017 considered significant, respectively. Next, to analyze the overall effects of temperature on these response variables at 10 °C, 20 °C with MC, and 30 °C with MC, we applied a generalized linear model (*glm* function, *stats* package) with temperature as a continuous independent variable. To test for interactions between temperature and viscosity, we excluded the 10 °C treatment and used a 2x2 ANOVA (*Anova* function, *car* package) with temperature and viscosity as categorical independent variables. P values less than 0.05 were considered significant for these analyses.

To calculate the amount of power miracidia require to move through the water, we used the following equation for the metabolic cost of motility for ciliated organisms (Crawford, 1992):

$$P = 3\pi D v^2 \eta$$

where D is the organism's diameter (m), v is the organism's velocity (m/s), and η is the water viscosity (N*s/m^2). We did not apply the same equation to cercariae given their non-ciliated body morphology and unusual movement patterns.

To measure how strongly temperature affected miracidial and cercarial speed, we calculated Q_{10} values using the following equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10^{\circ}C}{(T_2 - T_1)}}$$

where R is the rate of the response variable and T is the temperature (°C).

To measure how strongly viscosity affected miracidial and cercarial speed, we calculated Reynolds number using the following equation:

$$Re = \frac{\rho u L}{\mu}$$

where ρ is the density of the fluid (kg/m^3), u is the velocity of the organism (m/s), L is the length of the organism (m), and μ is the dynamic viscosity of the fluid (N*s/m^2).

Lastly, to calculate the theoretical maximum distance miracidia and cercariae could travel, we extracted survival data at 10, 20, and 30 °C for miracidia from Anderson et al. (1982) and at 20 and 30 °C for cercariae from Lawson and Wilson (1980). Cercarial survival data at 10 °C was not available for extraction from the literature. We then multiplied longevity values by average swimming speed, assuming constant movement throughout miracidial and cercarial lifespans.

3. Results

3.1. Miracidial and cercarial speed, miracidial power, and cercarial tail-beat frequency

Temperature had a positive significant effect on miracidial speed (t = 4.71, df = 48, P < 0.0001, Table 1) and the 2x2 ANOVA showed a significant effect of temperature ($F_{1,88}$ = 25.92, df = 1, P < 0.0001) and viscosity ($F_{1,88}$ = 6.99, df = 1, P = 0.0097), but no interaction between those two factors ($F_{1,88}$ = 1.32, df = 1, P = 0.2534) (Fig. 2A). A Bonferroni-adjusted t-test revealed that mean miracidial speed was significantly greater at 30 °C with MC than 10 °C (P < 0.0001, Table 2). In fact, miracidia swam 2.5 times faster at 30 °C with MC than 10 °C, with a difference of 0.17 mm/s (95% Confidence Interval (CI): 0.10, 0.24). All other Bonferroni-adjusted t-tests were not significant (Table 2).

Temperature also had a positive significant effect on cercarial speed (t = 5.82, df = 25, P < 0.0001, Table 1). The 2x2 ANOVA indicated that there was a significant effect of temperature

Table 1
Speed, power, and tail-beat frequency for *Schistosoma mansoni* miracidia and cercariae as a function of temperature compared across 10 °C, 20 °C with methylcellulose (MC), and 30 °C with methylcellulose (MC). Models are shown below the response variable.

Generalized linear models	Intercept	Slope	Standard error
Miracidial speed			
Log_{10}(Speed + 1) ~ Temperature	0.014	0.008	0.002
Cercarial speed			
Log_{10}(Speed + 1) ~ Temperature	0.048	0.009	0.002
Miracidial power			
Power ~ Temperature	-1.257^{-12}	1.229^{-13}	3.391^{-14}
Cercarial tail-beat frequency			
Tail-beat frequency ~ Temperature	−5.215	1.135	0.018

($F_{1,40}$ = 69.31, *df* = 1, *P* < 0.0001) and viscosity ($F_{1,40}$ = 51.00, *df* = 1, *P* < 0.0001), but the interaction between those two factors was not significant ($F_{1,40}$ = 4.00, *df* = 1, *P* = 0.0525) (Fig. 2B). All Bonferroni-adjusted *t*-tests for cercarial speed were significant (Table 2).

