Contrasting temperature responses in the seasonal timing of cercarial shedding by two *Rhipidocotyle* trematodes

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Running title: Temperature-dependent seasonal cercarial release

SUMMARY

Global warming and the associated longer growing seasons are predicted to lengthen the seasonal duration of larval release by parasites. We exposed freshwater mussel hosts, *Anodonta anatina*, from two high latitude populations to high, intermediate and low temperature throughout the annual cercarial shedding period of the sympatric trematodes *Rhipidocotyle fennica* and *R. campanula*, sharing the same transmission pathway. At the individual host level, *R. fennica* (unlike *R. campanula*) started cercarial release earlier in the season, but also stopped earlier, at higher temperature. The mean length of the cercarial shedding period of both *Rhipidocotyle* species did not differ between the treatments at the individual host level, but the total shedding period among all mussels became longer with higher temperature for *R. fennica*. These results do not completely support the view that climate warming would invariably increase the seasonal duration of larval shedding by parasites, but emphasise species-specific differences in seasonal cercarial release and transmission with respect to climate change. *R. campanula* clearly started the cercarial release earlier in the season and at a lower temperature than *R. fennica*, suggesting that *R. campanula* should compete better than *R. fennica* in colder environments, but that climate warming should benefit *R. fennica* more than *R. campanula* in the future.

Key words: *Anodonta anatina,* Bucephalidae, cercaria, climate change, Digenea, mollusk, *Rhipidocotyle*, seasonality, temperature, transmission.

KEY FINDINGS

- At the host population level, the total cercarial shedding period of *R. fennica*, from the first to the last observation of emergence, lasted for 10-16 weeks in the high and intermediate temperature treatments, respectively, but for only 4-6 weeks in the low temperature treatment. In contrast, the total period of cercarial shedding by *R. campanula* ranged from 14 to 18 weeks in the low temperature treatment (which was longer than that of *R. fennica*), and varied from 8 to 18 weeks at the other temperatures.
- At the individual host level, however, temperature treatment did not affect the mean duration of the seasonal cercarial shedding period either for *R. fennica* or for *R. campanula*.
- At the individual host level, *R. campanula* clearly started the seasonal cercarial release earlier, at a lower temperature, and with less day-degrees, stopped the seasonal cercarial release earlier, and had a longer mean duration of cercarial release than *R. fennica*.
- The results for *R. fennica* at the host population level support the prediction that climate warming would increase the total duration of larval shedding by parasites.
- However, the results for *R. campanula* did not support this view; no indication of lengthening of the seasonal cercarial release period with temperature was observed either at the individual host level or at the population level.

INTRODUCTION

Marked seasonal fluctuation in temperature conditions is characteristic of high-latitude ecosystems. Seasonal temperature variation can affect trematode parasites in many ways, including the timing of cercarial emergence, so that the release of cercariae primarily occurs during the warm summer months in temperate and boreal zones (Chubb, 1979; Taskinen, 1998a; Karvonen *et al*. 2004). Field studies by Fingerut *et al*. (2003) revealed that the emergence of five trematode species from their common snail host was species-specific, and correlated significantly with monthly water temperature as the number of emerged cercariae increased remarkably during the warm summer months. Experimental studies have also reported increased release of cercariae with rising temperature (e.g. Fingerut *et al.* 2003; Thieltges and Rick, 2006; Studer *et al*. 2010; Shim *et al*. 2013), although neutral or even negative responses have also been reported (Koprivnikar and Poulin, 2009). Thus, the predicted climate warming (IPCC, 2007) and the associated warmer summers and longer growing season (e.g. Ruosteenoja *et al*. 2011) are likely to affect the seasonal pattern of cercarial release, by advancing the onset or delaying the cessation of cercarial emergence. A common expectation is that the seasonal duration of larval release by parasites will increase as a consequence of a longer thermal growing season (longer summer) (Marcogliese, 2001; Harvell *et al*. 2009). Such a lengthening of the cercarial release period has been observed in water bodies receiving thermal effluents (e.g. Aho *et al*. 1982). However, experimental long-term manipulations of temperature conditions over the seasonal cercarial release period are rare (for an

exception see Paull and Johnson, 2014). Furthermore, no studies have yet investigated whether such lengthening of the seasonal cercarial shedding period would be as a result of a longer cercarial shedding period at the individual host level or as a result of the seasonal variation between host individuals leading to an overall longer shedding period.

Cercaria larvae of trematodes emerge over species-specific temperature ranges (e.g. Fingerut *et al*. 2003). However, inter-specific comparisons of cercarial emergence from the molluscan host in varying temperature conditions that can reveal species-specific responses have been utilized quite rarely (Fingerut *et al*. 2003; Koprivnikar and Poulin, 2009). In addition, the importance of high-latitude areas has been highlighted in earlier studies (Morley and Lewis, 2013; Studer and Poulin, 2014), but, to our knowledge, experimental temperature manipulations have not been performed in relation to high latitude host–trematode associations at high latitudes (> 60°). Such a study would be timely, since climate change is predicted to be most pronounced at high latitude regions. For example, climate models predict an increase in annual temperature from 2 to 7° C by the 2080s in Finland compared to a 1961-1990 baseline period (Jylhä *et al*. 2004), warmer summer and longer growing season (e.g. Ruosteenoja *et al*. 2011).

The production of cercariae in the molluscan host is an important component of the complex life-cycle of trematodes, underpinning transmission of infection to the next host and hence influencing the total life-time reproduction success of the parasite. Cercariae of trematodes are transmitted to a variety of aquatic invertebrates and vertebrates. Consequently, trematode parasites may play an important role in the functioning of aquatic ecosystem (Kuris *et al*. 2008). Therefore, any change in the seasonal duration of the cercarial shedding period can affect different trophic levels in aquatic ecosystems by increasing/decreasing parasite burden. In the present long-term (5 months) study, we investigated the effect of three different temperature levels on the temporal aspects of the seasonal cercarial shedding traits of two closely related, sympatric trematodes, *Rhipidocotyle campanula* and *R. fennica,* in their common first intermediate host, *A. anatina*. The three temperatures reflect the natural temperatures occurring throughout the distribution range of *A. anatina* in Finland (60°-68°N). In addition to the first host, both parasite species use the same second intermediate host, the cyprinid fish *Rutilus rutilus* (Taskinen *et al*. 1991; Gibson *et al*. 1992). We were specifically interested in distinguishing the seasonal duration of cercarial shedding at individual host and host population levels. Our hypothesis was that, in both *Rhipidocotyle* species, the higher temperature (longer warm season) would result in a longer cercarial shedding period per host individual and per host population.

MATERIALS AND METHODS

Study species

The bivalve mollusc host, *Anodonta anatina*, is a common European freshwater mussel with maximum life span > 10 y, age of maturation 2-4 y and maximum length of 12 cm (Taskinen and Valtonen, 1995). *A. anatina* serves as the first

intermediate host of the bucephalid trematodes, *Rhipidocotyle campanula* and *R. fennica* (Taskinen *et al*. 1991, 1997; Müller *et al*. 2015), where the cercariae are produced asexually within the gonads of mollusc hosts by sporocysts. Whereas the prevalence of infection by *R. campanula* is usually less than 10% (Taskinen *et al*. 1991; Müller *et al*. 2015), that by *R. fennica* can be up to 50 % in littoral habitats (Taskinen *et al*. 1994). Pronounced seasonality in the developmental stages of cercariae and the number of sporocysts of the *Rhipidocotyle* species in *A. anatina* were observed by Taskinen *et al*. (1994). Both parasite species have been linked to decreased growth, survival and reproduction of *A. anatina* (Taskinen and Valtonen, 1995; Taskinen, 1998b; Jokela *et al*. 2005; Müller *et al*. 2015). The second intermediate host of both parasite species is the cyprinid fish *Rutilus rutilus*, in which *R. fennica* metacercariae encyst in the fins and *R. campanula* metacercariae encyst in the gills (Taskinen *et al*. 1991; Gibson *et al*. 1992). The definitive hosts for *R. campanula* are the percid fishes *Perca fluviatilis* and *Sander lucioperca* and the definitive host for *R. fennica* is the esocid fish *Esox lucius* (Taskinen *et al*. 1991; Gibson *et al*. 1992).

