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Jocelyn M. Choo

Connection between Temperature, Larval Production, Virulence and Geographical Distribution of *Rhipidocotyle* Parasites Infecting the Duck Mussel, *Anodonta anatina*

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ABSTRACT

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Diss.

In this thesis, two bucephalid trematode parasites *Rhipidocotyle campanula* and *R. fennica*, which use the same first (*Anodonta anatina*) and second intermediate (*Rutilus rutilus*) host were studied. The aim was to investigate the effect of temperature on one of the key processes in the transmission of these parasites: 1) the emergence of cercarial larvae from *A. anatina* over short (1 h) and 2) long (throughout the annual cercarial shedding period, from May to October) time periods as well, as on 3) mussel survival and 4) the seasonal timing of cercarial release. In addition, the aim was to study how the cercarial shedding traits are linked to the 5) geographical occurrence and abundance of the *Rhipidocotyle* species. In the experimental studies, the cercarial emergence by *R. fennica* increased significantly with increasing temperature over short and long time periods, while that by *R. campanula* was unaffected by temperature. *R. campanula* clearly started seasonal cercarial release earlier and at a lower temperature than *R. fennica*. Survival of mussels, especially cercariae-shedding mussels, was lower at higher temperature, and the shedding of *R. campanula* cercariae was associated with higher mussel mortality than the shedding of *R. fennica*. The average duration of the seasonal cercarial release period of both species was unaffected by temperature at the individual host level, but at the host population level the cercarial shedding period of *R. fennica* (but not of *R. campanula*) was longer at higher temperature. The field study showed that the occurrence, mean prevalence and abundance of *R. fennica* – in accordance with the experimentally observed association of cercarial release with high temperature – decreased from the south (61–64 °N) to the low north (65–66 °N), but this pattern was not detected in *R. campanula*.

Keywords: *Anodonta anatina*, cercarial production, latitudinal pattern, *Rhipidodotyle* parasites, *Rutilus, rutilus*, Unionidae, temperature, virulence.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals (I–IV).

Responsibilities of Jocelyn M. Choo in the articles of this thesis: In I, the experiment was planned together with JT. JMC carried out the experiment. Statistical analyses were performed by JT, and JMC wrote the article together with JT. In II and III, the planning of the experiments and collection of materials as well as statistical analyses were performed together with JT. JMC performed the experiments, and wrote the articles jointly with JT. In IV, the preliminary idea to carry out this study came from JT, who performed majority of the initial work. JMC contributed to material collection together with RK. HM organized and analysed the meteorological data. JMC contributed to the writing, and JT was mainly responsible for the statistical analyses and writing the article.

I Choo J.M. & Taskinen J. 2015. Effect of short-term temperature change on cercarial release by *Rhipidocotyle fennica* (Trematoda, Bucephalidae) from the freshwater bivalve host, *Anodonta anatina*. *Ecological Parasitology and Immunology* 4: doi:10.4303/epi/235932.

II Choo J.M. & Taskinen J. 2015. Temperature-dependent transmission and virulence of two *Rhipidocotyle* species parasitising a molluscan host. Submitted manuscript.

III Choo J.M. & Taskinen J. 2015. Contrasting temperature responses in the seasonal timing of cercarial shedding by two *Rhipidocotyle* trematodes. Submitted manuscript.

IV Taskinen J., Mäkelä H.M., Kortet R. & Choo J.M. 2015. Latitudinal distribution and abundance of the trematode parasites *Rhipidocotyle fennica* and *R. campanula*. Manuscript.

1 INTRODUCTION

1.1 Temperature and its impact on host–parasite systems

One of the most common interactions between species has been found to occur between parasites and their hosts (Bush *et al*. 2001). However, parasites, hosts and/or their interaction can be affected in different ways by different environmental factors including climate warming and the associated longer thermal growing season. Temperature is considered an especially influential factor, because it not only affects parasites directly during each of the different stages in their life cycle (Chubb 1979), but also indirectly via alterations in the distribution and abundance of their hosts (Marcogliese 2001). However, there is considerable interspecific variation in these responses. For the most part, the effect of increased temperature, as demonstrated regarding the infective larval stages (cercariae) of trematode parasites, includes rapid maturation, increased transmission and virulence (Mouritsen 2002, Thieltges and Rick 2006, Paull and Johnson 2011), at least up to an optimum temperature level (Studer *et al*. 2010), as well as decreased survival (McCarthy 1999). However, neutral or even negative temperature effects have also been reported (Koprivnikar and Poulin 2009). Host species are also subject to temperature-driven increase in susceptibility and reduced immune-competence (Seppälä and Jokela 2010), thereby leading to enhanced parasite-induced host mortality.

Range expansion or shifting patterns in the distribution of species towards higher latitudes, and changes in the timing of seasonal events (phenology) by parasites and hosts are among the important impacts of global climate warming (Lindgren and Gustafson 2001, Root *et al*. 2003, Kutz *et al*. 2005, Parmesan 2006). This is already evident in studies showing the northward movement of the parasite *Perkinsus marinus,* which causes disease in oysters, from its original southeast coastal range to the northeast coast in Sweden as a result of a warming trend in the north (Cook *et al*. 1998). In addition, the seasonal duration of larval release by parasites is expected to increase as a result of the longer thermal growing season (longer summer) (Marcogliese 2001) associated with

climate warming. Such lengthening in the seasonal cercarial transmission window will extend the risk of parasitism for target hosts.

Thus, changes in temperature and the associated thermal growing season are likely to affect host–parasite dynamics in different ways. Because parasites are influenced by different biotic and abiotic factors, as well as the abundance, distribution and condition of their hosts, for their basic life-history functions (e.g. transmission, reproduction and dispersal), predicting the implications of climate warming for parasite species and their hosts becomes very complex and context-dependent (Marcogliese 2001). Some parasite species will benefit from the warming through increased production and range extension, while others may experience range contractions or become locally extirpated.

In high latitude areas, temperature-mediated influence on host–parasite systems is particularly evident because parasite occurrence, reproduction and transmission show strong seasonality mediated by seasonal temperature fluctuations in the environment (Rantanen *et al*. 1998, Taskinen 1998a, Fingerut *et al*. 2003, Karvonen *et al*. 2004, 2010, Hakalahti *et al*. 2006). For instance, a greater part of each year is unsuitable for parasite growth, reproduction and transmission. Accordingly, the parasite life cycle is completed within narrow temporal limits, namely, warm summer months (e.g. Chubb 1979, Karvonen *et al*. 2004). High latitude parasites have evolved under seasonal temperature constraints. However, their high sensitivity to temperature (Poulin 2006) suggests that ongoing climate warming, which is projected to be stronger in high latitude areas (IPCC 2007), and the associated warmer summer and longer growing season (e.g. Ruosteenoja *et al*. 2011) are likely to alter the seasonal window for parasite growth by advancing its onset, delaying its cessation or lengthening the duration of the seasonal growing period for parasites. The importance of studying temperature-driven influence on host–parasite systems at high latitudes has been highlighted in previous studies (Morley and Lewis 2013, Studer and Poulin 2014), but experimental temperature manipulations have not been performed in relation to high latitude host–trematode associations at high latitudes (> 60°).

1.1.1 Cercarial emergence

The emergence of the infective larval stages (cercariae) from the molluscan host is an important feature in the trematode life cycle permitting transmission of infection from the first to the second intermediate or definitive host, thus contributing to the life-time reproductive success of the parasite. The emergence of cercariae is often initiated in response to different environmental factors, among which temperature is the most important factor (Poulin 2006). The cercarial production of trematodes in the molluscan host is receiving increased attention, because in addition to the link between cercarial production and temperature, it comes with a cost to the hosts as a result of the utilization of host tissues and energy reserves for larval production (Jokela *et al*. 1993). Moreover, cercariae are transmitted to different aquatic vertebrates and invertebrates, and the large numbers of cercariae that emerge into the aquatic

environment may play important secondary roles in the functioning of aquatic ecosystems in terms of biomass and energy flow (Thieltges *et al*. 2008, Morley 2012, Preston *et al*. 2013). Furthermore, a number of cercarial species transmitted to humans are of public health and medical importance (Lewis and Tucker 2014).

For *Rhipidocotyle* parasites, transmission from the first (*A. anatina*) to the second (*R. rutilus*) intermediate host takes place via free-swimming cercarial larvae. The cercarial release from the freshwater unionid mussel host (*A. anatina*) is strongly temperature-dependent, and it occurs in nature during the summer months (Taskinen *et al*. 1994, 1997, Taskinen 1998a). This is a common trend in temperate and cold climatic zones (Chubb 1979, Taskinen 1998a), suggesting that ambient temperature is one of the important determinants of cercarial production in trematodes. In addition, by increasing temperature, the release of *Rhipidocotyle* cercaria can be induced even during winter, outside of the natural shedding period (Taskinen *et al*. 1991). The water temperature and accumulated day-degrees required for the start of seasonal cercarial emergence by both *Rhipidocotyle* species are different, being much lower for *R. campanula* than for *R. fennica* (Taskinen *et al*. 1994, 1997). Whereas *R. campanula* starts cercarial release in early June, *R. fennica* does not start until 3–4 weeks later (in mid-July) (Taskinen *et al*. 1994, 1997, Taskinen 1998a). Consequently, the two parasites have different seasonal timing for cercarial emergence. In the laboratory, the cercarial shedding of *R. campanula* responds quickly to increased temperature, but the response of *R. fennica* is much slower (Taskinen *et al*. 1991).

However, the relationship between temperature and cercarial emission might not be straightforward, since neutral or even slightly negative temperature effects have also been reported for other trematode species (Poulin 2006). Results by Morley and Lewis (2013) and Studer and Poulin (2014) indicate that temperature effects on cercarial emergence are complex, depending on the host–parasite system, temperature range, acclimation, host size and latitude. Almost all of what is known about temperature–host–parasite interaction is derived from studies on snail hosts, with marine trematodes being the most studied (e.g. Fingerut *et al*. 2003, Thieltges and Rick 2006, Koprivnikar and Poulin 2009, Studer *et al*. 2010, Studer and Poulin 2014). The bivalvian mussel hosts remain poorly studied (Lyholt and Buchmann 1996, Morley *et al*. 2010). This is surprising because freshwater mussels are worldwide in distribution (e.g. Graf and Cummings 2006, Bogan 2008) and serve as intermediate hosts for many species of larval trematodes (e.g. Taskinen *et al*. 1991, Gibson *et al*. 1992, Grizzle and Brunner 2009). Understanding the response of different trematode–host associations to changing temperature will provide a more robust grasp of temperature influence on different host–parasite systems.

1.1.2 Latitudinal species diversity and richness gradients

Latitudinal gradients in species diversity and richness are one of the well-documented universal patterns in the distribution of organisms in nature (Rohde 1992, Rosenzweig 1995, Gaston and Blackburn 2000). Although there are

a few exceptions, decreases in diversity and richness in relation to increasing latitude have been demonstrated for many animals and parasites at the regional, continental and global levels (MacArthur 1972, Hawkins and Porter 2001, Rohde 2002, Guernier *et al*. 2004, Kuklinski *et al*. 2006, Hof *et al*. 2008, Griffiths *et al*. 2014). Among the exceptions are the marine trematodes of snail hosts in Europe, which show no latitudinal gradient in species richness (Thieltges *et al*. 2011), and endoparasites of the marine teleost fish across distinct geographical areas, from the Antarctic to the tropics, which also show no latitudinal gradient in relative species diversity and abundance (Rohde and Heap 1998).

A recent meta-analysis by Kamiya *et al*. (2014) indicates that the relationship between parasite species richness and latitude is weak, but mainly positive, with richness increasing with latitude. Thus, the latitudinal gradients in parasite diversity may differ from those of free-living taxa. More research, especially on the factors influencing the latitude dependence of parasite species occurrence is required (Kamiya *et al*. 2014).

