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Research Article

Effect of Short-Term Temperature Change on Cercarial Release by *Rhipidocotyle fennica* (Trematoda, Bucephalidae) from the Freshwater Bivalve Host, *Anodonta anatina*

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Abstract Cercarial release from the first intermediate host is an important stage in the transmission of trematode parasites. Besides long-term (seasonal) temperature fluctuations, short-term temperature changes can also influence cercarial emergence. We tested the response of the bucephalid trematode, *Rhipidocotyle fennica* (*R. fennica*), acclimatized to 17 °C, to an abrupt temperature change. As the natural cercarial shedding by this parasite takes place annually during the warmest season, we expected a positive effect of temperature increase. Monitoring during one hour after the transfer from 17 °C to 20 °C revealed a significant increase in *R. fennica* cercarial release compared to the preceding one hour period. In contrast, cercarial release decreased in clams transferred to 14 °C, while no change was observed in control clams transferred from 17 °C to 17 °C. This shows that the cercarial release by *R. fennica* is sensitive to short-term temperature change, and, as predicted, responds positively to warming and negatively to cooling. The result emphasizes the importance of (i) temperature on the cercarial production of trematodes, and (ii) the need to carefully control temperature conditions when studying factors influencing the cercarial production of trematodes.

Keywords Cercaria; host-parasite relationship; mussel; parasite; temperature; transmission; trematodes; Unionidae

1. Introduction

Transmission of trematode parasites from the first intermediate (mollusk) host to the second intermediate host is achieved by cercarial larvae. Thus, production of cercariae is an important stage in the trematode life cycle and contributes to the life-time reproductive success of the parasite. Within mollusk hosts, trematodes multiply asexually and produce large numbers of cercariae, which usually emerge to find the next hosts [1]. For example, peak cercarial production rate of the bucephalid trematode *Rhipidocotyle fennica* (*R. fennica*) can reach over 20.000 larvae d^{-1} [2] and that of the diplostomatid species *Diplostomum (pseudo) spathaceum* almost 40.000 larvae d^{-1} [3]. This comes with a cost to the host, since host tissues and energy reserves are utilized for parasite larval production [4]. In the case of *R. fennica*, the reproduction, growth, and survival of the mollusk host *Anodonta anatina* (*A. anatina*) are greatly reduced by infection [5,6,7]. In addition to the costs to host fitness, the large numbers of trematode cercariae that emerge into the aquatic environment may play important roles in the functioning of aquatic ecosystems in terms of biomass and energy flow [8,9,10]. Furthermore, because trematodes are harmful, and as there are many medically important trematodes that are transmitted to humans by cercariae, the study of cercarial release is also a subject of interest in applied science [11,12].

Temperature conditions are well known to affect cercarial release by trematodes [13]. The emergence of cercariae from the mollusk hosts may be triggered by an increase or a decrease in temperature so that the effect of temperature is often species-specific [14]. Therefore, when studying the cercarial release by trematodes, an important methodological question is how short-term temperature change can influence the release of cercariae from the first intermediate host. In addition, if temperature change can affect trematode activity and cercarial production, it is possible that the mollusk hosts could actively change their microhabitat to regulate their ambient temperature, in order to counter the deleterious effects of trematode parasitism [15,16].

Thus far, regarding trematode response to short-term temperature change, Paull et al. [17] reported that trematode-infected snails transferred to a higher temperature (i.e., 3 °C $>$ acclimation temperature) released more parasites 12 h after the temperature shift than before, while those moved to a lower temperature (3 °C $<$ acclimation temperature) released fewer cercariae than before the shift. Studer et al. [18] also reported that infected snails exposed for one hour to a 4 °C–5 °C temperature boost showed significantly increased cercarial output at all temperature levels investigated. Taskinen et al. [19] and Taskinen [2] showed seasonal changes in emergence of *R.*

Table 1: Temperature treatment group specific total number (N_{tot}), number infected (N_{inf}), and number of cercariae-releasing clams (N) during the experiment, and the mean (±SE) number of *R. fennica* cercariae released h^{-1} $clam^{-1}$ at 17 °C (before temperature change) and after the clams were transferred to one of the three temperature treatments (14 °C, 17 °C, and 20 °C).