Temperature had a positive significant effect on miracidial power (*t* = 3.62, *df* = 48, *P* = 0.0007, Table 1) (Fig. 3A). The 2x2 ANOVA showed a significant effect of temperature ($F_{1,88}$ = 16.31, *df* = 1, *P* = 0.0001), but not viscosity ($F_{1,88}$ = 0.42, *df* = 1, *P* = 0.5206) or the interaction ($F_{1,88}$ = 0.36, *df* = 1, *P* = 0.5526). The amount of power generated at 30 °C with MC was three times greater than that at 20 °C with MC (*P* = 0.0150, Table 2) and more than 18 times greater than that at 10 °C (*P* = 0.0019, Table 2). All other Bonferroni-adjusted *t*-tests were not significant (Table 2).

Temperature also had a positive effect on cercarial tail-beat frequency (*t* = 62.90, *df* = 149, *P* < 0.0001, Table 1). The 2x2 ANOVA indicated there was a highly significant effect of temperature ($F_{1,251}$ = 1725.95, *df* = 1, *P* < 0.0001), but not viscosity ($F_{1,251}$ = 0.28, *df* = 1, *P* = 0.5944) or the interaction ($F_{1,251}$ = 2.67, *df* = 1, *P* = 0.1034) (Fig. 3B). All Bonferroni-adjusted *t*-tests for cercarial tail-beat frequency were significant (Table 2).

3.2. Q_{10}, Reynolds number, and maximum distance travelled

For miracidial and cercarial speed, Q_{10} values were between 1.05 and 2.65, with cercariae exhibiting the widest range of values. Q_{10} values for miracidial speed were greater than two in treatments where viscosity was not manipulated but decreased to less than two when MC was added. Q_{10} for cercarial speed exhibited similar trends (Table 3). Reynolds numbers for miracidial and cercarial speed were less than one across all treatments, but increased with temperature and decreased with viscosity (Table 3). That is, treatments with MC had smaller Reynolds numbers compared to treatments at the same temperature where viscosity was not manipulated for both larval stages.

Despite dying sooner at higher temperatures, the estimated maximum distance miracidia and cercariae could travel increased significantly with temperature (Fig. 4). When MC was added, the distance miracidia could travel decreased from 39 and 52 m to approximately 33 and 34 m at 20 and 30 °C, respectively (Fig. 4A). The distance cercariae could travel decreased from approximately 122 and 127 m to 100 and 81 m at 20 °C and 30 °C, respectively (Fig. 4B). Overall, the degree to which viscosity decreased the distance cercariae could travel at 30 °C versus 30 °C with MC was much greater than the decrease between 20 °C versus 20 °C with MC.

Table 2
Speed, power, and tail-beat frequency for *Schistosoma mansoni* miracidia and cercariae compared at 10 °C, 20 °C with methylcellulose (MC), and 30 °C with methylcellulose (MC). A Welch's *t*-test was conducted for groups with unequal variance. All treatments are listed in descending order of *P* values and are bolded for significance according to sequential Bonferroni corrections.

Comparison	*t*	*df*	*P*
Miracidial speed			
20 °C with MC & 30 °C with MC	1.90	30.00	0.0674
10 °C & 20 °C with MC	2.50	11.51	0.0287
10 °C & 30 °C with MC	4.93	22.10	**<0.0001**
Cercarial speed			
20 °C with MC & 30 °C with MC	3.19	16	**0.0057**
10 °C & 20 °C with MC	3.25	16	**0.0050**
10 °C & 30 °C with MC	5.36	14	**0.0001**
Miracidial power			
10 °C & 20 °C with MC	2.15	10.15	0.0570
20 °C with MC & 30 °C with MC	2.61	24.88	**0.0150**
10 °C & 30 °C with MC	3.58	20.04	**0.0019**
Cercarial tail-beat frequency			
10 °C & 20 °C with MC	69.75	82.64	**<0.0001**
10 °C & 30 °C with MC	53.84	53.14	**<0.0001**
20 °C with MC & 30 °C with MC	26.69	59.32	**<0.0001**