Amongst many factors (e.g., dispersal ability and colonization probability), climatic factors, especially temperature, have been the most cited aspect influencing the observed latitudinal diversity pattern in both parasitic and free-living (host) organisms (Rohde 1992, Poulin and Rohde 1997, Rohde and Heap 1998, Guernier *et al*. 2004, Smith *et al*. 2010, Knouft and Page 2011, Griffiths *et al*. 2014). This is because temperature is one of the key determinants in the timing of seasonal events (e.g. growth and reproduction), development and transmission in a variety of parasitic and non-parasitic organisms (see Stenseth and Mysterud 2002, Ložys 2004, Ficke *et al*. 2007, Studer *et al*. 2010, Paull and Johnson 2011). Therefore the decrease in species richness and diversity towards high latitudes is not surprising. There is a decline in thermal growing season length, an increase in seasonal and interannual variability in temperature and greater strength of winter frost towards higher latitudes (e.g. Pau *et al*. 2011). Few species can physiologically tolerate these "harsh" conditions.

In addition to cooler temperature as a limiting factor in high latitude regions, parasites (and hosts) may not have had enough time to recolonize the high northern areas after the last glaciation. Furthermore, sporadic occurrence and declines in host richness and abundance towards higher latitude areas severely limits host availability and parasite transmission regardless of the suitability of ambient temperature. This is because of the strong positive correlation between parasite and host species richness and abundance (Watters 1992, Hechinger and Lafferty 2005, Krasnov *et al*. 2007). Hosts serve as habitat and dispersal agent for parasites. Therefore, for parasites with complex, multi-host life cycles such as *Rhipidocotyle* trematodes, one might expect the parasites to decrease in abundance with increasing latitude, and to show latitudinal pattern in occurrence and abundance that match those of their hosts.

The seasonal pattern and temperature sensitivity of many parasites suggest that ongoing global climate warming, which is projected to be greatest at higher latitudes (IPCC 2007), and the associated warmer summer and longer

growing season (Ruosteenoja *et al*. 2011) are likely to change the typical latitudinal pattern of parasites, causing range expansion or shift of some parasites towards higher latitudes. The relaxation of temperature constraints that affect some life-history traits (e.g. development, transmission) of parasites and hosts in northern ecosystems as a result of climate warming could facilitate the introduction and establishment of species previously unknown in the north.

1.2 The importance of trematode parasites

Trematodes are an important group of parasites, interacting within different trophic levels/members of a community during their complex life cycle, which involves different transmission processes between hosts. Many trematode species infect vertebrates and invertebrates. They are a ubiquitous part of freshwater and marine food webs, and they can play important roles in the functioning of aquatic ecosystems via food web and energy transfer (Kuris *et al*. 2008, Preston *et al*. 2013). Some trematodes can cause major veterinary or health problems (Morgan *et al*. 2001, Lewis and Tucker 2014). The influence of trematodes on host life-history traits, as well as behaviour and thermal preference, has been reported in many studies (e.g. Latham and Poulin 2002, Moore 2002, Żbikowska 2004).

Trematodes of the Bucephalidae family are known to parasitize a wide range of hosts, ranging from molluscs to fishes to birds (e.g. Taskinen *et al*. 1991, Gibson *et al*. 1992, Grizzle and Brunner 2009). In the mussel first intermediate host, the gonad is the primary target of infection and sporocyst proliferation. This infestation represents a serious risk for the host, as it leads to decreased growth, physiological condition and survival. Furthermore, it will disable mussel gametogenesis, leading to parasitic castration (Taskinen and Valtonen 1995, Jokela *et al*. 2005, Gangloff *et al*. 2008, Müller *et al*. 2015) and possible mussel death (da Silva *et al*. 2002). Valtonen *et al*. (1997) found the prevalence of infection by *R. fennica* and *R. campanula* to be very high in roach (*Rutilus rutilus*) from 4 lakes: 92–95 % and 53–70 %, respectively. Hoffmann *et al*. (1990) found that infection by bucephalid cercariae caused a mass mortality of their fish second (*R. rutilus)* hosts as the result of a sudden increase in water temperature from 12 to 20 ˚C. Parasite-induced pathology can be amplified at warmer temperatures (Paull and Johnson 2011), as a result of temperature-facilitated parasite production and virulence (Mouritsen and Jensen 1997, Kocan *et al*. 2009), or temperature-suppressed host immune responses (Seppälä and Jokela 2010). Bucephalid infection itself is a stressor. Thus, any additional temperature effect on the level of bucephalid parasitism is likely to have substantial consequences for host individuals.

1.3 Study species

Rhipidocotyle campanula and *R. fennica* (Trematoda: Bucephalidae, Digenea), need three host species to complete their life cycles, and they possess two aquatic free-swimming stages (Fig. 1). The adult worms are intestinal parasites mainly of the northern pike *Esox lucius* (*R. fennica*) and the European perch *Perca fluviatilus* (*R. campanula*) (Taskinen *et al*. 1991). Adult worms reproduce sexually, producing eggs that are released into the water. Eggs hatch free-swimming miracidia. Miracidium larvae of both species penetrate the first intermediate host, *A. anatina* where they develop into sporocysts. The sporocysts invade (mainly) the gonads of the mussel host (Taskinen *et al*. 1997), asexually producing large numbers of free-swimming cercarial larvae, which emerge primarily during the summer months to infect the common second intermediate hosts, the cyprinid fish *R. rutilus* (Taskinen *et al*. 1991, Gibson *et al*. 1992). Whereas the emerged cercariae of *R. fennica* attach and encyst as metacercariae mainly in the fins of *R. rutilus*, those of *R. campanula* encyst in the gills (Taskinen *et al*. 1991). The life cycle is completed when an infected second intermediate host is consumed by a definitive host where the metacercariae excyst and transform into adult worms.

In natural *A. anatina* populations, the prevalence of infection by *R. campanula* is usually not high (< 5%) and the parasite destroys on average 90 % of *A. anatina* gonad tissue, while infections by *R. fennica* are common (20–90%) and lead to an average of 30 % gonad destruction (Taskinen *et al*. 1991, 1994). Both parasites more often infect older and female mussels (Taskinen and Valtonen 1995, Müller *et al*. 2015). Pronounced seasonality in the developmental stages of cercariae and the number of sporocysts of the *Rhipidocotyle* species in *A. anatina*, and no clear seasonality in the prevalence of infection were observed by Taskinen *et al*. (1994). Both parasite species have been linked to decreased growth, longevity and reproduction of *A. anatina* as well as their ability to survive environmental stress (Taskinen and Valtonen 1995, Taskinen 1998b, Jokela *et al*. 2005, Müller *et al*. 2015).

The northernmost known locations of *R. fennica* and *R. campanula* are at 62 N° and 65 °N, respectively (Taskinen *et al*. 1994). Both parasites appear to be widespread in European freshwaters (for a review see Petkevičiūtė *et al*. 2014). However, their distributions have not been well-mapped. The only known first and second intermediate hosts for both parasite species in Finland are the unionid mussel *A. anatina* and the cyprinid fish *R. rutilis*, respectively. However, there are records in other European countries of other unionid species such as *Unio crassus* and *U. pictorum* (Baturo 1977, Ivantsiv and Chernogorenko 1984, Petkevičiūtė *et al*. 2014), and fish species (Baturo 1977, Ivantsiv and Chernogorenko 1984) harbouring *R. campanula* (= *illense*). Thus far, the only record of *R. fennica* occurrence in *R. rutilus* second intermediate host is in Finland (Taskinen *et al*. 1991, Gibson *et al*. 1992). In Finland, *A. anatina* and roach have been found up to 68 °N (Oulasvirta *et al*. 2008, Hayden *et al*. 2013),

and perch and pike occur throughout Finland up to 70 °N, although more sporadically and in low numbers at the highest latitudes (Hayden *et al*. 2013, 2014).

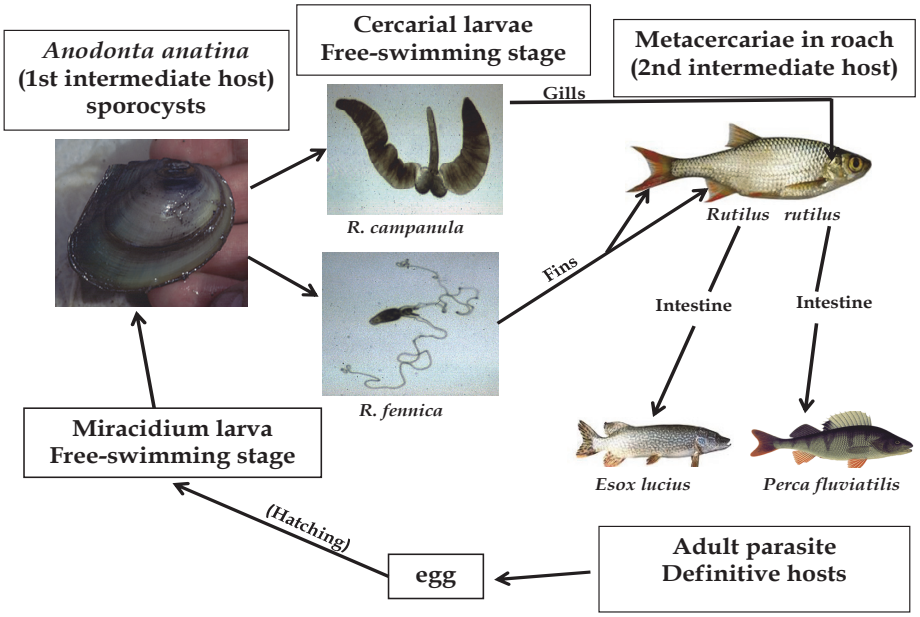

FIGURE 1 Life cycle of *Rhipidocotyle fennica* and *R. campanula*.

Anodonta anatina Nilss. (Mollusca Bivalvia, Unionidae) (= *A. piscinalis*) is a common and abundant dioecious freshwater bivalve mollusc inhabiting freshwaters in Europe. It is mature at 2–4 years of age, reaching a maximum life span of about 15–20 years and length of 12 cm (Økland 1963, Negus 1966). In Finland, the development of glochidia on the outer gill blades of female *A. anatina* takes place between July and August (Jokela *et al*. 1991). The glochidia are stored in the gills over the winter, and they are released the following spring (Negus 1966, Jokela *et al*. 1991, Pekkarinen 1993). After release, glochidia attach to a fish host (e.g. *R. rutilus* or *P. fluviatilis*) for about 4 weeks before benthic life begins (Jokela *et al*. 1991). Usually immature mussels (i.e. ≤ 2 years) are not infected, but after maturity the prevalence of infection in mussels increases with host age and size (Taskinen and Valtonen 1995, Taskinen *et al*. 1997). Concurrent infection (i.e. *R. campanula* and *R. fennica* occurrence in the same individual) is possible (Taskinen *et al*. 1991).

The cyprinid fish, roach *R. rutilus* is the second intermediate host for *R. fennica* and *R. campanula* (Taskinen *et al*. 1991). Roach has a ubiquitous distribution across western Eurasia and is found in different freshwater habitats (Kottelat and Freyhof 2007). They spawn during the spring in large groups, mainly in shallow waters. Individuals usually migrate to spawning sites (Mills 1991, Kestemont *et al*. 1999) such as littoral areas, bays, creeks and small ponds,

in which water warms early in the spring. Breeding sites can vary between populations and locations (Mills 1991). Spawning is observed mainly at temperatures above 12–16 °C (see Graham and Harrod 2009), indicating that the population recruitment of roach is strongly related to higher temperature. Roach have been found to harbour different protozoan and metazoan parasite species (Valtonen *et al*. 1997, Vainikka *et al*. 2009), but *R. fennica* and *R. campanula* are among the most common and abundant species (Valtonen *et al*. 1997, Vainikka *et al*. 2009). In Finnish waters, the transmission of *Rhipidocotyle* parasites to a roach is temporarily limited to occur between mid-June and September (Taskinen *et al*. 1994). Roach eurythermal characteristics allow them to survive a broad range of water temperatures (e.g. between 4 and > 30 °C) (Cocking 1959, Graham and Harrod 2009), but with a distinct preference for warmer temperatures. Roach growth mainly occurs above 12 °C (van Dijk *et al*. 2002) and juvenile growth is maximal between 20–27 °C (Hardewig and van Dijk 2003). Thus, roach are likely to profit from numerous aspects of the predicted climate change (Lehtonen 1996).