N_{tot}	N_{inf}	N	Before temperature change			After temperature change		
			Temp.	N	*R. fennica*	Temp.	N	*R. fennica*
22	13 (59%)	11	17 °C	2	54.5 ± 39.0	14 °C	10	32.7 ± 73.2
20	14 (70%)	14	17 °C	6	58.6 ± 34.6	17 °C	13	201.4 ± 64.9
20	16 (80%)	12	17 °C	3	33.3 ± 37.3	20 °C	12	323.3 ± 70.0

fennica cercaria with peak emergence during the warmest summer. In addition, by increasing temperature, release of *R. fennica* cercaria could be induced even during winter, outside the natural shedding period [20]. However, the response of *R. fennica* cercarial production to temperature has not been studied experimentally. Therefore, we investigated the effect of short-term (one hour) temperature change on the cercarial release by *R. fennica* from the first intermediate host, the freshwater clam *A. anatina*, by transferring the clams from the acclimatization temperature of 17 °C to one of the following three temperatures: 20 °C, 17 °C or 14 °C. We predicted that temperature increase would promote cercarial emergence, while decrease of temperature would slow down cercarial release.

2. Materials and methods

2.1. Study species

The life cycle of *R. fennica* includes three host species. The parasite matures in the definitive host, the esocid fish *Esoxlucius* [20,21], where the adult worms reproduce sexually, producing eggs that are released to the water. Miracidia larvae hatch from the eggs and penetrate the first intermediate host, *A. anatina*. Sporocysts of the parasite invade (mainly) the gonad of the host clam [22], producing cercarial larvae asexually. A specific diurnal pattern of cercarial release is exhibited by *R. fennica*, such that the main shedding period is during the day time, with the peak cercarial emergence occurring between 8 AM and 10 AM [20]. Emerged cercariae float in the water with the aid of their long furcae and attach to the fins of the second intermediate host, the cyprinid fish *Rutilus rutilus* [20].

The first intermediate host, *A. anatina*, is a common European freshwater bivalve clam with maximum life span > 10 y, age of maturation 2 y–4 y and maximum length of 100 mm–200 mm [5,23,24]. Female *A. anatina* develop glochidia larvae in July in their outer gill blades, where they are stored over winter to be released the following spring [22,25,26]. Glochidia are parasitic on freshwater fishes [25,27] before they detach and start their benthic life.

2.2. Clam collection and experimental design

A total of 62 *A. anatina* individuals were collected by snorkeling on 25th August 2014 from the River Haajaistenjoki in Finland (63°63′ N, 26°99′ E)—a small, shallow river having a dense population of *A. anatina* with a high prevalence of *Rhipidocotyle* parasites. The clams were transported to Lake Jyväsjärvi (62°14′ N, 25°47′ E), by the city of Jyväskylä, where they were kept in a cage measuring $120 \times 80 \times 100\,cm^3$ for two days prior to the experiment. On 27th August, the clams were brought to the laboratory where three experimental clam groups were established; each group included randomly selected clams of all size groups ($n = 20$ to 22 clams $group^{-1}$; Table 1). Older clams (i.e., ≥ 3 years of age) were used in the experiment as younger clams are normally not infected [5]. The water temperature of Lake Jyväsjärvi at the time of clam collection was 17 °C, which was the same as that in the River Haajaistenjoki.

The experiment was designed so that the number of cercariae released by each clam was first counted at the acclimatization temperature (17 °C) and again after a temperature change to one of the three new temperatures (14 °C, 17 °C, and 20 °C). Throughout the experiment, aerated, aged underground water (kept in the laboratory for 24 h) was used. Each of the 62 clams was first placed individually in a 4 L transparent plastic box filled with 2 L of water at 17 °C from 8 AM to 9 AM on the 27th of August. After one hour at 17 °C, clams were transferred to one of the three new temperatures such that clams from each clam group were individually assigned to 14 °C (decreased), 17 °C (control) or 20 °C (increased temperature) for another one hour from 9 AM to 10 AM. The clams were then removed from the boxes and stored for dissection. Meanwhile, the number of cercariae shed by each clam after one hour at 17 °C and after one hour at the new temperature was counted from a 50 mL sample of well-mixed cercarial suspension. The 50 mL water sample was examined microscopically and the number of cercariae found was multiplied by 40 to obtain the total number of cercariae released into the 2 L water volume in the experimental box.

