Journal of Helminthology

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Determining acute thermal limits of trematode cercariae using the CT_{max} approach

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Short Communication

Cite this article: Zenke L, Jolma ER, Born-Torrijos A and Thieltges DW (2026). Determining acute thermal limits of trematode cercariae using the CT_{max} approach. *Journal of Helminthology*, **100**, e83, 1–4
https://doi.org/10.1017/S0022149X26101424

Received: 17 December 2025
Revised: 16 March 2026
Accepted: 17 March 2026

Keywords:
Climate change; heatwaves; pulse warming; parasitism; thermal biology

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Abstract

Short-term thermal stress during heatwaves can potentially affect the transmission of parasites by increasing mortality of infective stages. Using the critical thermal maximum approach (CT_{max}), commonly used in free-living species, we exposed trematode cercariae of three marine species (*Cryptocotyle lingua, Himasthla elongata, Renicola parvicaudatus*) to a temperature ramping rate similar to realistic heatwave scenarios in the field and determined their acute temperature limits. The median CT_{max} values of all cercariae were much higher than the typical temperature extremes observed during heatwaves in the field. CT_{max} values slightly differed among the three trematode species and were higher than the thermal limit of their first intermediate host, the periwinkle *Littorina littorea.* This suggests that heatwave effects on trematode cercariae are likely not direct but more indirect, resulting from effects on the survival of their first intermediate host. Given its straightforward methodology and the potential for standardized comparisons of thermal limits among species and ecosystems, the CT_{max} approach holds promise for ecological research on the thermal biology of parasites.

Introduction

The transmission of parasites from one host to the next is often temperature-dependent (Rohr et al. 2011). This has been particularly well studied in trematode cercariae, the free-living infective stages between first and second intermediate hosts. An increase in temperature usually leads to an elevated release of cercariae from first intermediate hosts and a higher infectivity in second intermediate hosts (Morley and Lewis 2015; Poulin 2006). However, cercarial survival usually decreases with increasing temperature due to a faster depletion of the finite energy reserves of cercariae in the form of glycogen (Pechenik and Fried 1995). The typical experimental approach to study temperature effects on cercarial stages is the exposure to different levels of constant temperature treatments (e.g. Koprivnikar and Poulin 2009; Studer et al. 2010). While this approach can be informative about the general thermal biology of cercariae (Morley 2011; Morley and Lewis, 2013, 2015), it does not reflect short-term heat stress in the form of pulse warming such as heatwaves, which are becoming more frequent with climate change (Claar and Wood 2020). Warming rates during heatwaves can be very rapid in habitats such as shallow pools in freshwater systems or intertidal habitats during low tide (Desforges et al. 2023; Helmuth 1998; Jolma et al. 2026). During these rapid warming events, temperatures may potentially reach or exceed the upper thermal limits of cercarial stages. However, while upper thermal limits have been extensively studied in free-living species (e.g. Cereja 2020; Raby et al. 2025), they have rarely been investigated in parasites.

Here, we use the critical thermal maximum approach (CT_{max}) to study the acute thermal limits of the cercarial stages of three marine trematode species. In this approach, organisms are experimentally exposed to a gradual temperature ramping (rather than constant temperature treatments) until a sublethal endpoint is reached, at which motor control is lost (loss of equilibrium, Becker and Genoway 1979; Desforges et al. 2023; Raby et al. 2025). This approach can inform about short-term pulse warming effects but can also be used more generally as an indicator of the thermal limits of organisms and for comparisons among species (Bates and Morley 2020). In habitats where temperature can rise rapidly during heatwaves, the temperature ramping approach may also realistically reflect the situation in the field (Desforges et al. 2023). The three trematode species studied here use the common periwinkle *Littorina littorea* as their first intermediate host. This host species is very common in the intertidal zone of our study area and lives on hard substrate structures such as dykes and mixed mussel and oyster beds. When the latter are exposed during low tide, temperature can increase very rapidly (>20°C h^{-1} in extreme cases) during heatwaves and reach up to 36°C (Jolma et al. 2026). By exposing cercariae to a temperature ramping rate similar to that observed in the field, we determined the acute temperature limits of cercariae of *Cryptocotyle lingua, Himasthla elongata*, and *Renicola*

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parvicaudatus (previously *R. roscovita*). The first species uses fish, while the latter two species use bivalves as second intermediate hosts; all three species use birds as definitive hosts (Werding 1969). The aim of our study was to investigate whether the acute thermal limits of the three species might be exceeded during heatwaves and whether these differ among the three trematode species.