The European perch *P. fluviatilis* is the definitive host for *R. campanula* (Taskinen *et al*. 1991). It is a temperate mesotherm, cool-water, freshwater fish (Hokanson 1977), which is common in lakes, ponds and slow-flowing rivers across most of Europe and Asia. Perch spawn soon after the ice melts in April or May, mainly in littoral habitats. Perch co-occurs with roach and pike (*Esox lucius*). Perch are carnivorous and undergo dietary shifts that correspond with size. Wang and Eckmann (1994) have shown that the development and hatching success of perch eggs is most efficient at temperatures between 12 and 20 °C.

The Northern pike *E. lucius* is the definitive host for *R. fennica* (Taskinen *et al*. 1991). It is a large (< 130 cm) predatory freshwater fish (e.g. rivers, lakes and weakly saline waters), which is widely distributed around the northern hemisphere (e.g. Raat 1988, Crossman 1996). In northern areas, pike spawn in shallow water over vegetation immediately after the ice breaks in the spring, when water temperatures are between 8 and 12 °C (Casselman and Lewis 1996). After hatching, the larvae remain in vegetation for a few days (4–6 d) up to a month (Franklin and Smith 1963, Kennedy 1969). The pike migrates up tributaries and mainly reproduces in calm, sheltered and shallow waters with macrophyte vegetation (Craig 1996, Lappalainen *et al*. 2008).

1.4 Aims of the study

The aim of this thesis was to investigate the effect of temperature on one of the key process in the transmission of the trematodes *Rhipidocotyle campanula* and *R. fennica* from the first (*Anodonta anatina*) to the second (*Rutilus rutilus*) intermediate hosts, the emergence of cercarial larvae, over short (1 h) and long (20 weeks) time periods, *A. anatina* survival, and the seasonal timing of cercarial release. The aim was also to study how the cercarial shedding traits and mussel

host availability are linked to the latitudinal occurrence, prevalence and abundance of the *Rhipidocotyle* parasites at the northern boundary of their range. To document these, both field studies and laboratory experiments were performed. The response of *Rhipidocotyle* species to temperature has not been studied experimentally. Such a study is thus timely and should be of particular relevance due to ongoing climate change, especially because climate models predict an increase in annual air temperature from 2 to 7 °C in Finland by the 2080s, compared to a 1961–1990 baseline period (Jylhä *et al*. 2004).

Specific aims of the study:

1) Aside from seasonal temperature fluctuations, short-term temperature changes can also influence cercarial emergence. The aim was to investigate the effect of short term (1 h) temperature change on cercarial emergence by *R. fennica* from the first intermediate bivalve host *Anodonta anatina* in the laboratory (I).
2) To test the hypothesis that warming is associated with increased transmission (measured as cercarial output) and increased virulence (parasite-induced host mortality) of parasites, *A. anatina* were exposed to low, intermediate and high temperature throughout the annual cercarial shedding period (May–October). The cercarial release from mussel host and host survival were studied over a period of 20 weeks (II).
3) By utilizing the data from (II), the influence of temperature on the seasonal timing of the cercarial release by *R. fennica* and *R. campanula* was investigated. The specific interest was to distinguish the seasonal duration of cercarial shedding at the individual host level and the host population level (III).
4) To study the occurrence, prevalence and abundance of *Rhipidocotyle* parasites along a latitudinal gradient in Finland, by examining the first (*A. anatina*) and second (*R. rutilus*) intermediate hosts. The latitudinal occurrence of *A. anatina* was also studied by examining the fish hosts, *Perca fluviatilis* and *R. rutilus* (IV).

2 MATERIALS AND METHODS

2.1 Study system

The study species were collected from different locations in Finland. *A. anatina* for laboratory experiments were collected from the following rivers: Haajaistenjoki (August 25, 2014; 63° 63 'N, 26° 99 'E) (I), Kuusaankoski (May 17, 2011; 62° 25 ’N, 26° 00 ’E) and Haajaistenjoki (May 22, 2011) (II and III). For field study, populations of *A. anatina*, *R. rutilus* and *P. fluviatilis* were collected from three geographic regions along a latitudinal gradient: south (61–64 °N), low north (65–66 °N) and high north (67–69 °N).

2.2 Laboratory experiments (I, II, III)

2.2.1 Cercarial emergence – effects of temperature

To test the hypothesis that global warming and the associated longer thermal growing season will increase the transmission (measured as cercarial output) and virulence (parasite-induced host mortality) of parasites as well as the seasonal duration of larval release by parasites (Marcogliese 2001, Harvell *et al*. 2002), three laboratory experiments were performed at the University of Jyväskylä (I) and Konnevesi Research Station (II and III). *Rhipidocotyle* species–*A. anatina* system was used as a model.

2.2.2 Responses of *Rhipidocotyle fennica* to short-term temperature change (I)

A. anatina mussels were collected from the River Haajaistenjoki during late August by snokeling, a period when most cercariae are fully developed and ready to emerge (Taskinen *et al.* 1994). In the laboratory, mussels were individually monitored for cercarial emergence at 17 °C (acclimatization temperature), which was the same as the ambient temperature, for 1 h and then

after temperature change, when mussels were individually moved from 17 °C to one of three new temperatures (14, 17 and 20 °C) for another 1 h. Mussels were removed from the boxes and the shed cercariae were identified, following Taskinen *et al*. (1991). The number of cercariae shed by each cercariae-shedding mussel after 1 h at 17 °C and after 1 h at the new temperature was counted from a 50 ml sample of well-mixed cercarial suspension.

2.2.3 Long-term effects of temperature on cercarial emergence and host survival (II), and the seasonal timing of cercarial shedding (III)

A. anatina mussels from two populations were exposed to low, intermediate and high temperature throughout the annual cercarial shedding period of *Rhipidocotyle campanula* and *R. fennica*, during which time the cercarial release (transmission) and host survival (virulence) (II) were studied. Concomitantly, the seasonal timing of cercarial shedding by *Rhipidocotyle* parasites in relation to temperature (III) was also studied. The number of cercariae shed per cercariae-shedding mussel after 24 h was counted as well as mussel mortality at 14-d intervals over a period of 20 weeks, between May 31 and October 28. The temperature range in the three treatments paralleled the natural variation occurring within the distributional range of study organisms in Finland. From the date of collection until June 25, mussels were established in the laboratory in two 163 l tanks (48 x 60 x 70 cm) under flow-through conditions (i.e. allowing a continuous flow of new water, one population per tank). Each tank was filled with 5 cm of sand on the bottom and supplied with 10 l min^{-1} of running water from the hypolimnetic zone (9 m depth) of Lake Konnevesi. 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.

On June 25, the mussels were randomly assigned to one of the three temperature treatments (two replicates for each treatment), such that mussels from both populations and from all size groups were distributed evenly to each of the 6 tanks. The average water temperatures from June 25 to October 28 (when experiment was terminated) were 18 °C (range 7–24 °C), 15 °C (range 6.6–20 °C) and 13 °C (range 6–17 °C) in high, intermediate and low temperature treatments, respectively. The maximum daily water temperature was 24 °C on July 27 in high temperature and, 20 °C in intermediate and 17 °C in low temperature treatment on September 4. The experiments were terminated on October 28, 2011. At that point, cercariae shedding had ceased practically in all treatments. For *R. fennica* and *R. campanula*, the cercarial shedding season has been reported to occur between late May and early October (Taskinen *et al*. 1994, 1997, Taskinen 1998a).

2.3 Field studies on latitudinal distribution of *Rhipidocotyl*e parasites and their mussel host *A. anatina* (IV)

Materials were collected from 37 southern, 13 low northern and 7 high northern water bodies between 1989 (Taskinen *et al*. 1991) and 2015. The main focus was on three regions along a latitudinal gradient; south (61–64 °N), low north (65–66 °N) and high north (67–69 °N). The aim was to map the latitudinal pattern in the occurrence, prevalence and abundance of *Rhipidocotyle* parasites by examining the first and second hosts in the laboratory for occurrence of parasites. The frequency of occurrence of *A. anatina* was used as a measure of host availability. Climatological date from the years 1961–2014 obtainable from the Finnish Meteorological Institute, was used to construct a map with the number of days when the daily mean air temperature was ≥ 15 °C in order to evaluate the length of the seasonal thermal growing season of the parasites. This was used as a measure of transmission potential. In addition, the latitudinal occurrence of *A. anatina* was also studied by examining roach and perch for parasitic glochidium larvae of *A. anatina*, which are suitable hosts for *A. anatina* glochidia (Jokela *et al*. 1991).

2.4 Statistical analyses

Differences in the cercarial output between temperature treatments were tested using one-way ANCOVA (I) and two-way ANOVA (II). To determine whether the mean cercarial output was different between mussel groups one-way ANOVA (I). To compare the proportions of mussels shedding cercariae at different temperatures χ^2-tests were used (II). Differences in survival between mussels shedding and those that did not shed cercariae were determined using logistic regression (II). Differences between treatments with respect to seasonal cercarial shedding traits were analysed using two-way ANOVA, with each parasite being analysed separately with treatment and populations as fixed factors (III). Differences between species with regard to seasonal cercariae traits were analysed using one-way ANOVA and Kruskal–Wallis tests. Differences between the three regions with regard to the occurrence frequency of *R. fennica* or *R. campanula* were analysed using χ^2-tests or Fisher's exact tests. The relationship between latitude and the mean prevalence or abundance of the *Rhpidocotyle* parasite was studied using Spearman rank correlation analysis. Statistical analyses were performed with IBM SPSS statistics version 22.0. (I) and PASW Statistics 18 (II, III and IV).

3 RESULTS AND DISCUSSION

3.1 Temperature-mediated cercarial emergence (I and II)

Owing to the temperature-dependence of many trematodes (Poulin 2006), any change in temperature is likely to affect cercarial emergence. The effect of temperature on cercarial emergence from the molluscan host varies among trematode species, both under field conditions and in the laboratory (Fingerut *et al*. 2003, Koprivnikar and Poulin 2009). In this study, differences in cercarial emergence between two closely related, sympatric *Rhipidocotyle* parasites in response to different temperatures, were observed, over short and long time periods (I, II and III). The cercarial release of *R. fennica* (but not of *R. campanula*) was sensitive to both short and long term changes in temperature (I and II). The proportion of mussels shedding *R. fennica* as well as cercarial output, was significantly higher at higher temperatures compared to low temperatures over short (I) and long time periods (II).

The cercarial release by *R. fennica* from mussels transferred from 17 °C to 20 °C increased significantly during the 1 h monitoring when compared to the preceding 1 h period, while that from mussels transferred to 17 °C (control) remained unchanged, and that from mussels transferred to 14 °C decreased (I). Results consistent with observations from the present study have also been found for other trematodes by Studer *et al*. (2010) and Paull *et al*. (2015). Higher temperature not only triggers the emergence of cercariae, it can also speed up cercariae production within the mollusc host. However, while the abrupt temperature increase in the short-term experiment probably triggered the release of already mature cercariae from the mussel host, leading to a burst of cercariae emergence, it did not accelerate cercarial maturation within the sporocysts. Cercariae of *R. campanula* did not emerge neither before nor after temperature change (I). This result was unexpected, because, the thermal requirements of *R. campanula* are less demanding than those of *R. fennica*, it can start seasonal cercarial release at a lower temperature than *R. fennica* (Taskinen *et al*. 1994, 1997). Furthermore, cercarial shedding of *R. campanula* responds

quickly to increased temperature, but that of *R. fennica* is much slower (Taskinen *et al*. 1991). This is because, fully mature cercariae of *R. campanula*, which are readily available to emerge when suitable temperature is attained are found in the sporocysts in high proportions throughout the year (Taskinen *et al*. 1994). In contrast, mature cercariae of *R. fennica* readily available to emerge are found only during the cercarial shedding period (July to September). The cercarial release by *Rhipidocotyle* parasites has a diurnal periodicity (Taskinen *et al*. 1991). Whereas the emergence of *R. fennica* cercariae is greatest between 8 and 10 a.m., that of *R. campanula* peaks between 4 p.m. and 4 a.m. Therefore, the present experiment (I) was performed at the time of highest daily cercarial shedding by *R. fennica*. These results highlight the importance of short-term temperature change on the cercarial shedding of trematodes, the need to carefully control temperature conditions when studying factors influencing the cercarial emergence of trematodes, and the importance of other cercarial emergence-controlling factors (e.g. time of day), regardless of ambient temperature suitability.