Experimental set-up

Altogether 281 *A. anatina* mussels were collected from the River Kuusaankoski (May 17, 2011; 62°25'N, 26°00'E) and 290 mussels from the River Haajaistenjoki (May 22, 2011; 63°63'N, 26°99'E), Finland. At the Konnevesi Research Station, University of Jyväskylä, the mussels were individually marked and measured. Average shell length ± s.e. for the River Haajaistenjoki mussels was 61.8 ± 0.6 (range 33.0-92.6 mm), and for the River Kuusaankoski mussels 77.8 ± 0.6 (range 51.7-101.7 mm). From the date of collection to 25th June, mussels were kept in the laboratory in two flow-through tanks filled with 5 cm of sand at the bottom and supplied with incoming water from the hypolimnetic zone (9 m depth) of Lake Konnevesi at up to 10 l min^{-1} flow rate. Water temperatures in both tanks were the same throughout this period ranging from 10.5 °C on May 31 to 11.7 °C on June 25 (Fig. 1). On June 25, the mussels were randomly assigned to one of the three temperature treatments: high, intermediate or low temperatures, with two replicate tanks per treatment. The maximum daily water temperature was attained on July 27 in high temperature (24 °C), and on September 4 in the intermediate (20 °C) and low temperature treatments (17 °C). The Mussels from both populations and from all size groups were distributed evenly to each of the six tanks (for mussel numbers $tank^{-1}$, see Table 1). There was no length difference between mussels allocated to the 3 temperatures (Two-way ANOVA; $F_{1, 565} = 0.040$, $P = 0.961$) and no interaction between population and treatment ($F_{1, 565} = 0.728$, $P = 0.484$).

The water temperature ranges in the different temperature treatments corresponded to the natural extreme temperature variations currently occurring throughout the distributional area of *A. anatina* in Finland, from 60° to 68°N and represent the maximum summer temperatures varying from about 17 to 24°C, respectively. The number of days when the average daily water temperature in the different treatments was ≥ 15 °C, a measure of the length of the warm/growing season, was 74, 62 and 26 days in high, intermediate and low temperature treatments, respectively. The temperature treatments were established as follows. (1) High temperature tanks were placed in outside shelter/shade and supplied with running water from the littoral zone (< 2m depth) of Lake Konnevesi. (2)

Intermediate temperature tanks were kept indoors and supplied with heated hypolimnetic water from Lake Konnevesi. (3) Low temperature tanks were kept indoors and supplied with (unheated) hypolimnetic water from Lake Konnevesi. *Anodonta* mussels are filter feeders utilising phytoplankton, bacteria and fine organic particles (Jorgensen *et al*. 1984), thus a continuous flow of lake water was necessary to provide the mussels with food. Due to logistic constraints, differences other than temperature existed between the treatments. Mussels in the high temperature treatment were subject to a larger daily fluctuation of temperature than those in the intermediate or low temperature treatments (Fig. 1), as the littoral water and outdoor tanks were used. In addition, the seasonal profile varied such that the water temperature in the high temperature treatment tanks peaked in late July (24 °C), and in the intermediate (20 °C) and low temperatures (17 °C) it peaked in early September (Fig. 1). However, results by Roushdy (1984) indicate that cercarial release does not differ between constant and diurnally variable temperatures.The indoor tanks were illuminated by artificial light with the photoperiod set to correspond with the natural rhythm. The outdoor tanks received natural light but the shelter above the tanks provided effective cover against direct sun light. However, during the 24 h cercarial release monitoring period, similar artificial light was used for all mussels to provide equal light conditions (see below). Water flow into the tanks was adjusted such that it was higher in the intermediate and low temperatures (10 l min^{-1}) than in the high temperature tanks (5 l min^{-1}). This was to compensate for the probable higher food density in the high temperature tanks that received littoral water, than the intermediate and low temperature tanks that received hypolimnetic water. A submersible temperature logger was placed in one replicate tank per treatment to measure water temperature every 4 h from June 25 to October 28 (end of experiment).

Cercarial release from each mussel was followed over a period of 20 weeks by counting the number of cercariae released per *A. anatina* at 14-day intervals between 31 May and 28 October, during a total of 12 monitoring sessions. On each monitoring day, individual mussels were placed in a 4 l transparent plastic box (length 26.5 cm, width 19 cm and height 13.6 cm) filled with 2 l of filtered lake water for 24 h (dead mussels were removed at this stage) after which mussels were removed and returned to their respective holding tanks. The number of cercariae in the box was counted visually (when numbers were low, < 20 cercariae), or microscopically from a 50 ml of well mixed subsample (when cercariae numbers were high > 20 cercariae). The water temperatures in the monitoring boxes during the 24 h period of cercarial shedding were adjusted to correspond with those in the respective holding tanks and, when necessary, a temperature-controlled room was used. Light conditions were also set to correspond with the natural day length and rhythm because the cercarial release of *Rhipidocotyle* species is diurnal (Taskinen *et al*. 1991).

The experiment was terminated on October 28, 2011, when cercarial release approached zero in practically all treatments. Survival of the mussels through the experiment was 64.2 % (see Figures S1 and S2). Results relating to the total annual cercarial output and host survival at different temperatures are not included here, but will be published separately.

Data analysis

Statistical analyses were performed using PASW Statistics 18. Mussels that did not shed cercariae, those that shed both *R. campanula* and *R. fennica* cercariae (double infected), and those that were infected by *Phyllodistomum* sp., were not included in the statistical analyses. Before the final analyses, data from replicate tanks were combined, as prior tests revealed no differences between replicates for any measured variable.

To compare differences between treatments and populations with regard to the mean start date, water temperature and day-degrees required for the start, as well as the stop date and the mean duration of cercarial emergence, two-way ANOVA was applied separately for *R. campanula* and *R. fennica* with treatment and population as fixed factors (Table 2).

When testing differences between the parasite species, treatments were analysed separately to satisfy ANOVA assumptions. Start date, (cube root transformed), day-degrees and water temperature for the start, stop date and duration of cercarial shedding were, one at a time, the response variables, while parasite species and population were the fixed factors (Table 3). For start date of cercarial shedding at high temperature and stop date at high and intermediate temperatures, populations were analysed individually to meet ANOVA assumptions. However, the non-parametric Kruskal-Wallis test was applied when analysing differences between parasite species with respect to the stop date of cercarial shedding at high and intermediate temperatures for the River Haajaistenjoki, (Table 3). To account for multiple tests, a Bonferroni correction was applied to p-values when analysing differences between parasite species (Table 3). Whenever ANOVA indicated a significant effect of temperature treatment, the differences between treatments were analysed with Tukey's b Post Hoc tests. Means are given with ± 1 standard error (s.e.). Relationship between the mean number of cercariae released and temperature during each monitoring session was studied using correlation analysis with data from the two populations and three treatments combined.

RESULTS

Cercarial release at the total host population level

At the host population level, the total cercarial shedding period (from the first to the last observation of emerged cercariae) of *Rhipidocotyle* cercariae varied between species and across treatments. It ranged from 12 July to 28 October, whereas that of *R. campanula* ranged from 31 May to 3 October (Table 1; Fig. 1). The total period of cercarial shedding by *R. fennica* lasted for 10 to16 weeks in the high and intermediate temperature treatments, respectively, but for only 4 to 6 weeks in the low temperature treatment (Table 1; Fig. 1). In contrast, the total period of cercarial shedding by *R. campanula* in the low temperature treatment lasted for 14 to 18 weeks, which was longer than that of *R. fennica*, and varied from 8 to 18 weeks at the other temperatures (Table 1). Water temperature at the time of the first emergence of *R. fennica* cercariae varied from 16.5 to 21.5 °C , which was clearly higher than the 10.5 °C observed for *R. campanula* (Table 1; Fig. 1). The exact timing of the first and last

observations of emerged cercariae for each mussel shedding cercariae is given in Fig. S1 and Fig. S2.

Seasonal cercarial release with respect to temperature

The peak cercarial release by *R. fennica* co-occurred with the seasonal thermal maximum, but that of *R. campanula* was not clearly restricted to the period of highest temperature (Fig. 1) Cercarial shedding by *R. fennica* increased substantially at temperatures above 15 °C (Fig. 2) but high numbers of *R. campanula* cercariae were released as soon as the temperature exceeded 10 °C (Fig. 2). A positive relationship between the mean cercarial release by both *R. fennica* and *R. campanula* with the ambient temperature at the time of monitoring was found among the seasonal monitoring sessions (Spearman's rho = 0.636, $P < 0.001$, n = 36 and Spearman's rho = 0.615, $P < 0.001$, n = 36, respectively). During the months of the highest temperatures (July, August and September) a positive relationship between the mean cercarial release and the ambient temperature at the time of monitoring was evident for *R. fennica*, (Spearman's rho = 0.898, $P < 0.001$, n = 18 Fig. 2), but not for *R. campanula* (Spearman's rho = 0.261, $P = 0.295$, n = 18, Fig. 2).