Monitoring boxes for each temperature treatment were placed next to each other during the cercarial monitoring period in order to maintain specific water temperatures. The average (minimum-maximum) water temperature in the boxes measured at the end of each of the one hour cercarial monitoring periods was 17 °C (16.9 °C–17.0 °C) before temperature change, and after the temperature change

was 19.9 °C (19.8 °C–20.0 °C), 16.9 °C (16.8 °C–17.0 °C) and 14.2 °C (14.0 °C–14.5 °C) in increased, control, and decreased temperature treatments, respectively. Natural light conditions prevailed in the window-equipped laboratory and during each one hour cercarial monitoring period boxes were placed at the same distance from the window to ensure that the light conditions were equal for all boxes. After the experiment, all the clams were dissected and their gonads were examined for *Rhipidocotyle* parasites and their quantity [19]. Ages were determined for a subsample of clams from each clam group by counting the annual growth rings on the shell.

One-way ANOVA was used to determine whether the mean cercarial output was different between the three clam groups after one hour at 17 °C, prior to the transfer to the new temperatures. The number of cercariae released was used as the dependent variable and the clam group as a fixed factor. The effect of temperature treatment (increased, control or decreased) on the cercarial output was studied using one-way ANCOVA with the change in the cercarial production (i.e., the number of cercariae shed after temperature shift minus the number of cercariae shed before temperature shift) during the experiment as a response variable, treatment as a fixed factor, and the number of cercariae released before the temperature change as a covariate. Statistical analyses were performed using IBM SPSS statistics version 22.0. Means are given with ± 1 standard error (SE).

3. Results

The proportion of clams infected by *R. fennica* was 69%, with no significant difference between the three temperature treatment groups (χ^2-test, df $= 2$, $\chi^2 = 2.161$, $P = .339$; see Table 1). A high proportion of the infected clams (86%, 37 out of 43) released cercariae, with no significant differences between the temperature treatment groups (χ^2-test, df $= 2$, $\chi^2 = 1.470$, $P = .141$; see Table 1). Cercarial shedding was not related to the intensity of infection, as the proportion of *A. anatina* clams shedding cercariae did not differ whether infected with a low amount, moderate amount or a large amount of parasite sporocyst material in the host gonad, respectively (χ^2-test, df $= 2$, $\chi^2 = 1.058$, $P = .589$).

Prior to the transfer, the average cercarial output of *R. fennica* per clam over the one hour shedding period at 17 °C did not differ between the three clam groups (one-way ANOVA; $F_{2,34} = 0.136$, $P = .873$), being on average 49 ± 21 cercariae h^{-1} $clam^{-1}$ (Figure 1). After the transfer, cercarial release was differentially affected by temperature treatment. There was a statistically significant difference in the change of cercarial production between the temperature treatments (one-way ANCOVA, "treatment": $F_{2,33} = 5.515$, $P = .009$). The change in cercarial release was the highest when the clams were transferred from 17 °C to 20 °C, with