The proportion of mussels shedding cercariae and the total annual cercarial output per cercaria-shedding mussel increased significantly with temperature for *R. fennica*, resulting in 200–500 fold increase in annual cercarial output at high temperature compared with low temperature in the long-term experiment. These traits were unaffected by temperature for *R. campanula* (II), suggesting a fundamental interspecific difference in the temperature-cercarial emergence relationship between these two closely related sympatric parasites. Interspecific variations in cercariae release have been reported by Koprivnikar and Poulin (2009); the shedding of the *Maritrema novaezealandensis* trematode seems to decrease with increased temperature, while the shedding of *Acanthoparyphium sp.* increased with rising temperature. These contrasting results in cercarial shedding patterns emphasize the importance of context-dependency when predicting global warming-driven effects on the dynamics of host-parasite systems: some parasites are very sensitive to temperature, others are not, and the direction of the responses observed (increased or decreased output or no change) differs among species.

3.1.1 Virulence (II)

Many parasites display greater virulence (host-induced mortality) at higher temperature (e.g. Paull and Johnson 2011). Mussel mortality was greatest at higher temperature. However, the mortality of cercariae-shedding mussels was considerably greater at higher temperature (II), indicating that the virulence associated with cercarial emergence increases with temperature. Jokela *et al*. (1999) have demonstrated that the mortality of cercariae-shedding snails is higher than that of non-shedding snails under stress conditions. This is not surprising, because cercarial production in mollusc host is achieved at the expense of the host (e.g. Jokela *et al*. 1993), with excessive depletion of host energy reserves and excessive tissue damage during larval production. It has been predicted that with increasing temperature, the virulence of parasites (i.e.

parasite-induced host mortality) increases (Marcogliese 2001, 2008, Harvell *et al*. 2002). The shedding of *R. campanula* cercariae was associated with higher host mortality than the shedding of *R. fennica* (II). Jokela *et al*. (2005) also observed lower survival of *Rhipidocotyle*-infected mussels under stress; the virulence of *R. campanula* appears to be higher than that of *R. fennica*. During the cercarial shedding season, the host exploitation rate, measured as the proportion of host gonad tissue replaced by parasite sporocysts is considerably higher for *R. campanula* (~ 90 %) than for *R. fennica* (~ 30 %) (Taskinen *et al*. 1994). It is not possible to conclude that the difference between these two species is due solely to variation in host exploitation rate, but this is definitely an attractive hypothesis that needs to be investigated further.

3.2 Seasonal cercarial emergence (III)

The predicted changes in temperature and the associated longer thermal growing season are expected to affect the timing of parasite life-cycle stages. A common expectation is that the seasonal duration of larval release by parasites will increase in length as a consequence of a longer thermal growing season (longer summer) (Marcogliese 2001, Harvell *et al*. 2009). Lengthening of the seasonal cercarial shedding period in thermally altered sites but not in ambient sites has been documented in investigations of other trematode species (Aho *et al*. 1983, Camp *et al*. 1982). However, experimental long-term manipulations of temperature conditions over the seasonal cercarial shedding period are rare (but see Paull and Johnson 2014 for an exception).

At the individual host level, 3 seasonal cercarial shedding traits (i.e. start time, temperature and day-degrees at the start) changed significantly with temperature change for *R. fennica*, but not for *R. campanula*. *R. fennica* started cercarial release earlier in the season with a high temperature and high day-degrees in the high temperature treatment; these traits remained constant across treatments for *R. campanula.* Seasonal cercarial shedding ceased earlier at high temperature for both species, but temperature had no effect on the mean duration of the seasonal cercarial shedding period of either of the *Rhipidocotyle* species.

At the host population level, the total shedding period by *R. fennica* from the first to the last observation of emergence lasted for 10–16 weeks in high and intermediate temperature treatments, respectively, and for 4–6 weeks at low temperature. In contrast, that of *R. campanula* ranged from 14 to 18 weeks at low temperature, (which was remarkably longer than that for *R. fennica*), and it varied from 8 to 18 weeks at high and intermediate temperatures. Thus, the total shedding period at the host population level was longer in the high than in the low temperature treatment for *R. fennica*. Within the temporal and thermal range of the present experiment, only results for *R. fennica* at the host population level support the view that climate warming would increase the duration of larval shedding by parasites (Marcogliese 2001, Harvell *et al.* 2009).

The lengthening of the total period of cercarial shedding by *R. fennica* with temperature was due to seasonal variation in the cercarial release between host individuals resulting in a longer total shedding period among all mussels (III). Lengthening of the cercarial release period of other trematodes has been observed in water bodies receiving thermal effluents (Aho *et al.* 1982).

The seasonal cercarial release peaked concomitantly with the seasonal thermal maximum for *R. fennica*, but not for *R. campanula*, which confirms previous observations of *R. fennica* (Taskinen 1998a). Cercarial shedding by *R. fennica* increased substantially at temperatures above 15 °C but high numbers of *R. campanula* cercariae were released as soon as the temperature exceeded 10 °C. During the months of the highest temperatures (July, August and September), a positive relationship between mean cercarial release and ambient temperature at the time of monitoring was evident for *R. fennica* but not for *R. campanula*. These experimental results are in accordance with previous field observations by Taskinen *et al*. (1994). Together these observations indicate that *R. fennica* is thermophilic (I, II and III). The projected climate warming in high latitudes and the associated earlier and warmer spring and longer summer (Tietäväinen *et al*. 2010, Ruosteenoja *et al*. 2011), should have a greater impact on *R. fennica* than on *R. campanula* in the future (I, II and III), favouring *R. fennica* more than R. *campanula*.

3.3 Latitudinal pattern

The frequency of the occurrence of *R. fennica*, as well as the mean prevalence of infection in *A. anatina* and the average site-specific mean abundance of metacercariae in roach decreased significantly from the southern to the low northern region, but this pattern was not detected in *R. campanula*. Moreover, both *Rhipidocotyle* parasites and their first intermediated mussel host were completely absent in the high northern region.

These contrasting results suggest that transmission factors such as temperature comprise, the most important determinant of the northern range border for *R. fennica* but not for *R. campanula.* Cercarial release by *R. fennica*, but not by *R. campanula*, increased significantly with increasing temperature (I), and *R. campanula* starts seasonal cercarial emergence much earlier and at a lower temperature than *R. fennica* (Taskinen *et al*. 1994). In addition, seasonal cercarial shedding by *R. fennica* started 30 to 50 days after the rise of water temperature to 15 °C, while that by *R. campanula* started immediately (Taskinen *et al*. 1994). Therefore, a shorter summer, lower temperature and shorter thermal growing season in the low northern region will constrain cercarial shedding, and hence the transmission, of *R. fennica* in this region but not of *R. campanula*. However, the complete absence of both parasites in the high northern region suggest that factors other than temperature may be limiting the transmission of these parasites in this region, such as host availability (as also the first intermediate host, *A. anatina*, was not found in the high northern region). *A. anatina* is the

only known first intermediate host for both *Rhipidocotyle* species in Finnish waters (Taskinen *et al*. 1991).

4 CONCLUSIONS

Species-specific variation in the emergence of trematode cercariae from the mollusc host and seasonal timing of cercarial emergence at different temperatures was confirmed in this study, emphasizing the importance of context-dependency when predicting climate warming-mediated influence on host–parasite systems. Temperature strongly, but differently, affected cercarial shedding by *Rhipidocotyle* parasites (I, II and III). Even closely related sympatric parasite species that share the same transmission pathway can respond very differently to temperature change. These species-specific differences in temperature responses between two *Rhipidocotyle* species in their cercarial shedding traits, have likely contributed to the present geographical distribution of the parasites and will probably affect their future occurrence and abundance in the high latitude regions where climate warming is predicted to be greatest.

The good performance of *R. fennica* at higher temperature, given its observed need for higher temperature and longer warm period for the start of seasonal cercarial release (I, II and III), suggest that *R. fennica* is likely to profit from numerous aspects of the predicted climate change in Finland: increased temperature, warm summer and longer growing season especially in the north. For instance, the occurrence frequency and abundance *R. fennica* may increase in the low north region as a result of longer and warmer summers predicted for this region. However, this may also have important implications for roach and *A. anatina*, because bucephalid infections have been reported to cause mass mortality of roach and *Rhipidocotyle* parasites are well-known to decrease the growth, survival and reproduction of *A. anatina*, as well as the ability of *A. anatina* to survive environmental stress.

While these results indicate a clear increase in cercarial output and the seasonal duration of larval shedding by *R. fennica* at higher temperatures (I, II and III), further studies examining the survival and infectivity of these parasites in relation to temperature would provide a better understanding of the transmission of *Rhipidocotyle* species from the first to the second host. Cercarial emergence from the mussel host is an important feature in the transmission dynamics of infection in trematode life cycle, however, cercarial survival and

infectivity, both of which are temperature-dependent are also crucial to the transmission process of *Rhipidocotyle* parasites. Cercarial survival generally decreases with increasing temperature (McCarthy 1999, Mouritsen 2002), likely owing to a faster depletion of their energy reserves, and cercarial infectivity increases with temperature, at least up to an optimum temperature (McCarthy 1999, Studer *et al*. 2010). Therefore, increased cercarial emergence and lengthening of the seasonal duration of larval release with increasing temperature and the associated longer thermal growing season, will not necessarily translate into increased transmission success if the cercariae have lower survival and infectivity at high temperature. Further studies on the effects of mussel exposure to higher temperatures than those used here will be needed to better understand how *Rhipidocotyle* spp.–*A. anatina* system will respond to predicted temperature rise in Finland.

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YHTEENVETO

Lämpötilan, toukkatuotannon, virulenssin ja maantieteellisen levinneisyyden väliset yhteydet pikkujärvisimpukan *Rhipidocotyle*-loisilla

Loiset vaikuttavat isäntiinsä haitallisesti heikentämällä niiden kasvua, lisääntymistä ja säilyvyyttä. Tätä kautta loisilla voi olla tärkeä rooli sekä isäntiensä populaatiodynamiikkaan että evoluutioon vaikuttavana tekijänä. Monet loiset vaikuttavat ihmisiin joko käyttämällä meitä tai hyötyeläimiämme tai -kasvejamme isäntänään aiheuttaen terveydellisiä, taloudellisia ja sosiaalisia ongelmia.

Lämpötilalla voi olla tärkeä merkitys loisten menestymiselle ja lois–isäntäsuhteen luonteelle. Loiset voivat esimerkiksi tuottaa enemmän jälkeläisiä ja/tai olla haitallisempia korkeammassa lämpötilassa. Tästä syystä käynnissä oleva ilmaston lämpeneminen saattaa aiheuttaa muutoksia loisten runsaudessa, loisten maantieteellisessä levinneisyydessä ja loisten aiheuttamissa haitoissa. Näitä muutoksia ja niiden lajikohtaisia eroja on kuitenkin tutkittu vähän, etenkin korkeilla leveysasteilla, vaikka tutkimustulokset olisivat ensiarvoisen tärkeitä yritettäessä ennustaa ilmastonmuutoksen vaikutuksia niin itse loisten kuin loisten aiheuttamien haittojen esiintymiseen.

Rhipidocotyle fennica ja *R. campanula* ovat Bucephalidae-heimoon ja Trematoda-luokkaan kuuluvia loisia, joilla on epäsuora elämänkierto. Ensimmäisessä väli-isännässä, pikkujärvisimpukassa (*Anodonta anatina*), loisten sporokystivaiheet tuottavat suvuttomasti kerkaria-toukkia ja heikentävät simpukan kasvua sekä alentavat niiden lisääntymis- ja elinkykyä. Kerkaria-toukat tarttuvat toiseen väli-isäntään, särkeen (*Rutilus rutilus*), ja muuntuvat metakerkaria-toukiksi, jotka ovat kysteissä evillä (*R. fennica*) tai kiduksilla (*R. campanula*). Kun loisittu särki tulee pääisännän, hauen (*R. fennica*) tai ahvenen/kuhan (*R. campanula*), syömäksi, loiset aikuistuvat pääisäntänsä suolessa ja tuottavat suvullisesti munia, jotka päätyvät veteen ja kehittyvät mirakidium-toukiksi, jotka tarttuvat edelleen pikkujärvisimpukkaan.