Cercarial release at the individual host level, and differences between temperature treatments

At the individual host level, three seasonal cercarial shedding traits, start date of cercarial release, water temperature and day-degrees at the start of release, showed significant differences between temperature treatments for *R. fennica* (Table 2). Tukey's b post hoc test and paired Mann-Whitney confirmed that all paired differences between the treatments with respect to each of these traits were significant ($P < 0.05$).Thus, *R. fennica* started cercarial release on average earlier, at a higher temperature and with a lower sum of day-degrees in the high temperature treatment than in the lower temperatures (Figs. 3-5). However, for *R. campanula* these cercarial shedding traits were unaffected by temperature (Table 2; Figs. 3-5). The stop date of cercarial release and the water temperature on that date for both species differed significantly between the treatments (Table 2). Tukey's b post hoc tests and paired Mann-Whitney U tests indicated that all paired differences between the treatments with respect to the stop date and water temperature on that date were significant ($P < 0.05$). Hence cercarial shedding by both parasites ceased earlier and at a higher temperature in the high temperature treatment than in either intermediate or low temperatures (Figs. 1, 3 and 4).

Finally, the duration of the seasonal period of cercarial shedding by both species did not differ between treatments (Table 2; Fig. 6). This suggests that the seasonal period of cercarial shedding, at least by *R. fennica*, shifted according to prevailing temperature conditions, occurring earlier in high and later in low temperature treatment, rather than increasing or decreasing in duration. None of the cercarial shedding traits were affected by mussel population or by the treatment × population interaction (Table 2). This indicates that all the six seasonal cercarial shedding traits were independent of mussel population, and that the afore-mentioned temperature effects were equal in both populations, within both parasite species.

A positive relationship between the mean cercarial release by *R. fennica* and the ambient temperature at the time of monitoring was found among the 12 seasonal monitoring sessions in the high temperature treatment (Spearman's rho = 0.814, P = 0.001, n = 12) and in the intermediate temperature (Spearman's rho = 0.614, P = 0.034, n = 12) but not in the low temperature (Spearman's rho = 0.179, P = 0.577, n = 12). The mean cercarial release by *R. campanula*, also, correlated positively with the temperature at the time of monitoring, within the high temperature (Spearman's rho = 0.700, P = 0.011, n = 12), the intermediate temperature (Spearman's rho = 0.716, P = 0.009, n = 12) and the low temperature (Spearman's rho = 0.746, P = 0.005, n = 12) treatments.

Cercarial release at the individual host level, and differences between parasite species

There was a significant difference between the parasite species with respect to the six cercarial shedding traits studied: start date, water temperature and day-degrees at the start, stop date and duration of cercarial release (Table 3). The release of cercariae by *R. fennica* clearly started later than that by *R. campanula*, the difference varying from 42 to 87 d, with the largest difference found among the River Haajaistenjoki mussels in the low temperature treatment (Fig. 3). The mean water temperature at the start of seasonal release of cercariae was clearly higher for *R. fennica* (15-20 °C) and lower for *R. campanula* (10-12 °C) (Figs. 1 and 3). Accordingly, the cercarial release also started with a much higher sum of day-degrees for *R. fennica* than for *R. campanula*, with a five-fold difference among River Kuusaankoski mussels in the high temperature treatment, for example (Fig. 5). In addition, although the cercarial release by *R. campanula* ended earlier in the season (Fig. 4), the total duration of cercarial release was longer for *R. campanula* than for *R. fennica* (Fig. 6). A three-fold difference in the average duration of cercarial shedding period between the species was observed in the low temperature treatment (Fig. 6). For the five traits considered here, the effects of population and treatment ×population interactions were not significant, indicating that the traits were independent of mussel population, and that the afore-mentioned temperature differences between parasites were equal for both mussel populations.

DISCUSSION

Ongoing and predicted increases in global temperatures and the associate thermal growing season will have important implications for many host-parasite systems, including the timing of parasite life cycle stages (Marcogliese, 2001; Kutz *et al.* 2005). Some parasites will advance their date of emergence, some that were previously active only in the summer may become active year-round (Kutz *et a*l. 2005). A common expectation is that the seasonal duration of larval release by parasites will increase as a consequence of increased thermal growing season (longer summer) (Marcogliese, 2001; Harvell *et al*. 2009). In the present study, the total period of cercarial shedding (from the first to the last observation of emergence) by *R fennica* at the host population level, supported this view, as the shortest seasonal shedding period occurred in the low temperature treatment. However, the total period of seasonal cercarial shedding by *R campanula* was unaffected by temperature at the

host population level. Such a lengthening of the total period of seasonal cercarial shedding by *R. fennica* at higher temperature was due to the seasonal variation between host individuals resulting in a longer total shedding period among all mussels. In addition, at the individual host level the mean length of the seasonal cercarial shedding period of *R. fennica* and *R. campanula* did not differ between the temperature treatments. Therefore, within the temporal and thermal range of the present experiment, only the results for *R. fennica* at the host population level support the prediction that climate warming will increase the duration of larval shedding by parasites.

The results also revealed that closely related, sympatric parasite species that infect the same first (*A. anatina*) and second (*R. rutilus*) intermediate host species displayed different seasonal cercarial shedding patterns with respect to thermal conditions. *R. fennica* brought forward the start of seasonal cercarial release and started cercarial release with lower day-degrees in the high temperature treatment, but *R. campanula* did not. Furthermore, *R. campanula* clearly started the seasonal cercarial release earlier, at a lower temperature, with less day-degrees, and also stopped the seasonal cercarial release earlier, but had a markedly longer total seasonal duration of cercarial emission than *R. fennica*. The results further suggested a threshold temperature of 15 °C and 10 °C for abundant cercarial release by *R. fennica* and *R. campanula*, respectively, which was manifested in a clear seasonal association of the peak release of *R. fennica* cercariae to the warmest months. These experimental results were in accordance with previous field observations by Taskinen *et al*. (1994). Based on these observations, it can be predicted that *R. campanula* should thrive better than *R. fennica* in colder, more northern, short-summer environments, where early onset of cercarial release is presumably advantageous. The short summers (and low temperature) of northern regions should constrain *R. fennica*, which started cercarial emergence later in the season and required high temperatures to trigger the release, as well as higher day-degrees to start cercarial shedding. Therefore the projected climate warming in high-latitudes, with earlier and warmer spring and longer summer (Tietäväinen *et al*. 2010; Ruosteenoja *et al*. 2011), should benefit *R. fennica* more than *R. campanula* in the future.

The clearly earlier start of the seasonal cercarial release by *R. campanula* is difficult to explain by the transmission dynamics, as the two species share the same current (the bivalve *A. anatina*) and next (the fish *R. rutilus*) host in their life cycles (Taskinen *et al*. 1991; Gibson *et al*. 1992). It is also worth noting that both *R. fennica* and *R. campanula* are specific only to *A. anatina* as their first intermediate host in the study area (Taskinen *et al*. 1991; Gibson *et al*. 1992). The definitive hosts of *R. fennica* and *R. campanula* are the predatory fishes northern pike (*E. lucius*) and perch / pikeperch (*P. fluviatilis* / *S. lucioperca*), respectively (Taskinen *et al*. 1991; Gibson *et al*. 1992). Thus, it is also possible that the timing of cercarial shedding could be an adaptation to increase transmission to the final hosts, such as the differential seasonal occurrence of the final hosts in the littoral zone and their feeding on roach that we are not aware of. However, it is difficult to believe that the earlier start of cercarial release by *R. campanula* could be an adaptation only to northern conditions (although it might facilitate occurrence there) because both *R. campanula* and *R.*

fennica occur as far south as the Ukraine (Taskinen *et al*. 1991; Petkevičiūtė *et al*. 2014; Stunžėnas *et al*. 2014; Müller *et al*. 2015).