Materials and methods

Cercariae from three trematode species (*Cryptocotyle lingua, Himasthla elongata*, and *Renicola parvicaudatus* (previously *R. roscovita*)) originated from a stock of infected snails collected in Mokbaai, Texel, in November 2023 and were maintained in aquaria in a climate chamber at 15°C. For the experiment in September 2024, 43 snails (n = 16 for *C. lingua*, n = 14 for *H. elongata*, and n = 13 for *R. parvicaudatus*) were placed individually into 6-well plates with filtered seawater and kept in an incubator at 23–25°C for up to 180 minutes to induce shedding of the parasites. Every 30 minutes, the wells were checked for free-swimming cercariae and in total 48 cercariae of *H. elongata* and *R. parvicaudatus* (3–6 cercariae per snail, depending on shedding rates) were collected and individually pipetted into 24-well plates (n = 4 plates) filled with 2 mL of freshly filtered seawater. As the cercariae of *C. lingua* were prone to losing their delicate tails during the experiment, either during pipetting or as a stress response to the heating itself, which critically injured them, a larger number (n = 52) of cercariae of this species (1–6 cercariae per snail, depending on shedding rates) were used in the assay (n = 6 plates). All cercariae were acclimated to 16°C for 30 minutes before the experiment started.

The temperature ramping assay was conducted by increasing the temperature of an incubator (VEVOR Digital Thermoelectric Incubator) by 6°C per hour in steps of 2°C every 20 minutes, starting at 16°C. The temperature inside the cabinet was logged by loggers placed next to the well plates (Electric Blue EnvLogger T7.3). Every 20 minutes, the well plates were briefly removed from the incubator and observed under a microscope to record the status of the cercariae as either active (cercariae moving, i.e., actively swimming or crawling on the well bottom) or non-active (cercariae sunken to the bottom of the well and not moving, even after probing with a metal dissection needle). The temperature at which cercariae became non-active was recorded as critical maximum temperature (CT_{max}). To control for the capacity of the cercarial glycogen storage during each run, three cercariae of each species were kept at 16°C for the length of the assay (approx. 7 hours) and their activity was tested at the end.

Data analysis was conducted in R 4.3.2. using RStudio (R Core Team, 2023). Linear mixed models including *snail identity* and *plate* as random effects were used to compare the CT_{max} of the different species, using the package *nlme* for statistical analysis (Pinheiro et al. 2025). The package *ggplot2* was used for visualization (Wickham 2016). All raw data are available in a public data repository (Zenke et al. 2026).

Results and discussion

Due to tail loss, 32 cercariae of *C. lingua* had to be excluded from the analysis and three cercariae of *R. parvicaudatus* were unexpectedly lost, resulting in critical maximum temperature (CT_{max}) values being obtained for 24 *C. lingua*, 48 *H. elongata*, and 45 *R. parvicaudatus* cercariae. While the mean CT_{max} of *R. parvicaudatus* (mean ± SE (standard error): 54.8°C ± 0.54) and *H. elongata* (54.5°C ± 0.38) were similar, *C. lingua* (53.0°C ± 0.54) showed a significantly lower critical temperature ($p < 0.05$) than the other two species (Table 1; Figure 1). All cercariae that were kept at 16°C remained active until the end of the experiment.

Table 1. Linear mixed model output with the mean CT_{max} per species

	Value	Standard Error	Degrees of freedom	t-value	p-value
(Intercept)	54.457	0.382	71	142.759	<0.001
Cryptocotyle lingua	−1.496	0.540	11	−2.769	0.018
Renicola parvicaudatus	0.335	0.538	11	0.622	0.547

The intercept is the trematode species *Himasthla elongata*.

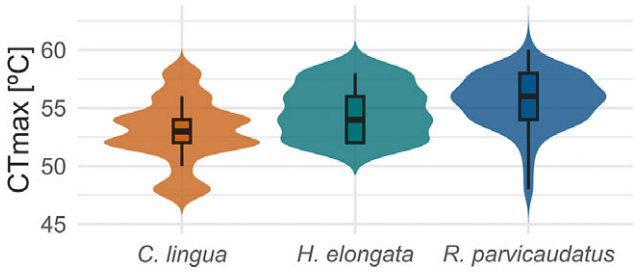


Figure 1. Violin boxplots showing critical maximum temperatures (CT_{max}) of cercariae of three trematode species. N = 24 for *Cryptocotyle lingua*, 48 for *Himasthla elongata*, and 45 for *Renicola parvicaudatus*.