Tässä työssä tutkittiin lämpötilan, toukkien tuotannon, virulenssin ja maantieteellisen levinneisyyden välisiä yhteyksiä *Rhipidocotyle*-loisilla. *Rhipidocotyle*-loisten oli aiemmin havaittu tuottavan kerkaria-toukkia lämpimimpään vuodenaikaan ja lisäävän simpukkaisännän kuolevuutta stressitilanteessa. Loisten kalaisäntien (särki, hauki/ahven) tiedettiin esiintyvän maantieteellisesti pohjoisinta Suomea myöten. *Rhipidocotyle*-pikkujärvisimpukka lajipari oli siten sopiva tutkimuskohde selvitettäessä seuraavia kysymyksiä: Kuinka lämpötila vaikuttaa loisten päivittäisen ja vuotuisen kerkaria-toukkien tuotannon määrään ja siihen, mihin aikaan vuodesta toukkia tuotetaan? Kuinka lämpötila vaikuttaa kerkaria-toukkia tuottavien ja ei-tuottavien simpukoiden säilyvyyteen ja loisten virulenssiin? Hypoteesina oli, että (i) loiset alkavat tuottaa toukkia aiemmin ja runsaammin lämpimämmässä ympäristössä ja että (ii) korkea lämpötila alentaa simpukoiden – erityisesti *Rhipidocotyle*-loisittujen simpukoiden –

säilyvyyttä. Lisäksi *Rhipidocotyle*-loisten on aikaisemmin havaittu eroavan kehitysnopeudessa siten, että *R. fennica* -loisen kerkaria-toukkien erittyminen simpukoista alkaa 30–45 vrk sen jälkeen, kun veden lämpötila on noussut alkukesällä 15 °C:een, kun taas *R. campanula* alkaa tuottaa toukkia lähes välittömästi lämpötilan noustua 15 °C:een. Tästä syystä tutkimushypoteesina oli myös, että (iii) *R. campanula* pystyy esiintymään pohjoisemmilla alueilla kuin *R. fennica*, koska pohjoisten alueiden lyhyt kesä rajoittaa *R. fennica* -loisen kerkaria-tuotantoa ja samalla loisen tarttumista simpukasta särkeen voimakkaammin kuin *R. campanula* -loisen tuotantoa.

Veden lämpötilan vaikutusta kerkaria-toukkatuotantoon tutkittiin lyhyessä ja pitkässä laboratoriokokeessa. Ennusteen mukaisesti lyhyessä laboratoriokokeessa *R. fennica* -loisen kerkaria-toukkien erittyminen kasvoi välittömästi, kun loisitut simpukat siirrettiin korkeampaan lämpötilaan (17 → 20 °C) ja väheni, kun simpukat siirrettiin alhaisempaan lämpötilaan (17 →14 °C). Pitkässä laboratoriokokeessa loisitut simpukat altistettiin luontaisen kaltaiselle veden lämpötilavaihtelulle toukokuusta lokakuulle kolmessa eri ryhmässä, joissa veden maksimilämpötilat olivat 24 °C (korkea), 20 °C (keskimääräinen) ja 17 °C (alhainen). Myös pitkässä laboratoriokokeessa korkea lämpötila lisäsi *R. fennica* -loisen kerkariaeritystä. Kerkaria-toukkia tuottavien simpukoiden osuus ja simpukan keskimäärin vuodessa tuottama kerkaria-määrä kasvoivat voimakkaasti lämpötilan noustessa – korkeassa lämpötilassa loisittu simpukkapopulaatio tuotti vuodessa 200–500-kertaisesti kerkaria-toukkia alhaiseen lämpötilaan verrattuna. Sitä vastoin *R. campanula* -loisella ei havaittu mitään eroja lämpötilakäsittelyjen välillä toukkien erittymisessä. Alhaisessa ja keskimääräisessä lämpötilassa *R. fennica* ja *R. campanula* -loisten vuotuiset kerkaria-tuotannot eivät poikenneet toisistaan, mutta korkeassa lämpötilassa *R. fennica* muodosti huomattavasti enemmän toukkia kuin *R. campanula*. Erittyneiden toukkien määrällä mitattuna *R. fennica* siis hyötyi korkeasta lämpötilasta, mutta *R. campanula* ei.

Keskimääräisessä kerkaria-toukkien erittymisen kestossa ei havaittu eroja eri lämpötilakäsittelyjen välillä. Koko isäntäpopulaatiossa kerkaria-toukkien erittyminen kesti *R. fennica* -loisella kuitenkin alhaisessa lämpötilassa, simpukkapopulaatiosta riippuen, vain 4–6 viikkoa, mutta korkeassa lämpötilassa 10–16 viikkoa. Sen sijaan *R. campanula* -toukkia erittyi pisimpään (14–18 vko) alhaisessa lämpötilassa. Keskimäärin *R. campanula* -toukkien erittyminen alkoi käsittelystä ja simpukkapopulaatiosta riippuen 42–87 vrk aikaisemmin ja huomattavasti alhaisemmassa lämpötilassa kuin *R. fennica* -toukkien, ja sen kerkaria-toukkia erittyi keskimäärin pidempään kuin *R. fennica* -toukkia. Siis myös toukkien erittymisen ajallisella kestolla mitattuna *R. fennica* hyötyi korkeasta lämpötilasta, mutta *R. campanula* ei.

Lämpötilan nousu alensi simpukoiden säilyvyyttä pitkässä laboratoriokokeessa. Kerkaria-toukkia erittävien simpukoiden kuolevuus oli suurempi kuin toukkia erittämättömien, erityisesti korkeassa lämpötilassa. Lisäksi *R. campanula* -toukkien tuottamiseen liittyvä kuolevuus oli suurempaa kuin *R. fennica* -toukkien tuottamiseen liittyvä kuolevuus. Tulokset kertovat *R. campanula* -loisen korkeammasta virulenssista.

Rhipidocotyle-loisten sekä niiden simpukkaisännän maantieteellistä levinneisyyttä ja runsautta selvitettiin tutkimalla vuosina 1996–2014 pikkujärvisimpukoita sekä niiden loisimia särkiä ja ahvenia Suomessa kolmella vyöhykkeellä pohjois–eteläsuunnassa: 61–64 °N (Etelä-Suomi; 37 järveä/jokea), 65–66 °N (Pohjois-Suomi; 13 järveä/jokea) ja 67–69 °N (pohjoisin Suomi; 7 järveä/jokea). Pikkujärvisimpukoista ja särjistä tutkittiin *Rhipidocotyle*-loisten esiintyminen ja särjistä sekä ahvenista tutkittiin pikkujärvisimpukan glokidium-toukkien esiintyminen. Lämpötilaolosuhteiden selvittämiseksi laskettiin Suomen alueelle 10x10 km^2 hilaruudukossa (yhteensä 3829 ruutua) vuosien 1961–2014 keskiarvo niiden vuorokausien lukumäärälle, jolloin ilman keskilämpötila oli ≥ 15 °C.

Etelä-Suomessa ≥ 15 °C vuorokausia oli keskimäärin 41–60 kpl, Pohjois-Suomessa 21–40 kpl ja pohjoisimmassa Suomessa alle 20 kpl. *R. fennica* -loisen esiintyvyys ja keskimääräinen runsaus laski Etelä-Suomesta Pohjois-Suomeen, mutta *R. campanula* -loisen esiintyvyys ja runsaus eivät eronneet Etelä- ja Pohjois-Suomen välillä. Kumpikaan *Rhipidocotyle*-lajeista ei esiintynyt pohjoisimmassa Suomessa. Jälkimmäinen tulos johtunee särki- ja ahventulosten perusteella ensimmäisen väli-isännän, pikkujärvisimpukan puuttumisesta pohjoisimmasta Suomesta. *R. fennica* -loisen huonompi menestyminen Pohjois-Suomessa *R. campanula* -loiseen verrattuna puolestaan voi johtua siitä, että alhainen lämpötila ja lyhyt kesä rajoittavat pohjoisessa *R. fennica* -loisen kerkaria-tuotantoa ja siten loisen tarttumista ensimmäisestä toiseen väli-isäntään).

R. campanula -loisella simpukassa elävän, kerkaria-toukkia tuottavan vaiheen, sporokystin, on aiemmassa tutkimuksessa todettu pysyvän aktiivisena ympäri vuoden. *R. fennica* -loisella sporokystit sen sijaan lakkaavat tuottamasta kerkaria-toukkia ja osittain hajoavat syksyllä ja alkavat jälleen kehittyä uudelleen keväällä lämpötilan noustessa. Tällöin kypsiä *R. fennica* -kerkaria-toukkia on sporokysteissä – toisin kuin *R. campanula* -loisella – vain toukkien parveiluaikaan. Tämä voi olla osasyynä siihen, että *R. campanula* -loisen virulenssi on korkeampi kuin *R. fennica* -loisen, mutta toisaalta tämä saattaa myös mahdollistaa sen, että *R. campanula* -loisen kerkaria-tuotanto voi alkaa paljon aikaisemmin ja alhaisemmassa lämpötilassa kuin *R. fennica* -loisen tuotanto. Tämän seurauksena *R. campanula* voi todennäköisesti myös menestyä paremmin pohjoisilla, lyhyen kesän alueilla kuin *R. fennica*.

Tämän työn tulokset viittaavat kytkökseen lämpötilan, toukkatuotannon, virulenssin ja maantieteellisen levinneisyyden välillä näillä kahdella pikkujärvisimpukan *Rhipidocotyle*-loisella. Tulosten perusteella *R. fennica* hyötyy ilmaston lämpenemisestä selvästi enemmän kuin *R. campanula*. Ilmastomallien ennustama 2–7 °C lämpötilan nousu vuosien 1961–1990 tasolta 2080-luvulle aiheuttanee erityisesti *R. fennica* -loisen runsastumista pohjoisessa Suomessa.

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ORIGINAL PAPERS

I

EFFECT OF SHORT-TERM TEMPERATURE CHANGE ON CERCARIAL RELEASE BY *RHIPIDOCOTYLE FENNICA* (TREMATODA, BUCEPHALIDAE) FROM THE FRESHWATER BIVALVE HOST, *ANODONTA ANATINA*

by

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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

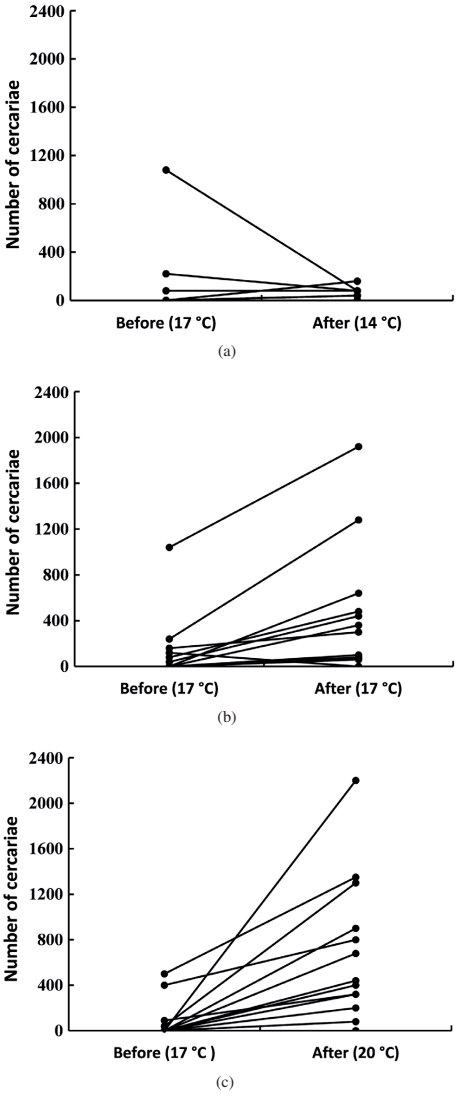

Figure 1: Release of *R. fennica* cercariae from each cercariae-shedding clam at 17 °C during a one hour period before transfer to a new temperature, and during a one hour period after transfer to a new temperature ((a) decreased 14 °C; (b) control 17 °C; and (c) increased 20 °C). Ambient holding temperature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

TEMPERATURE-DEPENDENT TRANSMISSION AND VIRULENCE OF TWO *RHIPIDOCOTYLE* SPECIES PARASITISING A MOLLUSCAN HOST

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Temperature-dependent transmission and virulence of two *Rhipidocotyle* species parasitising a molluscan host

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Abstract

Background
It has been suggested that global warming may increase both the transmission rate and the virulence of parasites. To test this hypothesis, we investigated the temperature-dependent transmission (measured as annual cercarial release) of two closely related, sympatric trematode species, *Rhipidocotyle fennica* and *R. campanula* and the molluscan host (*Anodonta anatina*) survival.