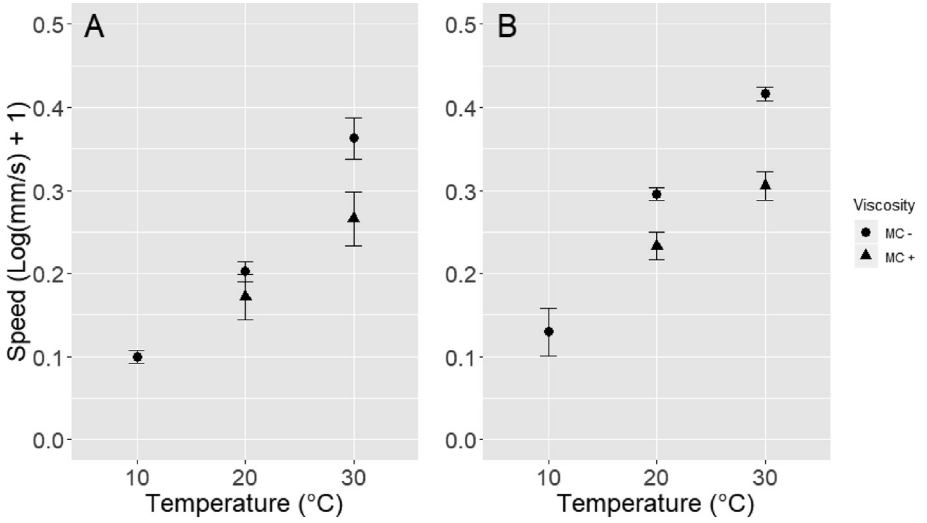

Fig. 2. Mean (±1 SE) *Schistosoma mansoni* miracidial speed (A) and cercarial speed (B) under various temperature and viscosity treatments. ● and ▲ represent no methylcellulose (MC-) and methylcellulose (MC+) treatments, respectively.

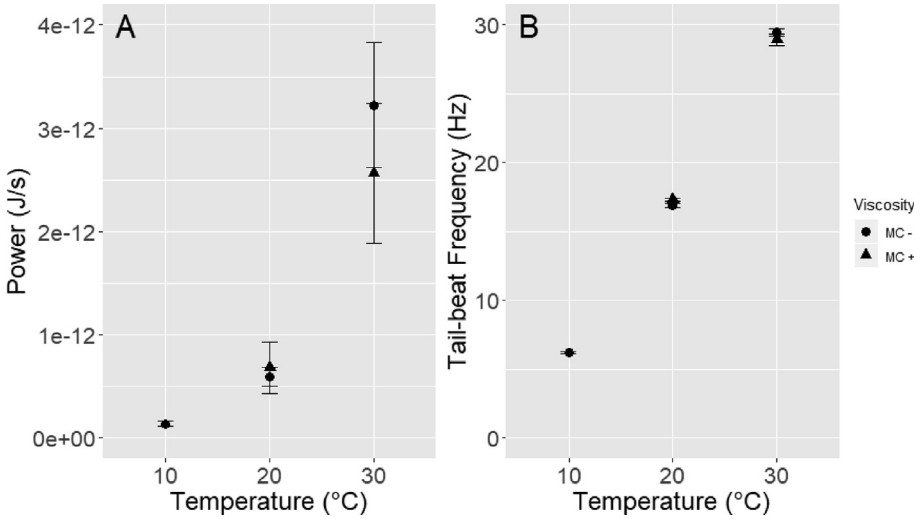

Fig. 3. Mean (±1 SE) *Schistosoma mansoni* miracidial power (A) and cercarial tail-beat frequency (B) speed across temperature and viscosity treatments. ● and ▲ represent no methylcellulose (MC-) and methylcellulose (MC+) treatments, respectively.

Table 3
Reynolds numbers and Q_{10} values for *Schistosoma mansoni* miracidial and cercarial speed across treatments with and without methylcellulose (MC).

	Miracidia		Cercariae	
Treatment	Reynolds number	Q_{10}	Reynolds number	Q_{10}
10 °C	0.030	–	0.071	–
20 °C	0.091	2.32	0.244	2.65
30 °C	0.271	2.35	0.504	2.09
20 °C MC	0.059	1.97	0.138	1.95
30 °C MC	0.109	1.91[a], 1.85[b]	0.196	1.05[a], 1.43[b]

[a] Compared between 30 °C MC and 20 °C.
[b] Compared between 30 °C MC and 20 °C MC.