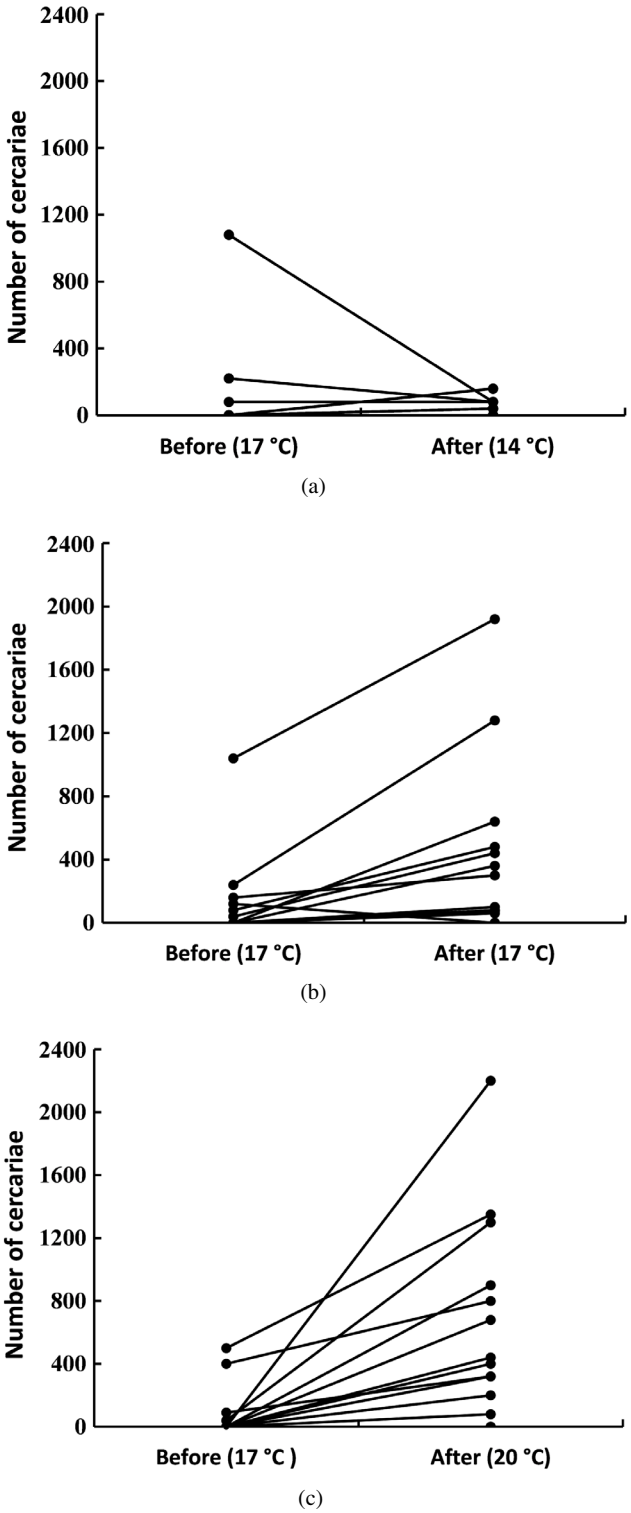

Figure 1: Release of *R. fennica* cercariae from each cercariae-shedding clam at 17 °C during a one hour period before transfer to a new temperature, and during a one hour period after transfer to a new temperature ((a) decreased 14 °C; (b) control 17 °C; and (c) increased 20 °C). Ambient holding temperature of the host clams, *A. anatina*, was 17 °C.

an increase of 290 ± 63 cercariae h^{-1} $clam^{-1}$. The second highest change, 143 ± 59 cercariae h^{-1} $clam^{-1}$, occurred in clams transferred from 17 °C to 17 °C. In contrast to the transfer to a higher or equal temperature, a negative change (-22 ± 66 cercariae h^{-1} $clam^{-1}$) in cercarial release was observed in the clams transferred from 17 °C to 14 °C (Figure 1). Post hoc comparisons revealed that the transfer to 17 °C did not differ from the other temperature treatments, but the transfer to 20 °C differed significantly from the transfer to 14 °C. The effect of the covariate "cercarial release before temperature change" was not significant ($F_{1,33} = 0.370$, $P = .547$), indicating that the change in cercarial shedding accompanying transfer to the new temperature was not affected by the shedding rate before the temperature change. These results suggest that the exposure to a higher temperature and to a lower temperature increased and decreased, respectively, the immediate cercarial release of *R. fennica* from *A. anatina*.

Inspection of the experimental clams for *Rhipidocotyle* parasites revealed that 7 out of 62 *A. anatina* were infected by *R. campanula*. However, only *R. fennica* cercariae emerged from the clams during the experiment. The mean age of the clams did not differ between treatment groups 14 °C (5.6 ± 0.6 y, $n_{\text{studied}} = 7$), 17 °C (5.8 ± 0.7 y, $n_{\text{studied}} = 6$), and 20 °C (6.1 ± 0.4 y, $n_{\text{studied}} = 8$) (one-way ANOVA, $F_{2,18} = 0.276$, $P = .762$).

4. Discussion

Many trematodes are strongly influenced by temperature conditions [18,28,29,30]. Thus, any change in the direction or magnitude of temperature is very likely to affect cercarial production, an important feature for the transmission success and maintenance of viable trematode populations within ecosystems. The present study experimentally investigated the effects of temperature change on the cercarial emergence of the *R. fennica* trematode. The results revealed strong effects of short-term (one hour) temperature change on the release of *R. fennica* cercaria by the clam hosts. As predicted on the basis of previous findings that *R. fennica* cercarial shedding takes place seasonally during the warmest months [2,19,22], the response of cercarial release by *R. fennica* to rapid temperature increase was positive. In turn, the decrease of temperature led to a decrease of cercarial shedding. These results, together, suggest that cercarial release by *R. fennica* is thermophilic.