Methods
Freshwater clam hosts, *A. anatina,* from two different populations, were exposed throughout the annual cercarial shedding period between May and October to high (max. 24 °C), intermediate (max. 20 °C) and low (max. 17 °C) temperatures, which paralleled the current temperatures occurring within the distributional range of study species in Finland (60-68°N). Clams were individually monitored for cercarial release and survival at 14-day intervals.

Results
The proportion of clams shedding cercariae and the total annual cercarial output of *R. fennica* per cercariae-shedding clam increased significantly with temperature, resulting in a 200-500 fold higher annual cercarial output at a high temperature compared with a low temperature. However, these cercarial shedding traits were unaffected by temperature in *R. campanula*. Annual cercarial output of *R. fennica* was higher than that of *R. campanula*, but, only in the high temperature treatment. Regardless of the cercarial-shedding status (shedding or not), clam mortality was higher at high temperature, although, the mortality of cercariae-shedding clams was remarkably higher. The shedding of *R. campanula* cercariae was associated with a higher clam mortality than the shedding of *R. fennica*.

Conclusions
The present results 1) indicate interspecific differences in transmission with respect to thermal conditions, 2) emphasise possible between-species differences in the effects of the projected climate warming, 3) indicate that virulence associated with cercarial shedding increases with temperature, 4) suggest that *R. fennica* should be relatively more prevalent in southern areas

and *R. campanula* in northern areas of the current high latitude region, and 5) suggest that the predicted future climate warming should favour *R. fennica* more than *R. campanula*.

Key words: *Anodonta anatina,* Bucephalidae, cercarial production, climate change, temperature, transmission, Trematoda, Unionidae, virulence.

BACKGROUND

Global warming is expected to have a profound influence on parasites and host-parasite relationships. It has been predicted that, along with increasing temperatures, both the transmission rate and the virulence (the parasite-induced host mortality) of parasites will increase [1-3]. The anticipated effects of climate change include geographical range expansion or the range shift of parasites, and, if other factors remain constant, an increase in local prevalence and abundance of parasitism, as a consequence of higher mean and maximum temperatures and the associated longer warm season annually [1-3]. The warming is projected to be the strongest at high latitudes of the globe [4-6].

Trematodes are an important group of parasites, interacting within different trophic levels during their complex 2-3 host life cycle. They can have important roles in the function of aquatic ecosystems. For example, the annual production of free-swimming trematode cercariae was greater than the bird biomass in coastal estuaries [7]. Trematodes also include species that cause major veterinary or health problems [8]. Cercarial release from the molluscan host is one of the key factors of trematode transmission success. In temperate and cold climatic zones, the cercarial release of trematodes from the molluscan hosts primarily takes place during the warm summer months [9, 10]. This suggests that environmental temperature is an important determinant of cercarial emergence. Indeed, in experimental studies, cercarial release has been shown to increase with rising temperature [11, 12]. As reviewed by [13], a 10 °C rise in temperature causes, on average, an 8-fold (up to 200-300 fold in some species) increase of cercarial production in snail hosts. However, the relationship between temperature and cercarial emission is not straightforward, because neutral or even slightly negative temperature effects have also been reported [13, 14]. More recently, the results of Morley and Lewis [15] and Studer and Poulin [16] indicate that thermal effects on cercarial emergence are complex, depending on the host-parasite system, temperature range, acclimation and latitude.

The importance of studying the temperature-cercarial shedding relationship at high latitudes has been highlighted in previous papers [15, 16], but, to our knowledge, experimental temperature manipulations have not been performed in relation to high latitude (> 60°) host-trematode associations. Thus, the aim of the present study was to investigate the effect of temperature on cercarial release and virulence (parasite-induced mortality) of two closely related, sympatric trematodes, *Rhipidocotyle campanula* and *R. fennica* (Bucephalidae), in their first intermediate molluscan host, *Anodonta anatina* (Bivalvia, Unionidae) in a northern latitude. In addition to sharing the same first host, these parasites also have the same second intermediate host, the cyprinid fish *Rutilus rutilus* [17, 18]. We used three temperature levels

covering the range of summer temperatures (15-25 °C) currently occurring in the distributional range of *A. anatina* at 60°-68°N in Finland.

Thus, our hypothesis was that the transmission, measured as annual cercarial production, of *R. fennica* and *R. campanula* would increase with temperature, whereas the survival of the host *A. anatina* would decrease, as their metabolic rate is expected to increase with temperature. Furthermore, as Jokela et al. [19] found *Rhipidocotyle* infection to increase the mortality of *A. anatina* under stress, our hypothesis was that the lowest host survival would be among those clams shedding cercariae at high temperature.

METHODS

Study species

The first intermediate host, *Anodonta anatina,* is a common, long-lived (> 10 years) and abundant freshwater bivalve in Europe. It is mature at 2-4 yr of age and can grow up to 12 cm in length [20, 21]. During July, female *A. anatina* develop glochidia larvae in the outer gill blades, where they are stored and maintained over winter to be released the following spring [22-24]. Before the start of their benthic life, the glochidia are ectoparasitic on a variety of freshwater fish species [22, 25].

The parasites, *Rhipidocotyle campanula* and *R. fennica* use *A. anatina* as their first intermediate host where the miracidium larvae develop into sporocysts. Branching sporocyst tubules of *Rhipidocotyle campanula* and *R. fennica* invade mainly the gonads of the freshwater bivalve clam *A. anatina* [17, 24, 26] producing asexually large numbers of free-swimming cercarial larvae. Emerged cercariae are transmitted to the second intermediate host, the cyprinid fish *Rutilus rutilus*, where they encyst as a metacercaria, and subsequently pass to the final host, the predatory fishes *Perca fluviatilis/Stizostedion lucioperca* and *Esox lucius*, respectively, in which the worms mature and produce eggs [17, 18]. In natural *Anodonta* populations, the prevalence of infection by *R. campanula* is usually less than 10% [17, 26], whereas that of *R. fennica* can be up to 50 % [27]. Both parasite species have been linked to the decreased growth, survival and reproduction of *A. anatina* [19, 21, 26, 28]. The mean (± s.e.) annual cercarial output of *R. fennica* was previously estimated to be 290,000 ± 26,000, with a maximum production of 440,000 cercariae y^{-1} [10].

Clam collection

A. anatina individuals were collected by snorkelling from the River Kuusaankoski (62°25'N, 26°00'E) and the River Haajaistenjoki (63°63'N, 26°99 'E) in southern Finland on May 17, 2011 and May 22, respectively (water temperature was 9 °C at both sites). At the Konnevesi Research Station, University of Jyväskylä, clams were individually marked and measured (shell length, height and width) on the date of collection and at the end of the study. Average shell length (± s.e.) in clams from the River Haajaistenjoki and River Kuusaankoski was 61.8 ± 0.6 mm (range 33.0 - 92.6 mm, n = 290) and 77.8 ± 0.6 mm (range 51.7 - 101.7 mm, n = 281), respectively. There was no length-difference between clams allocated to different temperature

treatments (see below) ($F_{1, 565}$ = 0.040, P = 0.961) and there was no interaction between population and treatment ($F_{1, 565}$ = 0.728, P = 0.484) in terms of clam length.

From the date of collection until June 25, clams were established in the laboratory in two 163 l tanks (48 x 60 x 70 cm) under flow–through conditions (i.e. allowing a continuous flow of new water) with one population per tank. Each tank was filled with 5 cm of sand on the bottom and supplied with running water (10 l min^{-1}) from the hypolimnetic zone (9 m depth) of Lake Konnevesi. Water temperatures in both tanks were the same throughout this period and ranged from 10.5 °C on May 31 to 11.7 °C on June 25 (Fig. 1).

Temperature treatments

From June 25, 2011, clams were randomly assigned to treatment groups of high, intermediate and low temperature; two replicate tanks per treatment. Clams from both populations and from all size groups were allocated evenly among the six tanks (48 River Haajaistenjoki clams per tank and 47 River Kuusaankoski clams per tank). The temperature range used in the three treatments (Fig. 1), were chosen to cover the natural variation experienced throughout the distribution area of *A. anatina* in Finland between 60° to 68°N, with the maximum summer temperatures varying from about 23° to 15°C, respectively. From June 25 to October 28 (when experiment was terminated because cercarial shedding had stopped practically in all treatments), the average water temperatures from June 25 to October 28 were 18 °C (range 7–24 °C), 15 °C (range 6.6–20 °C) and 13 °C (range 6–17 °C) in high, intermediate and low temperature treatments, respectively. 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).

Anodonta clams are filter feeders utilising phytoplankton, bacteria and fine organic particles [28], thus a continuous flow of lake water was necessary to provide the clams with food. To create the high temperature treatment, the tanks were placed in an outside shelter and supplied with running water from the littoral zone (< 2m depth) of Lake Konnevesi. Low and intermediate temperature tanks were kept indoors and supplied with the colder hypolimnetic water and heated hypolimnetic water from Lake Konnevesi, respectively. Due to logistic constraint, some differences between the treatments, in addition to temperature, could not be avoided. A submersible temperature logger, was placed in one replicate tank per treatment from June 25 to October 28 to measure water temperature every 4 h. In the high temperature treatment, the clams were subject to a larger daily fluctuation of temperature than the intermediate or low temperature (Fig. 1), as the littoral water and outdoor tanks were used. The indoor tanks were illuminated by artificial lights, however, the photoperiod was set to correspond with that outside. The outdoor tanks received natural light and the large shelter above the tanks provided effective cover against sunlight. However, during each 24 h cercarial release monitoring, similar artificial light was used for all clams to provide equal light conditions (see below). Water flow was set to be higher in the intermediate and low temperature tanks (10 l min^{-1}) than in the high temperature (5 l min^{-1}) to compensate for the likely higher food density in the high temperature tanks that received littoral water, as compared to the intermediate and low temperature tanks.

Cercarial emergence

The number of cercariae released per *A. anatina* was counted at 14-day intervals over a period of 20 weeks between 31 May and 28 October, 2011, during a total of 12 monitoring sessions. Each clam was placed individually into 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. Host mortality was assessed at this stage. After 24 h, clams were removed and shed cercariae were identified, following Taskinen et al. [17], and counted visually (when numbers were low) or from a 50 ml subsample with a microscope (when cercarial numbers were high). The temperature prevailing in each temperature treatment was maintained during monitoring – if necessary, a temperature regulated room was used. Similar artificial lights were used for all clams and light conditions were set to correspond with natural day length and rhythm, because the cercarial release of *Rhipidocotyle* species is diurnal [17]. The experiment was terminated on October 28, 2011. At that point cercariae emergence from *A. anatina* had ceased practically in all treatments. For *R. fennica* and *R. campanula*, the seasonal cercarial shedding period has been reported to occur between late May and early October [10, 24, 27].

Data analysis

Cercarial output for each 14-d interval was estimated/calculated by multiplying the cercariae release on each monitoring day by 14. To determine the total annual cercarial output, cercarial productions for all 14-d intervals were summed. All statistical analyses were performed using PASW Statistics 18. Clams that did not shed cercariae (except for the survival analysis), and those that shed both *R. campanula* and *R. fennica* cercariae (double infected), as well as those that were infected by *Phyllodistomum* sp. were not included in the analyses. Before the final analyses, data from two replicate tanks were combined, as prior analyses revealed no differences between replicates for any measured variables. Means are given with ± 1 standard error (s.e.).

χ^2-tests were used to compare the proportions of clams shedding *R. fennica* and *R. campanula* for the different temperature treatments. To compare temperature treatments and populations with regard to the log-transformed total annual cercarial output, 2-way ANOVA was applied for *R. campanula* and *R. fennica* with treatment and population as fixed factors.