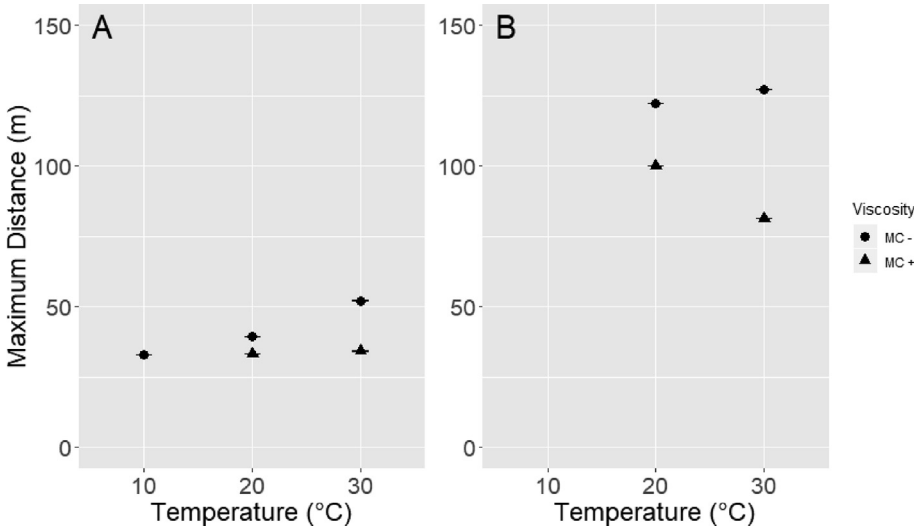

Fig. 4. Mean (±1 SE) estimated maximum distance traveled by *Schistosoma mansoni* miracidia (A) and cercariae (B) across temperature and viscosity treatments, based on active swimming speeds and longevity. Standard errors are derived from miracidial and cercarial speed exclusively because error estimates associated with longevity were not always provided in the original papers from which these data were extracted. ● and ▲ represent no methylcellulose (MC-) and methylcellulose (MC+) treatments, respectively.

4. Discussion

In this study, we decoupled the effects of temperature and viscosity over a natural seasonal temperature range on the aquatic larval stages of the human parasite *Schistosoma mansoni* to understand the physiological mechanisms underlying their movement. We found that temperature and viscosity have significant effects on miracidial and cercarial swimming speeds and the overall distance that these larval stages can travel. Cercarial tail-beat frequency and miracidial power were strongly affected by temperature but unaffected by changes in viscosity. Reynolds numbers for miracidial and cercarial speed were less than one

across all treatments, supporting the notion that both larval stages operate at low Reynolds numbers. Q_{10} values for miracidial and cercarial speed were greater than two when viscosity was not manipulated. These values decreased to values less than two when methylcellulose was added, indicating that the effect of temperature independent of viscosity was weaker than the effect of these factors combined. Said another way, the relative contribution of temperature to increasing miracidial and cercarial speed was overestimated when temperature and viscosity were coupled. Theoretical maximum distance calculations provided additional evidence that low viscosity at high temperatures strongly impact parasite locomotion.

Swimming speed is an important parameter for estimating exposure rates between the larval stages of *S. mansoni* and their respective hosts. If aquatic larval stages of parasites can swim faster and travel farther before mortality, then the likelihood of encountering and infecting a host increases. Here, we found that miracidial speed nearly doubled with every increase of 10 °C, which aligns with previous results that showed that the swimming rate of *S. mansoni* miracidia almost doubled from 4 to 12 °C, 12 °C to 22 °C, and 22 to 34 °C (Samuelson et al., 1984). Similarly, we found that cercarial speed more than doubled with every increase of 10 °C. These results could be used in mathematical models to more accurately reflect mechanisms underlying disease transmission. For example, a study conducted by Civitello and Rohr (2014) found that models separating exposure and infection rates of *S. mansoni* miracidia produced better predictions of prevalence in intermediate snail host populations compared with models that combined these processes. Incorporating exposure rate as a function of swimming speed and longevity may therefore produce more ecologically relevant predictions of disease risk.

Previous studies have used methylcellulose or similar agents to decouple the effects of temperature and viscosity. All noted that the locomotive ability of free-living organisms with low Reynolds numbers decreased when viscosity was increased at high temperatures; this trend was not seen for larger, high Reynolds number organisms that can more easily overcome viscous forces (Luckinbill, 1973; Linley, 1986; Podolsky and Emlet, 1993; Fuiman and Batty, 1997; Gemmell et al., 2013). Our results agree with these findings, as we found that the swimming performance of miracidia and cercariae was poor at 10 °C when viscosity was high. Surprisingly, miracidial speed did not significantly decrease in the 20 °C with MC treatment compared with the 20 °C treatment while cercarial speed did, suggesting that the larger cercarial stage may be more vulnerable to changes in viscosity at intermediate temperatures. This result was unexpected because miracidia generate thrust through multiple cilia and should be more strongly impacted by viscosity due to a higher surface area to volume ratio. Conversely, cercariae can only generate thrust from a single forked tail and should be less impacted by viscosity due to a lower surface area to volume ratio (Samuelson et al., 1984; Biewener, 2003). However, both miracidial and cercarial speed significantly decreased in the 30 °C with MC treatment compared with the 30 °C treatment, which demonstrates that free-living larval stages are strongly affected by changes in viscosity at high temperatures.