The mechanism enabling the early onset of cercarial release by *R. campanula* is that they have their cercarial production machinery 'on standby' throughout the year (Taskinen *et al*. 1994). Fully developed cercariae are found in *R. campanula* sporocysts in high proportions in all seasons, readily available for shedding when a suitable temperature is attained (Taskinen *et al*. 1994). *R. fennica* has a different seasonal growth and development, as mature, ready-to-emerge cercariae are only found during the cercarial shedding period, July-September (Taskinen *et al*. 1994). This probably means that it takes a relatively long time for *R. fennica* to respond to increasing water temperature in spring in terms of cercarial production, as the growth of sporocyst starts from practically zero in spring (Taskinen *et al*. 1994). Cercarial release by *Rhipidocotyle* spp can also be triggered outside the natural shedding period (even in the middle of winter) if transferred to high temperature in the laboratory, but also in that case the time needed for *R. campanula* to start shedding cercariae is much shorter than for *R. fennica* (Taskinen *et al*. 1991).

There were differences between the temperature treatments in terms of water flow and water source (littoral vs. hypolimnetic), light conditions, temperature fluctuation and in the seasonal temperature profile. Whereas the high temperature tanks were kept in an outdoor shelter and were subject to a diurnal temperature fluctuation and natural light, the intermediate and low temperature tanks were kept in an indoor tank hall and illuminated with artificial light. However, the photoperiod was equal in all treatments and corresponded to the natural rhythm. Even though we cannot completely rule out confounding factors other than temperature, we do not believe that the difference in water and light source, or temperature fluctuation, could explain the observed contrasting responses in the seasonal cercarial release by *R. fennica* and *R. campanula* between the temperature treatments. New infections of mussels during the experiment, via miracidia from unfiltered lake water, were unlikely due to the seasonal maturing of *Rhipidocotyle* trematodes in late autumn (Taskinen *et al*. 1991). Thus, the present results should reliably indicate temperature responses in the seasonal timing of cercarial shedding by *R. fennica* and *R. campanula*

Previous studies investigating the seasonal dynamics of trematode cercarial release include field observations showing significant increase in cercarial emergence during summer months (Taskinen *et al*. 1994; Taskinen, 1998a; Fingerut *et al*. 2003), a longer seasonal shedding period in water bodies receiving thermal effluents (Aho *et al*. 1982) and experimental evidence on the role of temperature in controlling the start and the duration of emergence (Taskinen *et al*. 1991; Fingerut *et al*. 2003; Paull and Johnson, 2014). In addition, long-term experimental studies like the present one are needed to understand better the specific interactions between climate change and emergence of trematode parasites.

To conclude, the results of this study do not completely support the view that climate warming would invariably increase the seasonal duration of larval shedding by parasites, but emphasises species-specific differences in the seasonal cercarial release and transmission with respect to warming (Marcogliese, 2001; Harvell *et al*. 2009). Research on the geographic distribution of the species is needed to determine

whether the observed temperature differences in cercarial shedding traits affect the current distribution and abundance of *Rhipidocotyle* species at the northern boundary of their occurrence. Due to the contrasting species-specific temperature-dependence, the *Anodonta anatina*-*Rhipidocotyle* spp. host-parasite relationship offers a unique system to study the effects of the ongoing and predicted climate warming, with earlier spring and longer, warmer summer (Tietäväinen *et al*. 2010; Ruosteenoja *et al*. 2011), at high-latitudes.

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TABLE 1 Total numbers of *Anodonta anatina* mussels (N) and numbers of mussels shedding cercariae (N_s; n shedding *R. fennica* / n shedding *R. campanula*) from the River Haajaistenjoki and the River Kuusaankoski kept in high (HT), intermediate (IT) and low temperature (LT). Start and stop dates represent the range between the earliest and latest observations of starting and cessation of cercarial emergence, respectively. Duration indicate the length (weeks) of the cercarial release at the host population level, from the first to the last observation of shedding. Start °C and Stop °C represent the water temperatures (°C) on the dates when the first and last cercariae emerged, respectively.

	N	**N_s**	**Start date**		**Stop date**		**Duration**		**Start °C**		**Stop °C**	
			R. fennica	*R. campanula*	*R. fennica*	*R. campanula*	*R. f*	*R. c.*	*R. f.*	*R. c.*	*R. f.*	*R. c.*
River Haajaistenjoki												
HT	96	33/8	12 Jul-8 Aug	31 May-27 Jun	27 Jul-18 Sep	14 Jun-18 Sep	10	16	21.5	10.5	16.0	16.0
IT	97	19/11	12 Jul-3 Oct	31 May-4 Sep	4 Sep-14 Oct	12 Jul-3 Oct	14	18	15.5	10.5	9.0	12.0
LT	97	6/20	4 Sep-3 Oct	31 May-8 Aug	18 Sep-14 Oct	14 Jun-3 Oct	6	18	16.5	10.5	11.0	11.0
River Kuusaankoski												
HT	93	37/3	12 Jul-8 Sep	31 May-14 Jun	8 Aug-18 Sep	14 Jun-27 July	10	8	21.5	10.5	16.0	23.0
IT	93	13/6	12 Jul-14 Oct	31 May-12 Jul	4 Sep-28 Oct	12 Jul-18 Sep	16	16	15.5	10.5	8.0	15.7
LT	95	2/8	4 Sep-3 Oct	31 May-8 Aug	4 Sep-3 Oct	12 Jul-4 Sep	4	14	16.5	10.5	11.0	16.5

TABLE 2 Two-way ANOVA statistics and standardized test statistics for Mann-Whitney U test for the effect of temperature treatment (and mussel population) on the cercarial shedding traits of *R. fennica* and *R. campanula*. Statistically significant effects are marked with an asterisk*. 'L' stands for Log_{10} transformation of the response variable. HT, IT and LT represent high, intermediate and low temperature treatments, respectively.

Parameter	**Analysis**	**Factor**	**Test statistics**	***P***
Start date, *R. fennica*	2-ANOVA	Treatment	$F_{2, 104} = 22.620$	< 0.001*
		Population	$F_{1, 104} = 0.530$	0.468
		Treatm. × Pop.	$F_{2, 104} = 0.843$	0.433
Start date, *R. campanula*	2-ANOVA	Treatment	$F_{2, 50} = 1.722$	0.189
		Population	$F_{1, 50} = 0.061$	0.806
		Treatm. × Pop.	$F_{2, 50} = 0.988$	0.379
Start temp., *R. fen*. River Kuus.	1-ANOVA	Treatment	$F_{2, 49} = 9.270$	< 0.001*
Start temp., *R. fen*. River Haaj.	Mann-Whit.	HT vs. IT	-6.222	< 0.001*
Start temp., *R. fen*. River Haaj.	Mann-Whit.	HT vs. LT	-4.268	< 0.001*
Start temp., *R. fen*. River Haaj.	1-ANOVA	IT vs. LT	$F_{1, 23} = 7.298$	0.013*
Start temperature, *R. campanula* (L)	2-ANOVA	Treatment	$F_{2, 50} = 5.647$	0.150
		Population	$F_{1, 50} = 0.050$	0.837
		Treatm. × Pop.	$F_{2, 50} = 0.378$	0.687
Day degrees to start, *R. fennica*	2-ANOVA	Treatment	$F_{2, 104} = 7.965$	< 0.001*
		Population	$F_{1, 104} = 1.818$	0.181
		Treatm. × Pop.	$F_{2, 104} = 0.423$	0.656
Day degrees to start, *R. campan.*	2-ANOVA	Treatment	$F_{2,50} = 1.673$	0.198
		Population	$F_{1, 50} = 0.050$	0.823
		Treatm. × Pop.	$F_{2, 50} = 0.976$	0.384
Stop date, *R. fennica*	2-ANOVA	Treatment	$F_{2, 104} = 62.583$	< 0.001*
		Population	$F_{1, 104} = 0.213$	0.646
		Treatm. × Pop.	$F_{2, 104} = 2.912$	0.059
Stop date, *R. campanula*	2-ANOVA	Treatment	$F_{2, 50} = 3.625$	0.034*
		Population	$F_{1, 50} = 3.728$	0.059
		Treatm. × Pop.	$F_{2, 50} = 0.527$	0.594
Stop temp., *R. fen.* River Haaj.	1-ANOVA	Treatment	$F_{2, 54} = 60.021$	< 0.001*
Stop temp., *R. fen.* River Kuus.	Mann-Whit.	HT vs. IT	-4.760	< 0.001*
Stop temp., *R. fen.* River Kuus.	1-ANOVA.	HT vs. LT	$F_{1, 37} = 11.234$	0.002*
Stop temp., *R. fen.* River Kuus.	1-ANOVA.	IT vs. LT	$F_{1, 13} = 0.162$	0.694
Stop temperature, *R. campanula* (L)	2-ANOVA	Treatment	$F_{2, 50} = 36.443$	0.027*
		Population	$F_{1, 50} = 0.269$	0.620
		Treatm. × Pop.	$F_{2, 50} = 0.096$	0.909
Duration, *R. fennica*	2-ANOVA	Treatment	$F_{2, 104} = 2.668$	0.074
		Population	$F_{1, 104} = 0.185$	0.194
		Treatm. × Pop.	$F_{2, 104} = 1.665$	0.194
Duration, *R. campanula*	2-ANOVA	Treatment	$F_{2, 50} = 0.564$	0.573
		Population	$F_{1, 50} = 3.050$	0.087
		Treatm. × Pop.	$F_{2, 50} = 0.031$	0.990

TABLE 3 Two-way ANOVA statistics and standardized test statistics for Mann-Whitney U test for the effect of parasite species (and mussel population) on the cercarial shedding traits. To account for multiple tests, the critical value for statistical significance was set to $P = 0.025$ (Bonferroni correction). Statistically significant effects are marked with an asterisk*. 'CR' stands for cube root transformation of the response variable.