To compare the two parasite species, the treatments were analysed separately to satisfy ANOVA assumptions. Total annual cercarial output was used as the response variable, while parasite species (*R. campanula*, *R. fennica*) and population (River Kuusaankoski, River Haajaistenjoki) were the fixed factors. To account for multiple tests, the Bonferroni correction was applied to p-values when analysing differences between parasite species. Whenever ANOVA indicated significant effect of temperature, the differences between treatments were analysed with Tukey's Post Hoc tests.

Survival differences with respect to cercarial-shedding status (shedding or not) were studied using logistic regression, with survival (survived throughout the experiment vs. did not survive) as the dependent variable and temperature treatment (TREAT), clam population (POPU) and cercarial-shedding status (CERC) as independent categorical covariates. The logistic regression model that best described the data was searched using an automated forward stepwise model construction procedure of PASW utilising likelihood ratio

significance tests for evaluating each explaining term, i.e. the three categorical covariates as well as all their possible interactions. It is worth noting that it was not possible to compare the survival of uninfected and infected clams, since the infection status of the dead individuals was not always clear.

Clams growth was analysed using 2-way ANOVA with population and temperature treatment as fixed factors and shell length increment from the start to the end of the experiment as the dependent variable; only the non-cercariea-shedding clams were included, since *Rhipidocotyle* infection reduces the growth of *A. anatina* [28].

RESULTS

Cercarial release

The total shedding period by *R. fennica* from the first to the last observation of emergence lasted for 10–16 weeks in high and intermediate temperature treatments, respectively, and for 4–6 weeks at low temperature. In contrast, that of *R. campanula* ranged from 14 to 18 weeks at low temperature, (which was remarkably longer than that for *R. fennica*), and it varied from 8 to 18 weeks at high and intermediate temperatures. The difference in the proportion of clams shedding *R. fennica* cercariae between high and intermediate temperatures was significant (χ^2-test, $p < 0.008$ in both rivers), as was that between high and low temperatures (χ^2-test, $p < 0.001$ in both rivers) and between the intermediate and low temperatures (χ^2-test, $p < 0.013$ for both rivers). Thus, the proportion of clams from both populations shedding *R. fennica* increased remarkably with temperature (Table 1A). In contrast, there were no differences in the proportion of clams shedding *R. campanula* cercariae between the three treatments, either in the River Haajaistenjoki (χ^2-test, $p = 0.315$) or the River Kuusaankoski (χ^2-test, $p = 0.765$) (Table 1A) material.

The effect of temperature on the total annual cercarial output of *R. fennica* was statistically significant but not of *R. campanula* (Table 2). The total cercarial output of *R. fennica* clearly increased with increasing temperature (Fig. 2), being 30 and 27 times higher at high compared to low temperature in both River Haajaistenjoki and River Kuusaankoski clams, respectively (all paired differences were significant using the Tukey's post hoc tests), whereas the annual cercarial output of *R. campanula* was constant with respect to temperature (Fig. 2).

Interspecific differences in annual cercarial output were highly significant at the high temperature, but not for the other temperature treatments (Table 3). In the high temperature treatment, the annual cercarial output of *R. fennica* was as much as 18 times that of *R. campanula* (Fig. 2). The effect of population and the treatment × population interaction , were not significant under any treatment (Table 3), suggesting that the annual cercarial output within each treatment group was independent of the population, and that the treatment specific effects for both parasite species were identical in both populations.

If host density is constant, the number of cercariae produced at the population level is a product of the mean annual cercarial output clam^{-1} and the proportion of shedding clams. Consequently, the estimated annual cercarial output of *R. fennica* at the population level at the high temperature was, respectively, 179 and 518 times higher than that at the low temperature

in both the River Haajaistenjoki and River Kuusaankoski clams (Table 4). In contrast, for these two populations, the estimated annual cercarial output of *R. campanula* at the high temperature was 1.8 and 9.8 times lower than that at the low temperature, respectively (Table 4).

Survival and growth of clams

When analysing the survival of the clams, the logistic regression model that best fits the data included the terms treatment (TREAT) (change in log likelihood of the model if term removed = 18.193, df = 2, $P < 0.001$) and cercarial production status (CERC) (change in log likelihood if term removed = 10.580, df = 2, $P = 0.005$) (Table 5). Within the term TREAT, the contrast that compared survival at high versus low temperature was significant with an odds ratio value of 0.538 (Table 5), indicating that clam survival at high temperature was only close to half that at low temperature (see also Table 1B). Within the term CERC, the contrast, comparing non-shedding clams to *R. fennica* shedding individuals, was not significant, whereas the contrast that matched non-shedding clams against *R. campanula* shedding individuals was significant (Table 5). This indicates that clam survival was lower among *R. campanula* shedding clams (but not among those shedding *R. fennica*) than among non-shedding individuals (see Table 1B). The odds ratio value suggests that the probability of survival throughout the experiment was 1.63-fold in non-shedding clams as compared to *R. campanula* shedding clams. Although the TREAT × CERC interaction was significant (change in log likelihood of the model if term removed = 11.768, df = 4, $P = 0.019$), none of the contrasts comparing temperature treatments / shedding groups were statistically significant *per se*. The proportion of surviving individuals among clams shedding *R. fennica* and *R. campanula* decreased significantly from 75 and 68 % to 31 and 18 %, respectively, from low to high temperature (χ^2 test, $P = 0.015$ and $P = 0.005$, respectively), while the respective decrease among the non-shedding clams was from 75 to 68 % (χ^2 test, $P = 0.227$) (populations combined). Thus, the TREAT × CERC interaction was probably due to a higher decrease of survival at high temperature in individuals shedding cercariae than in non-shedding ones.

The growth of *A. anatina* was not affected by temperature treatment (2-ANOVA, $F_{2, 221} = 0.526$, $P = 0.592$), population ($F_{1, 221} = 0.025$, $P = 0.873$) or by their interaction ($F_{2, 221} = 1.667$, $P = 0.191$). The mean shell length increment of the clams that did not release cercariae during the experiment, was 0.2 ± 0.1, 0.4 ± 0.3 and 0.1 ± 0.1 mm in high, intermediate and low temperatures (populations combined), respectively, equalling 0.1-0.7 % of their initial length.

DISCUSSION

Analyses related to the effects of global warming on host-parasite relationships predict (i) an increase in transmission and virulence of the parasites and (ii) species-specific variation (i.e. context dependency) in the transmission success of the parasites [1-3]. The results of the present study mainly support these predictions. The transmission of *R. fennica*, measured as the annual cercarial output, was remarkably higher at a high temperature compared with a low temperature treatment. However, that of *R. campanula* was unaffected by temperature

treatments, suggesting a fundamental interspecific difference in the temperature–cercarial shedding relationship between these two species. In addition, regardless of the cercarial-shedding status (shedding or not shedding), clam mortality was significantly higher at high temperature, although, the mortality of cercariae-shedding clams was considerably higher, which supports the prediction that parasite virulence increases with increasing temperature.

The contrasting temperature response in annual cercarial release of *R. fennica* and *R. campanula* suggest that the effect of climate warming on these two closely related, sympatric trematodes species, even though they utilise the same molluscan host and are transmitted to the same second intermediate host, may be remarkably different. The predicted climate warming of 2 to 7° C by 2080s in these high latitudes [30] should clearly favour *R. fennica* more than *R. campanula*. The present results also allow us to predict that the current latitudinal distribution of the species may change. If transmission of the other life cycle stages would remain the same, *R. fennica* should be relatively more prevalent in southern areas and *R. campanula* in northern areas of the current study region.

It would be tempting to suggest that the good performance of *R. campanula* in the low temperature treatment would be an adaptation to northern, high latitudinal conditions with low summer temperatures. However, the geographical distribution of this species in Europe does not support this view. Both *R. campanula* and *R. fennica* occur as far south as the Ukraine [17, 26, 31, 32]. The northernmost finding of bucephalid trematodes is that of *R. campanula* in Lake Kuivasjärvi, Finland (65 °N) [27], but a detailed mapping of occurrence and abundance of *R. fennica* and *R. campanula* in northern regions is required. It is also difficult to explain the observed difference between these *Rhipidoctyle* species in terms of transmission. Although the definitive hosts of the species differ (*E. lucius* and *P. fluviatilis* / *S. lucioperca*, respectively) [17, 18], it is not known how transmission between the various life cycle stages might explain the observed result. It is also worth noting that both *R. fennica* and *R. campanula* are restricted to only *A. anatina* as their first intermediate host in Finland [17, 18].

The total annual production of *Rhipidocotyle* cercariae measured in field conditions yielded clearly higher numbers than in the present study [10]. A plausible reason for this difference could be a resource limitation in the present study, which was carried out under laboratory conditions. This was indicated by the lower survival of cercariae-shedding clams (48.3-80.0 %, Table 1) and a lower growth (less than 1 %) in this experiment compared to 91.7 % survival and 3.4 % growth under field conditions [10, 28]. However, even though there were differences between the treatments in terms of water flow and water source (littoral or hypolimnetic), the equal growth of non-shedding clams in the different temperature treatments suggests that the treatments did not differ in terms of the abundance of food resources. The tanks also differed with respect to light conditions and temperature fluctuations. High temperature tanks kept in an outdoor shelter were subject to a diurnal temperature fluctuation and natural light, whereas the intermediate and low temperature tanks were located in an indoor tank hall and illuminated with artificial light, although the photoperiod rhythm was equal in all treatments. Even though we cannot completely rule out confounding factors other than temperature, we do not believe that differences in water and light source, or temperature fluctuation, would likely explain the observed contrasting responses in the cercarial release of *R. fennica* and *R. campanula* between the temperature

treatments. New infections, via miracidia from unfiltered lake water, were unlikely due to the seasonal maturing of *Rhipidocotyle* trematodes in late autumn [17]. Thus, the present results should reliably indicate the temperature responses of *R. fennica* and *R. campanula* in a long term experiment – an attribute that differentiates this study from most of the temperature/cercarial shedding studies that have been completed within relatively short period (days or weeks).

In the present study, the mortality of cercariae-shedding clams was remarkably greater at higher temperature, indicating that virulence associated with cercarial shedding increases with temperature. Jokela et al. [34] have demonstrated that the mortality of cercarial-shedding snails is higher than that of non-shedding snails under stress condition. This is not surprising because cercarial production is very demanding and costly for the host. The intensive production of cercariae is usually accompanied by excessive depletion of host energy reserves [35] and excessive tissue damage or even death of the hosts [36]. Moreover, the mortality of mollusc host has been reported to be highest during the cercarial shedding period [37]. The synergistic effect of cercarial shedding and increasing temperature is important to the molluscan host. The shedding of *R. campanula* cercariae induced higher host mortality than the shedding of *R. fennica* in the present study. This is in accordance with the previous result that infection by *R. campanula* increased the mortality of *A. anatina* under stress more than infection by *R. fennica* [19]. Furthermore, higher virulence was associated with the release of *R. campanula* cercariae even though the numbers of cercariae released were much higher in *R. fennica*. This can probably be explained by the more intensive host use by *R. campanula* in terms of the number of parasite sporocysts in the host clam, by a more frequent invasion of vital organs by sporocysts of *R. campanula*, as well as by the seasonal timing of cercarial emergence by *R. campanula* to a period that is more harmful for *A. anatina*, as compared to *R. fennica* [24].

Trematode parasites are a ubiquitous part of freshwater and marine food webs. These worms may account for a remarkable proportion of the biomass and production in an aquatic ecosystem [7, 38] and thus play important roles in aquatic food webs and energy transfer [39]. This is also true for *Rhipidocotyle* species. Cercariae of *R. fennica* may occur in densities of 1,000 larvae m^{-3} [10], *Rhipidocotyle* infections in the fish second intermediate host may have prevalences up to 95 % [40], and an infection with *Rhipidocotyle* parasites may sterilise *A. anatina* and reduce clam growth [21, 28]. Therefore the present study contributes to our understanding and helps with prediction of the effects of climatic warming on parasitism by species of *Rhipidocotyle*. Moreover, the current results does not completely support the prediction that climate change will generally benefit parasites, as warming was associated with an increased cercarial output only in the case of *R. fennica*, but completely support the complexity of climate change–parasitism interplay [1-3, 41, 42].