Results consistent with observations from the present study have also been found for other trematode species. Paull et al. [17] and Studer et al. [18] reported that the release of trematode cercariae increased temporarily in infected snails moved from lower to higher temperature, but decreased significantly in snails moved from higher to lower temperature. The boost in cercariae release from the clams moved to 20 °C in the present study could be explained as a simple consequence of increased host metabolic activity at higher temperature resulting in the greater energy resources available to the parasites [13]. Usually, higher temperature not only accelerates cercariae production within the mollusk hosts but also triggers the emergence of cercariae from the mollusk hosts [28,29]. In the current study, all the clams were collected from the field in late August when most cercariae are fully developed and ready to emerge [19]. Therefore, in a short-term study like the present one, the abrupt temperature increase probably triggered the release of already developed cercariae from the clam hosts, leading to a burst of cercariae emergence, and did not accelerate cercarial maturation within sporocysts.

The cercarial release by *Rhipidocotyle* trematodes has a diurnal periodicity such that the emergence of *R. fennica* cercariae takes place in the day time, peaking between 8 AM and 10 AM [20]. Thus, the present experiment was performed at the time of highest daily productivity of *R. fennica*. This could explain the high proportion (37/43) of infected individuals releasing cercariae, and the relatively high cercarial production by *R. fennica* in the present study. The mean cercarial outputs of above 200–300 h^{-1} $clam^{-1}$ at 17 °C and 20 °C are comparable to ca. 450–2,000 recorded at 22 °C [20]. This indicates that the experimental conditions for the clams and the parasite in the current study were not limiting the production of *R. fennica* cercaria. In contrast, the conditions for the cercarial release by *R. campanula* were presumably adequately met, leading to the total lack of shed cercariae for this species. The thermal requirements of *R. campanula* are not more demanding than those of *R. fennica*; it can start the cercarial release at a lower temperature than *R. fennica* [19]. However, *R. campanula* sheds cercariae mainly at night, with the period 8 AM–10 AM being the poorest [20]. Thus, we believe that the unsuitable time of the day mainly accounts for the non-emergence of *R. campanula* cercariae.

From the methodological point of view, the results highlight the critical role of temperature conditions when performing studies on cercarial release. The present observations on *R. fennica* show that abrupt changes in ambient temperature can substantially increase or decrease cercarial production. On the other hand, this can be utilized in studies where cercarial larvae of *R. fennica* are needed. The release of cercariae can be triggered by a slight increase in water temperature.

Besides methodology, the findings of the current study are also important when assessing the host-parasite relationship between the molluscan host and *R. fennica*. The production of cercariae by the parasite is achieved at the expense of the host. Thus, the present results indicate that the costs for the host clam (related to cercarial production and release) may be higher at higher temperature. The host clam, *A. anatina*, is capable of moving on the bottom

sediment. For example, a mean crawling track length of about 2 m was evident among *A. anatina* clams of Lake Saravesi, Finland [31]—a lake with 30% prevalence of *R. fennica* infection in *A. anatina* [5]. In theory, moving would enable infected *A. anatina* to influence its microhabitat by moving to deeper water to decrease environmental temperature. A thermal preference for colder microhabitat, a reverse fever, has been observed in trematode-infected snails, *Planorbarius corneus*, and explained as a defense response against the parasites [32]. Therefore, *A. anodonta* infected by *R. fennica* could also migrate to the deeper water to mitigate the adverse effects of the parasite. However, this hypothesis is not supported by the vertical distribution of clams infected by *R. fennica*. Prevalences of infection are found to be significantly lower in deeper water than in the littoral zone [5]. If infection by *R. fennica* has an impact on the vertical movements and thermal preference of *A. anatina*, could the parasite, *R. fennica*, manipulate the behavior of the host clam causing it to move to shallow, warm water, for the benefit of the parasite? These contrasting hypotheses remain to be studied in the future.

Climate warming has been recognized as one of the main factors affecting host-parasite relationships [13, 33]. Therefore, in the future the response of *R. fennica* to long-term changes in temperature should also be studied. Climate models predict a 2 °C to 7 °C increase in annual temperature by the 2080s compared to the 1961–1990 baseline period, in Finland, the present study region [34]. If long lasting (weeks, months) increases in temperature increase cercarial production by *R. fennica* as occurred in the current short-term study, this should have a major impact on the total annual larval production of this parasite species.

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Conflict of interest The authors declare that there are no conflicts of interest.

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