Parameter	Analysis	Factor	Test statistics	*P*
Start date, High temp., River Haaj.	1-ANOVA	Species	$F_{1, 39} = 65.981$	< 0.001*
Start date, High temp., River Kuus.	1-ANOVA	Species	$F_{1, 39} = 19.601$	< 0.001*
Start date, Interm. temp.	2-ANOVA	Species	$F_{1, 45} = 59.499$	< 0.001*
		Population	$F_{1, 45} = 0.075$	0.785
		Species × Pop.	$F_{1, 45} = 0.445$	0.508
Start date, Low temperature	2-ANOVA	Species	$F_{1, 32} = 74.525$	< 0.001*
		Population	$F_{1, 32} = 0.134$	0.716
		Species × Pop.	$F_{1, 32} = 1.371$	0.250
Start temperature, HT, River Haaj.	Mann-Whit.	Species	4.738	< 0.001*
Start temperature, HT, River Kuus.	1-ANOVA	Species	$F_{1, 38} = 106.84$	< 0.001*
Start temperature, IT, River Haaj.	Mann-Whit.	Species	3.490	< 0.001*
Start temperature, IT, River Kuus.	Mann-Whit.	Species	3.385	0.001*
Start temperature, Low temperature	2-ANOVA	Species	$F_{1, 32} = 188.70$	0.046
		Population	$F_{1, 32} = 9.480$	0.200
		Species × Pop.	$F_{1, 32} = 0.215$	0.646
Day-degrees to start, High temp. (CR)	2-ANOVA	Species	$F_{1, 77} = 138.31$	< 0.001*
		Population	$F_{1, 77} = 0.115$	0.736
		Species × Pop.	$F_{1, 77} = 2.275$	0.136
Day degrees to start, Interm. temp.	2-ANOVA	Species	$F_{1, 45} = 57.163$	< 0.001*
		Population	$F_{1, 45} = 0.075$	0.786
		Species × Pop.	$F_{1, 45} = 0.577$	0.451
Day-degrees to start, Low temp.	2-ANOVA	Species	$F_{1, 31} = 101.570$	< 0.001*
		Population	$F_{1, 31} = 1.287$	0.265
		Species × Pop.	$F_{1, 31} = 0.151$	0.701
Stop date, High temp., River Haaj.	Mann-Whit.	Species	4.783	< 0.001*
Stop date, High temp., River Kuus.	1-ANOVA	Species	$F_{1, 38} = 22.067$	< 0.001*
Stop date, Interm. temp., River Haaj.	Mann-Whit.	Species	3.763	< 0.001*
Stop date, Interm. temp., River Kuus.	1-ANOVA	Species	$F_{1, 17} = 49.168$	< 0.001*
Stop date, Low temperature	2-ANOVA	Species	$F_{1, 32} = 10.225$	0.003*
		Population	$F_{1, 32} = 0.789$	0.378
		Species × Pop.	$F_{1, 32} = 0.136$	0.715
Stop temperature, HT, River Haaj.	Mann-Whit.	Species	0.543	0.587
Stop temperature, HT, River Kuus.	Mann-Whit.	Species	-0.964	0.335
Stop temperature, IT, River Haaj.	Mann-Whit.	Species	0.368	0.713
Stop temperature, IT, River Kuus.	Mann-Whit.	Species	-2.174	0.030
Stop temperature, Low temperature	2-ANOVA	Species	$F_{1, 32} = 3.428$	0.315
		Population	$F_{1, 32} = 1.856$	0.403
		Species × Pop.	$F_{1, 32} = 1.771$	0.193
Duration, High temperature	2-ANOVA	Species	$F_{1, 77} = 5.628$	0.020*
		Population	$F_{1, 77} = 2.136$	0.148
		Species × Pop.	$F_{1, 77} = 0.194$	0.661
Duration, Intermediate temperature	2-ANOVA	Species	$F_{1, 45} = 5.470$	0.024*
		Population	$F_{1, 45} = 0.491$	0.487
		Species × Pop.	$F_{1, 45} = 2.577$	0.115
Duration, Low temperature	2-ANOVA	Species	$F_{1, 32} = 11.006$	0.002*
		Population	$F_{1, 32} = 1.098$	0.303
		Species × Pop.	$F_{1, 32} = 0.234$	0.632

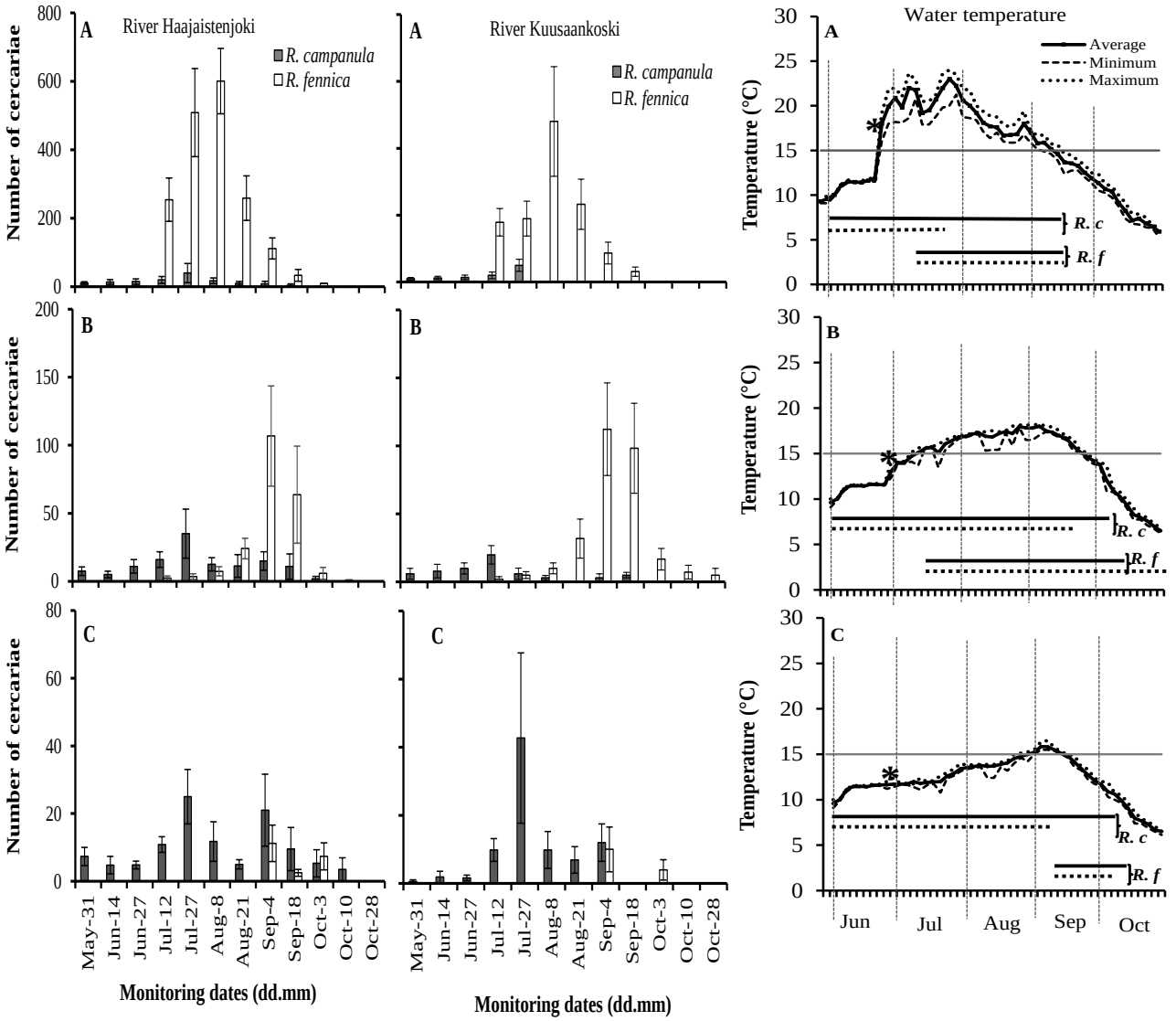

FIGURE 1 The daily (mean ± SE) cercarial release of *Rhipidocotyle campanula* (*R. c*) and *R. fennica* (*R. f*), water temperature profile at 3-day intervals and the total duration of cercarial release by mussels from the River Haajaistenjoki (straight line) and by mussels from the River Kuusaankoski (dotted lines), from 31 May to 28 October in the high, (A) intermediate (B) and low temperature treatments (C). An asterisk represents the day when the mussels were assigned to the different temperature treatments. Note the different scales on the y-axes.