Miracidia and cercariae have very different body morphologies and means of locomotion, making direct comparisons difficult. Cercarial tail-beat frequency and miracidial power are not directly comparable, but they do provide an indirect measure of the effect of temperature and viscosity on locomotive ability. Viscosity did not significantly hinder cercarial tail-beat frequency and miracidial power production across temperatures in this study. A recent study conducted by Krishnamurthy et al. (2016) found that cercariae generate thrust by constricting smooth muscles at the anterior and posterior ends of their tail. Importantly, the joints at these ends are flexible and allow cercariae to bend their tails in a way that generates enough thrust to overcome viscous drag. Similar adaptations are present among other low Reynolds number swimmers and could explain why tail-beat frequency and miracidial power are driven more by temperature, which influences metabolic rate and muscle contractions, than changes in viscosity (Crawford, 1992).

For miracidial and cercarial speed, Reynolds numbers increased with temperature and Q_{10} values were greater than two for treatments without MC, supporting the notion that the swimming mechanics of these organisms is temperature-dependent. However, Reynolds numbers for treatments with constant viscosity (i.e. 20 °C with MC and 30 °C with MC) were much smaller than treatments where viscosity was not manipulated (i.e. 20 °C and 30 °C), indicating that a decrease in viscosity associated with high temperatures allows miracidia and cercariae to move more efficiently than the effect of temperature alone. Similarly, Q_{10} values were less than two when MC was added, indicating that the influence of temperature on speed was likely overestimated when the effect of viscosity was ignored. This trend has been observed in other aquatic organisms (Podolsky and Emlet, 1993; Fuiman and Batty, 1997).

By independently manipulating temperature and viscosity, we were able to measure the relative effects of temperature and viscosity on the swimming performance of low Reynolds number swimmers. Coupling these findings with temperature-dependent longevity data allowed us to calculate the theoretical maximum distance miracidia and cercariae could travel as a function of energy availability and swimming speed, which decreased significantly with high viscosity (Fig. 4). These results make sense, as the change in viscosity from 30 °C ($\nu = 8.00 \times 10^{-7}$ m^2s^{-1}) to 10 °C ($\nu = 1.31 \times 10^{-6}$ m^2s^{-1}) is substantial (64% increase) and will have the greatest impact on small, low Reynolds number swimmers that move in a fluid regime dominated by viscous forces. In contrast, larger organisms would not be impacted greatly by such changes in kinematic viscosity due to the fact that they experience a primarily inertial fluid regime. Overall, our estimates of maximum distance are more easily applied to miracidia, as they are constant swimmers, but represent a conservative estimate for cercariae, as they spend time passively sinking in addition to actively swimming (Graefe et al., 1967; Nuttman, 1974; Brachs and Haas, 2008; Krishnamurthy et al., 2016). Cercariae are likely able to travel farther than our estimate of 127 m at 30 °C due to an alternating pattern of swimming and passive sinking in which the passive phase would require little of the stored metabolic energy. For both miracidia and cercariae, increasing temperature may indirectly increase the probability of encountering an intermediate snail host or a human host, respectively, because the cost of increased swim speeds and shorter lifespan is smaller than the cost of decreased swim speeds under viscous conditions. Taken together, these results suggest that the energetic cost of enhanced swimming performance for both larval stages is partially offset by low viscosity at high temperatures and the survival of these organisms may not be as negatively impacted by high temperatures as previously assumed.

We provide many lines of evidence showing that miracidia and cercariae display poor swimming performance due to the additive effects of low temperature, which decreases metabolic rate, and high viscosity, which decreases swimming efficiency. Conversely, low viscosity at high temperatures reduces the energetic cost of movement for both larval stages and allows them to travel farther than they can at low temperatures despite shorter lifespans. That is, the tradeoff between faster speed and shorter lifespan at high temperatures seems to result in a net benefit for miracidia and cercariae. As such, warming seasonal and diel temperature variation associated with global climate change might increase the exposure rate to potential hosts and thus increase disease transmission (Poulin, 2006; Lafferty, 2009; Mas-Coma et al., 2009). Biotic factors,

economic development, urbanization, human migration, mass drug administration, and other factors also influence disease transmission and should not be discounted (Thieltges et al., 2008; Johnson et al., 2009; Blum and Hotez, 2018), but the results presented here offer a novel perspective on why parasitic diseases may increase in the context of global climate change.

This study is one of only a few to apply principles of fluid mechanics to the aquatic larval stages of a human parasite to improve our understanding of how temperature and viscosity affect parasite transmission. Overall, this work provides a fundamental mechanistic understanding of parasite movement across temperatures and explains how increases in seasonal temperature variation might increase disease transmission in host-parasite systems that have free-living larval stages.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.12.003.

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