The CT_{max} values recorded for all three trematode species in our experiment (53.0 to 54.8°C) were much higher than the temperature extremes observed during heatwaves in typical habitats of their first intermediate gastropod host, such as mixed oyster and mussel beds. In particular, in our study area, temperatures can reach up to 36°C during low tide in the tide pools on these beds (Jolma et al. 2026). Such tide pools can be hot spots of infections for trematode cercariae as the remaining water volume during low tide is small, increasing the exposure of second intermediate hosts to infective cercarial stages. Although the increased temperature of these pools during heatwaves could compromise the survival of cercariae, our experiment based on realistic temperature ramping suggests that the acute thermal limits of the three trematode species are not exceeded during typical temperature extremes experienced during heatwaves in our study area.

In contrast to the cercarial stages, the thermal limit of their first intermediate host, the periwinkle *Littorina littorea*, is much lower, at about 30°C (determined in snails collected from the field with unknown infection status; Clarke et al. 2000). This is in line with experimental studies based on constant temperature treatments, which showed that the survival of *L. littorea* infected with *H. elongata* strongly declined above 28°C, suggesting an increased mortality of infected periwinkles (Díaz-Morales et al. 2022). Given the similar CT_{max} values of the three trematode species and their competence in having a higher thermal limit than that naturally reached in tide pools, it is thus likely that heatwaves may generally have stronger negative effects on parasites indirectly via increased first intermediate host mortality rather than directly via effects on cercarial survival.

Although the CT_{max} values of the three trematode species were very similar, we found significant differences among the three

https://doi.org/10.1017/S0022149X26101424 Published online by Cambridge University Press

species. As a general rule, thermal limits are often higher in smaller organisms, likely due to higher metabolic rates or more efficient surface-to-volume ratios allowing for a faster acclimation compared to larger individuals (Cohen et al. 2017; Rohr et al. 2018). Likewise, in our experiment, the species with the smallest cercaria, *R. parvicaudatus* (body and tail length: 240 µm, 148 µm; Werding 1969), had the highest CT_{max} (54.8°C) compared to the larger species *H. elongata* (54.5°C, 632 µm, 577 µm; Werding 1969) and *C. lingua* (53.0°C, 232 µm, 389 µm; Werding 1969). However, in contrast to the expectation, the largest species (*H. elongata*) did not have a lower CT_{max} than the intermediate-sized species (*C. lingua*). Hence, the underlying mechanisms of the subtle but significant differences among the three species remain unclear. It also remains to be determined why the CT_{max} values of the three trematode species were generally higher than those of free-living species, which are usually well below 40°C in temperate species (Cereja 2020). Heat shock proteins may play a role, as they are known to be important in parasite-host interactions, sometimes leading to a high CT_{max} as a side effect (He et al. 2023; Polla 1991). It is also possible that the high CT_{max} values are, at least in part, methodological artefacts. The temperature loggers placed in the cabinet confirmed the temperature ramping, with variations of less than 1°C, and due to the small water volumes in the wells (2 mL) their temperature was presumably tightly following the cabinet temperature. However, it is possible that the short removal from the cabinet for observations led to a slight decrease in temperature in the wells. In addition, for the CT_{max} determination, the temperature at the end of a 20-minute 2°C ramping interval was used, while some individuals may have lost their mobility already at the beginning of the interval and thus at a 2°C lower temperature. This suggests that the actual CTmax of the three species may be a few degrees lower. While a potential overestimation of the CT_{max} does not compromise the main conclusions of our study, more precise temperature measurements would be desirable for comparisons among different studies.

Given its straightforward methodology and its potential for standardized comparisons of thermal limits among species and ecosystems (e.g., Ruthsatz et al. 2024), the CT_{max} approach holds promise for ecological research on the thermal biology of parasites. Detailed recommendations on increasing the comparability of thermal limit measurements exist from studies on free-living species (e.g., Desforges et al. 2023, Raby et al. 2025) and can easily be adjusted for parasitological research. As in free-living species, the approach may be useful for a variety of research questions, such as identifying the sensitivity of parasites to short-term thermal stress on small scales and exploring how thermal tolerances drive current and future distributions of parasites on larger scales.

Acknowledgements. We thank the two reviewers for their constructive comments.

Financial support. Financial support for the contribution of Ana Born-Torrijos was provided by the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 101027941. Open access funding provided by University of Groningen.

Competing interests. The authors declare none.

Data availability statement. All raw data are available in https://data.4tu.nl/datasets/187e1f6a-8152-415f-8ff3-4ca9f15bb058 (Zenke et al. 2026).

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