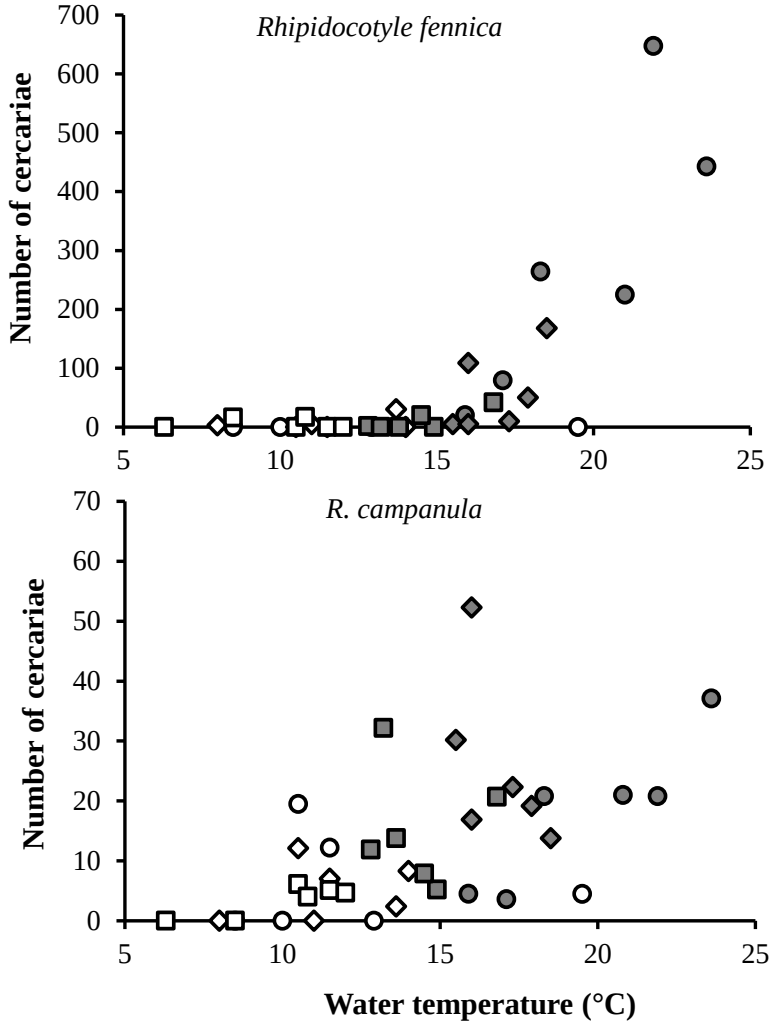

FIGURE 2 Mean number of cercariae released by *Rhipidocotyle fennica* and *R. campanula* in relation to water temperature at each of the twelve cercarial release monitoring sessions from mussels assigned to high (circles, n = 12), intermediate (diamonds, n = 12) and low temperature (squares, n = 12). Filled symbols represent the months of the highest water temperature – July, August and September. River Haajaistenjoki and River Kuusaankoski populations combined.

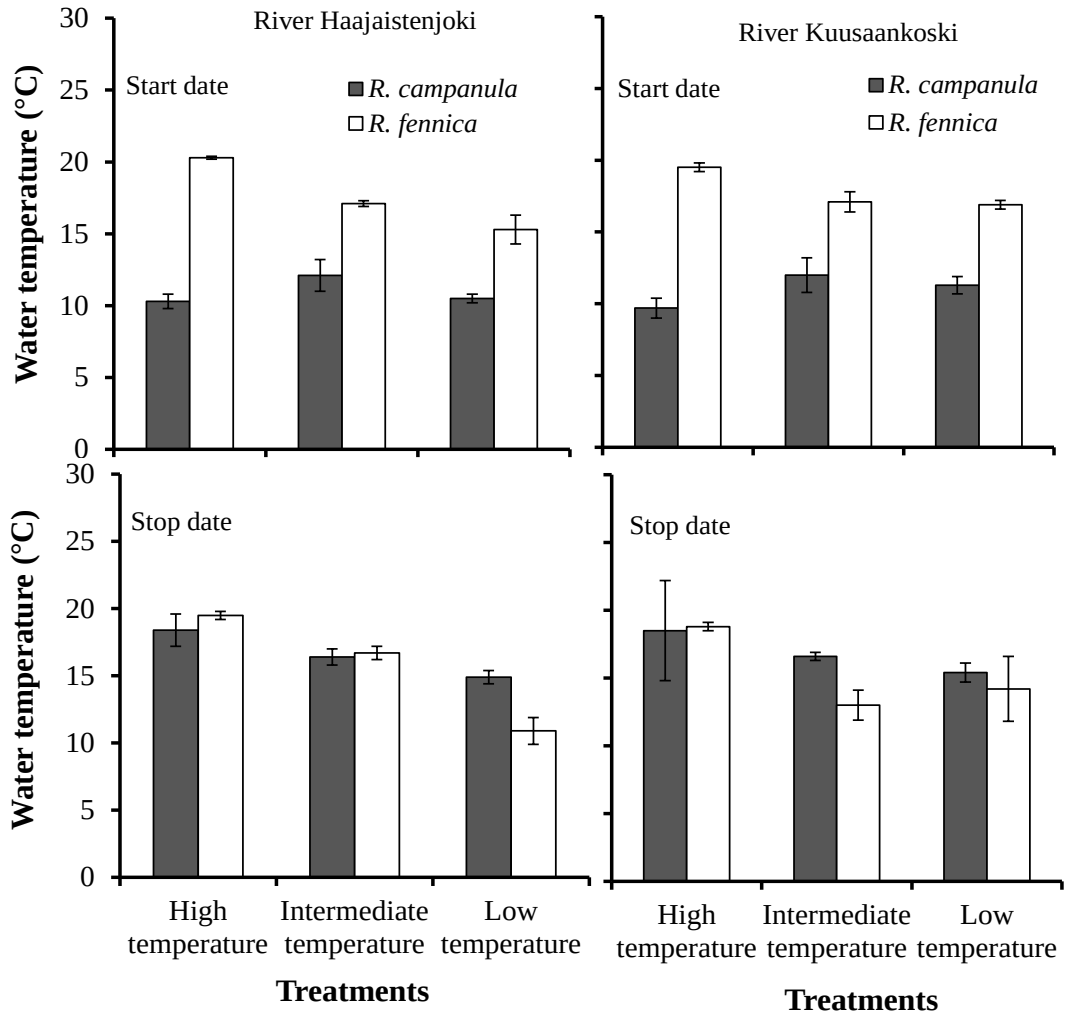

FIGURE 3 Water temperatures (mean ± S.E.) in the three temperature treatments on the dates of the first and last observation of emerged cercariae.

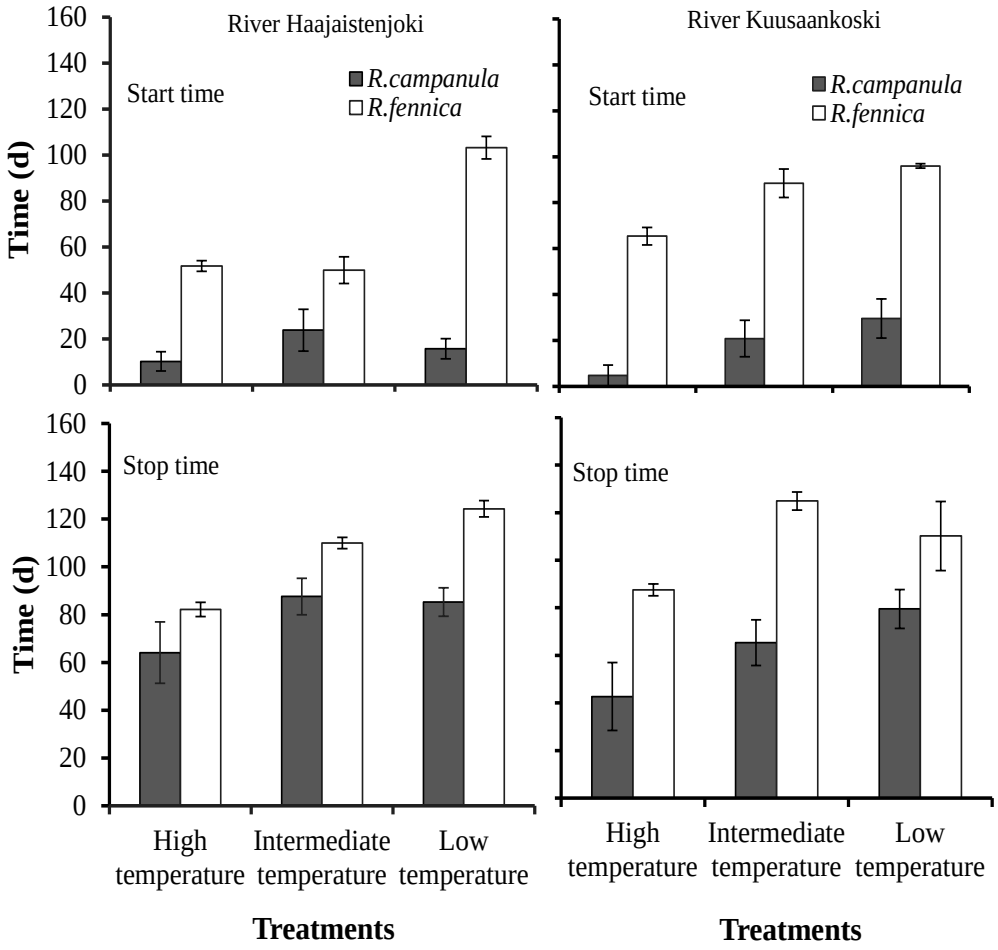

FIGURE 4 Time (days; mean ± S.E.) from the beginning of the experiment (May 31) to the start and stop of cercarial shedding in the different temperature treatments.

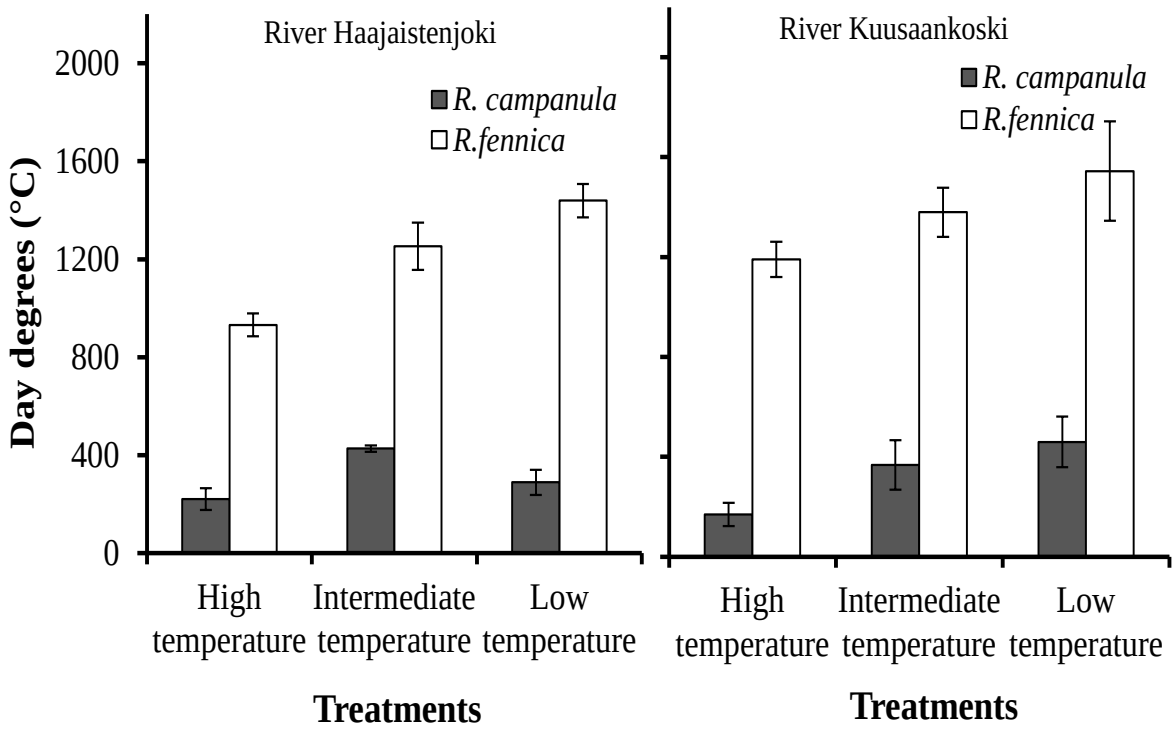

FIGURE 5 Mean (± S.E.) sum of day-degrees (°C) from May 17 to the start of cercarial release in the different temperature treatments.

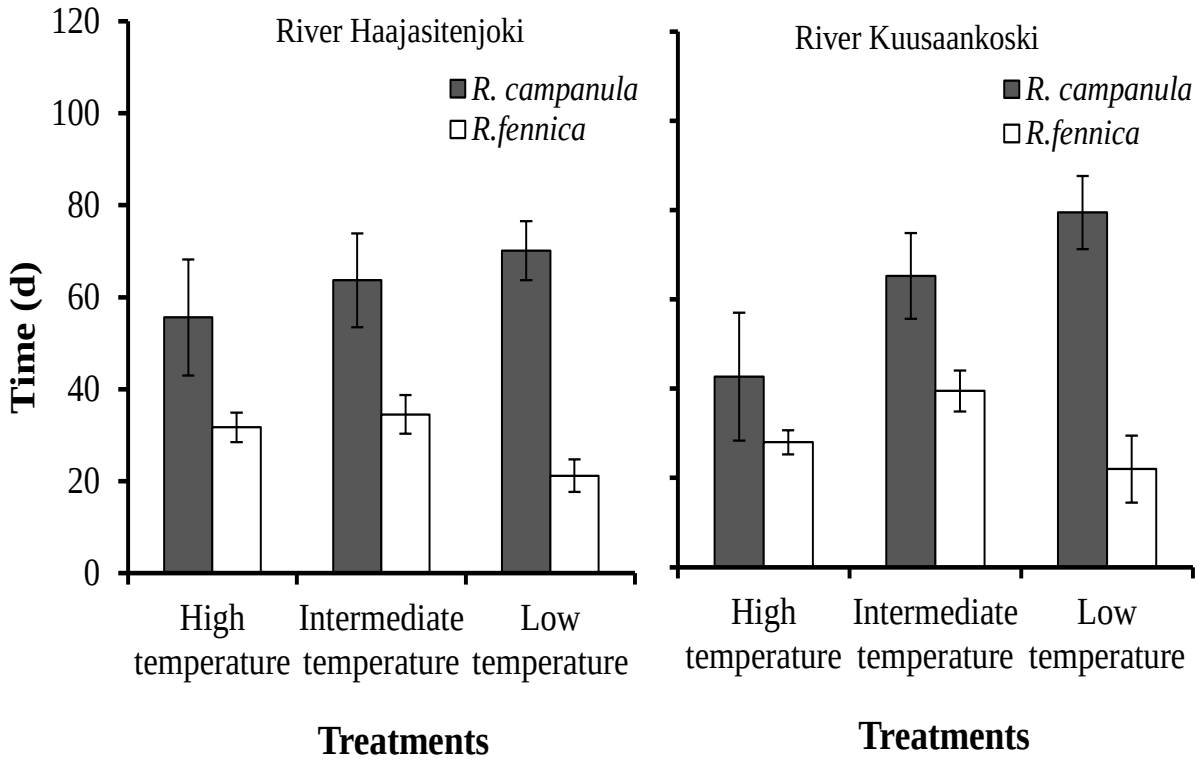

FIGURE 6 Duration of cercarial shedding (mean ± S.E.) of *R. fennica* and *R. campanula* in the different temperature treatments.

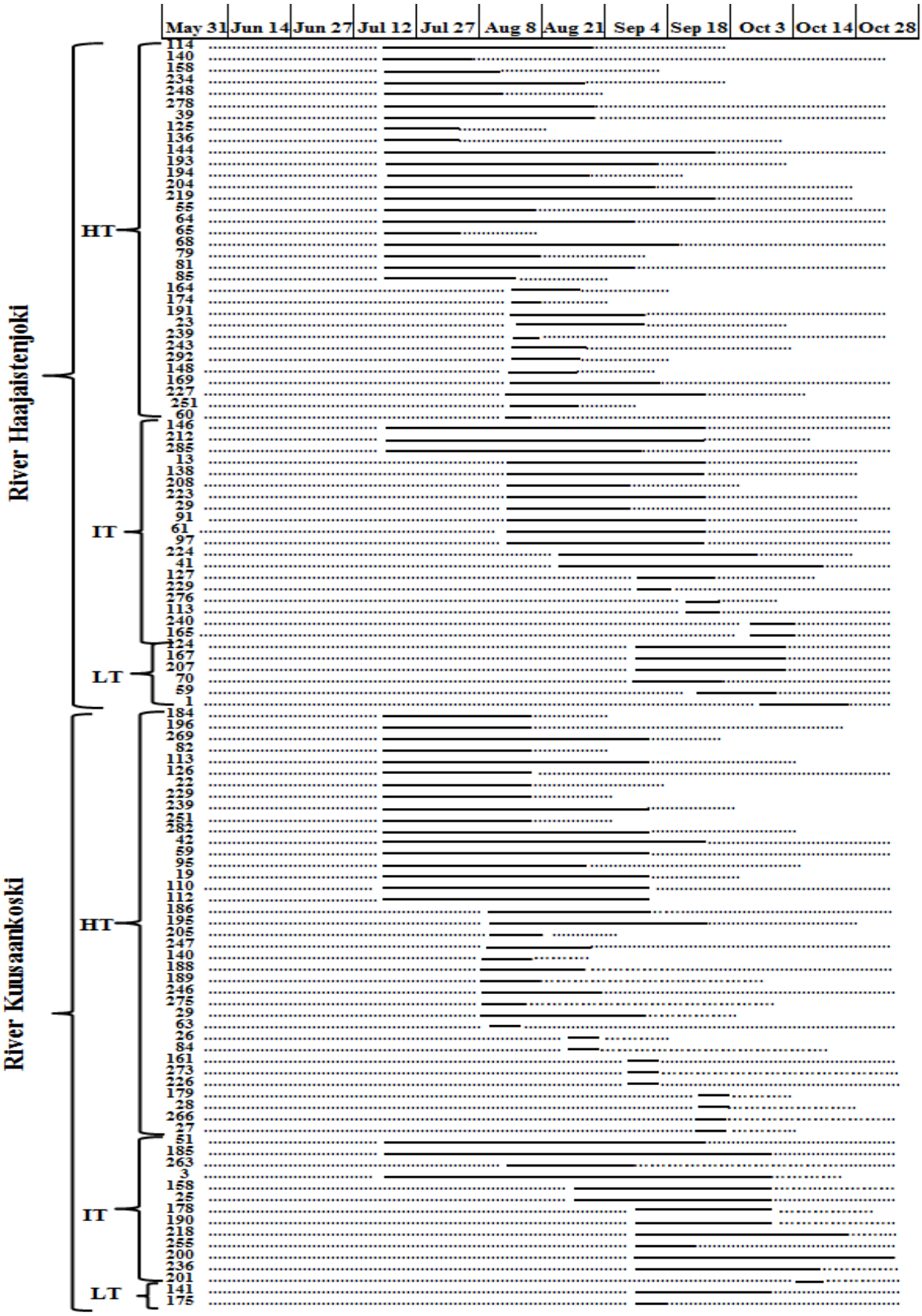

FIGURE S1 The patterns of individual *A. anatina* mussels shedding *R. fennica* at high temperature (HT), intermediate temperature (IT) and low temperature (LT). Solid line indicates the cercarial shedding period, dotted line represents the non-shedding period and the solid line ends when the mussel died or the experiment ended.

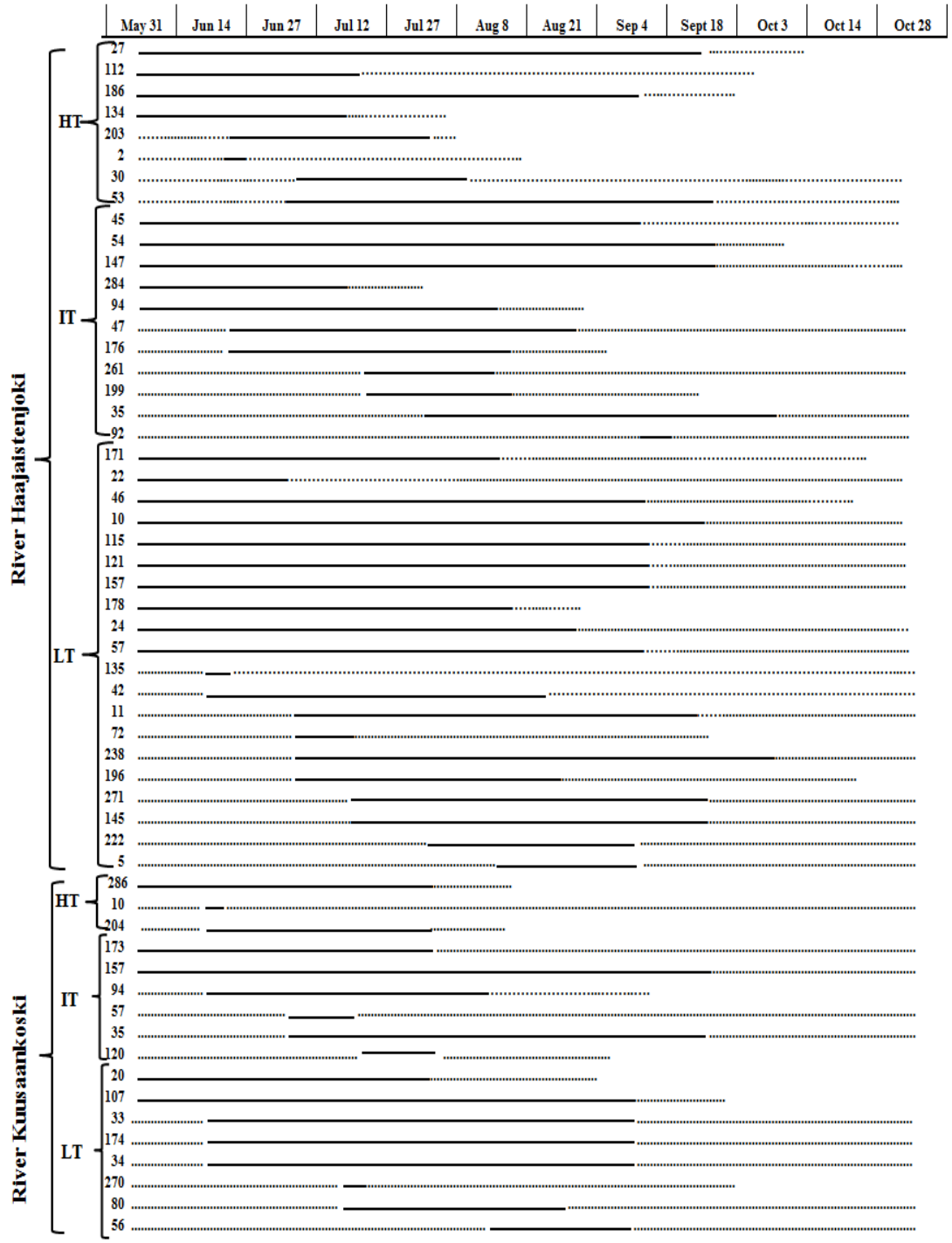

FIGURE S2 The patterns of individual *A. anatina* mussels shedding *R. campanula* at high temperature (HT), intermediate temperature (IT) and low temperature (LT). Solid line indicates the cercarial shedding period, dotted line represents the non-shedding period and the solid line ends when the mussel died or the experiment ended.