Although, these results indicate a clear increase in cercarial output by *R. fennica* at higher temperatures, further studies examining the survival and infectivity of these parasites in relation to temperature would provide a better understanding of the transmission of *Rhipidocotyle* species from the first to the second host. Our study focused on cercarial emergence from the clam host, which is an important feature in the transmission dynamics of infection in trematode life cycle, however, cercarial survival and infectivity, both of which are

temperature-dependent are also crucial to the transmission process of *Rhipidocotyle* parasites. Cercarial survival generally decreases with increasing temperature (McCarthy 1999, Mouritsen 2002), likely owing to a faster depletion of their energy reserves, and cercarial infectivity increases with temperature, at least up to an optimum temperature (McCarthy 1999, Studer *et al*. 2010). Therefore, increased cercarial emergence and lengthening of the seasonal duration of larval release with increasing temperature and the associated longer thermal growing season, will not necessarily translate into increased transmission success if the cercariae have lower survival and infectivity at high temperature. Further studies on the effects of clam exposure to higher temperatures than those used here will be needed to better understand how *Rhipidocotyle spp.-A. anatina* system will respond to predicted temperature rise in Finland.

COMPETING INTERESTS
Both authors in this research paper declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS
JMC and JT contributed equally to the planning and implementation of the study, including the writing. Both authors read and approved the final manuscript.

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Table 1. Non-shedding and cercariae-shedding *Anodonta anatina* clams, and their survival at different temperatures. A - Numbers and proportions (%) of *A. anatina* clams that did not release cercariae (non-shedding), released *R. campanula* or *R. fennica* cercariae. B - Numbers and proportions of individuals which survived among the respective categories over a period of 20 weeks.

	A. Number of clams		B. Number of clams surviving	
	R. Haajaiste.	R. Kuusaan.	R. Haajaiste.	R. Kuusaan.
High temperature				
Non-shedding	46 (52.9%)	53 (57.0%)	32 (69.6%)	35 (66.0%)
R. fennica	33 (37.9%)	37 (38.8%)	9 (27.3 %)	13 (35.1%)
R. campanula	8 (9.2%)	3 (3.2%)	1 (12.5%)	1 (33.3%)
Total	87 (100%)	93 (100%)	42 (48.3%)	49 (52.7%)
Intermediate temp.				
Non-shedding	66 (68.8%)	74 (79.6%)	46 (69.7%)	51 (68.9%)
R. fennica	19 (19.8 %)	13 (14.0%)	10 (52.6%)	11 (84.6%)
R. campanula	11 (11.5%)	6 (6.5%)	6 (54.5%)	4 (66.7%)
Total	96 (100%)	93 (100%)	62 (64.6%)	66 (71.0%)
Low temperature				
Non-shedding	69 (72.6%)	85 (89.5%)	46 (66.7%)	69 (81.2%)
R. fennica	6 (6.3%)	2 (2.1%)	4 (66.7%)	2 (100%)
R. campanula	20 (21.1%)	8 (8.4%)	14 (70.0%)	5 (62.5%)
Total	95 (100%)	95 (100%)	64 (67.4%)	76 (80.0%)

Table 2. Differences between treatments in the total annual cecarial output. 2-way ANOVA statistics for the effect of temperature treatment and clam population on (log-transformed) the annual cercarial release of *R. fennica* and *R. campanula*. Statistically significant effects are marked with an asterisk*.

Parasite	Factor	Statistics	*P*
R. fennica	Treatment	$F_{2, 104} = 36.386$	< 0.001*
	Population	$F_{1, 104} = 0.428$	0.514
	Treatm. x Pop.	$F_{2, 104} = 3.887$	0.024*
R. campanula	Treatment	$F_{2, 50} = 1.057$	0.355
	Population	$F_{1, 50} = 11.421$	0.002*
	Treatm. x Pop.	$F_{2, 50} = 5.227$	0.009*

Table 3. Interspecific difference in the total annual cercarial output. Results of 2-way ANOVA testing the effect of parasite species (*R. fennica* vs. *R. campanula*) and clam population on the annual cercarial release in each temperature treatment. Statistically significant effects are marked with an asterisk*.

Treatment	Factor	Statistics	*P*
High temperature	Species	$F_{1, 77} = 9.501$	0.003*
	Population	$F_{1, 77} = 1.125$	0.292
	Species x Pop.	$F_{1, 77} = 0.699$	0.416
Intermediate temp.	Species	$F_{1, 45} = 3.118$	0.081
	Population	$F_{1, 45} = 0.255$	0.616
	Species x Pop.	$F_{1, 45} = 2.037$	0.160
Low temperature	Species	$F_{1, 32} = 1.316$	0.260
	Population	$F_{1, 32} = 0.197$	0.660
	Species x Pop.	$F_{1, 32} = 0.026$	0.873

Table 4. Annual cercarial output at the population level. The number of cercariae shed at the populations level is a product of the mean numbers of cercariae released annually, the proportion of cercariae-shedding clams and the calculated total annual cercarial output per 1000 clams (with 95 % confidence interval in parentheses) based on the proportion of cercariae-shedding clams and the mean number of cercariae shed per clam during the experiment.

	Cercariae individual^{-1}	% shedding	Cercariae population^{-1}
Rhipidocotyle fennica			
River Haajaistenjoki			
High temperature	28266 (11922-36611)	37.9 (28.5-48.4)	10713 (3398-17720)
Intermediate temp.	4004 (1945-6063)	19.8 (13.1-28.9)	792 (255-1752)
Low temperature	947 (36-1858)	6.3 (2.9-13.1)	60 (1-243)
Total	17492 (11771-23213)	20.9 (16.5-26.0)	3656 (1942-6035)
River Kuusaankoski			
High temperature	15617 (10249-20985)	39.8 (30.4-49.9)	6216 (3116-10472)
Intermediate temp.	5346 (1241-9451)	14.0 (8.4-22.5)	748 (104-2126)
Low temperature	585 (0-1411)	2.1 (0.6-7.4)	12 (0-104)
Total	12471 (8347-16596)	18.5 (14.4-23.5)	2307 (1202-3900)
Rhipidocotyle campanula			
River Haajaistenjoki			
High temperature	2005 (470-3539)	9.2 (4.7-17.1)	184 (22-605)
Intermediate temp.	3483 (1384-5582)	11.5 (6.5-19.4)	401 (90-1083)
Low temperature	1535 (857-2213)	21.1 (14.1-30.3)	324 (121-671)
Total	2181 (1459-2902)	14.0 (10.4-18.6)	305 (152-540)
River Kuusaankoski			
High temperature	371 (0-1624)	3.2 (1.1-9.1)	12 (0-15)
Intermediate temp.	672 (0-1614)	6.5 (3.0-13.4)	44 (0-216)
Low temperature	1366 (342-2390)	8.4 (4.3-15.7)	115 (15-375)
Total	946 (402-1489)	6.1 (3.8-9.5)	58 (15-141)

Table 5. Logistic regression statistics of clam survival during the experiment. Categorical variables explaining survival were temperature treatment (TREAT; high, intermediate and low temperature), cercarial shedding status (CERC; non-shedding, shedding *R. campanula* and shedding *R. fennica*) and clam population (POPU; River Haajaistenjoki and River Kuusaankoski). TREAT contrasts survival at high temperature to that at intermediate or low temperature. CERC contrasts survival of non-cercarial-shedding clams to that of *R. campanula*-shedding and *R. fennica*-shedding individuals.

Best model	ß ± s.e.	Wald	df	*P*	Odds ratio
TREAT		14.763	2	0.001	
High vs. Intermediate temp.	-2.50 ± 0.20	1.561	1	0.212	0.78
High vs. Low temperature	-6.20 ± 0.24	6.779	1	0.009	0.54
CERC		10.041	2	0.007	
Non-shedding vs. *R. fen.*	0.03 ± 0.24	0.020	1	0.888	1.03
Non-shedding vs. *R. cam.*	0.49 ± 0.25	3.828	1	0.050	1.63
TREAT x CERC		10.862	4	0.028	
Variables not in the best model		Score	df	*P*	
POPU		3.005	1	0.083	
POPU x TREAT		1.217	2	0.544	
CERC x POPU		2.123	2	0.346	
CERC x POPU x TREAT		5.236	4	0.264	

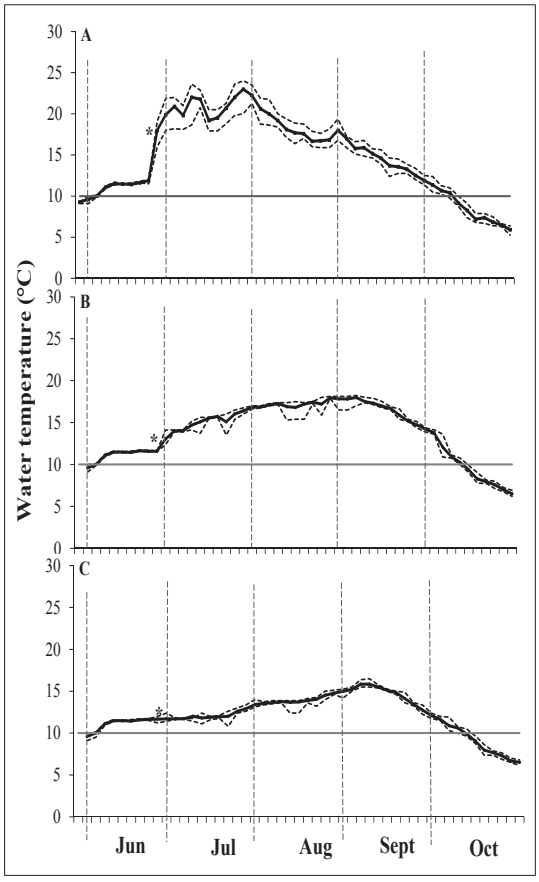

Figure 1. Water temperature profile in the three temperature treatments from June 25 to October 28. A – high temperature. B – intermediate temperature. C – low temperature. A submersible temperature logger, was placed in one replicate tank per treatment from June 25 to October 28 to measure water temperature every 4 h. Average (solid line), minimum and maximum (dotted lines) temperatures for every third day experienced by clams. Asterisks represents the day when clams were assigned to the different temperature treatments.

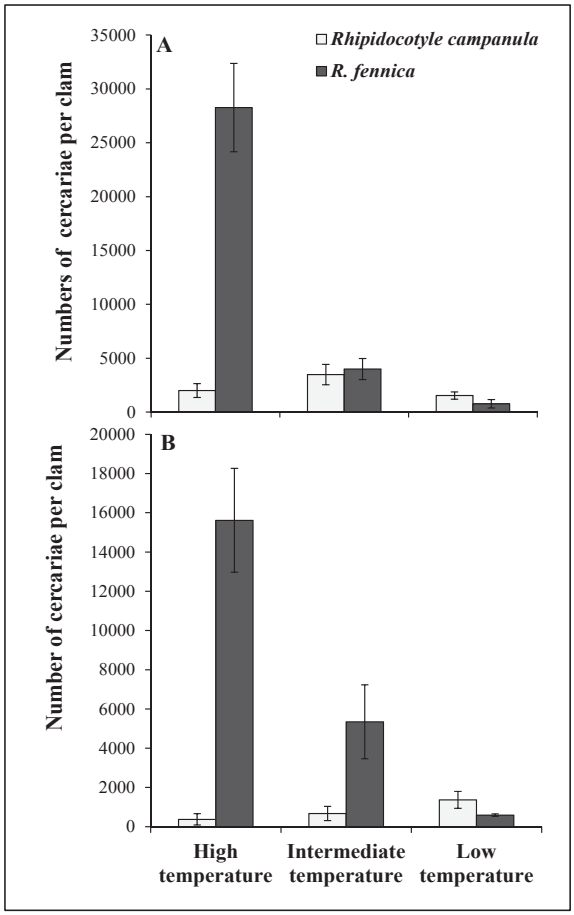

Figure 2. Total annual cercarial output (mean ± s.e.) of *R. campanula* and *R. fennica* at different temperatures. A – total annual average cercarial output from the molluscan host, *Anodonta anatina,* originating from the River Haajaistenjoki. B - total annual average cercarial output from *A. anatina,* originating from the River Kuusaankoski. For numbers of clams, see Table 1A.

III

CONTRASTING TEMPERATURE RESPONSES IN THE SEASONAL TIMING OF CERCARIAL SHEDDING BY TWO *RHIPIDOCOTYLE* TREMATODES

by

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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

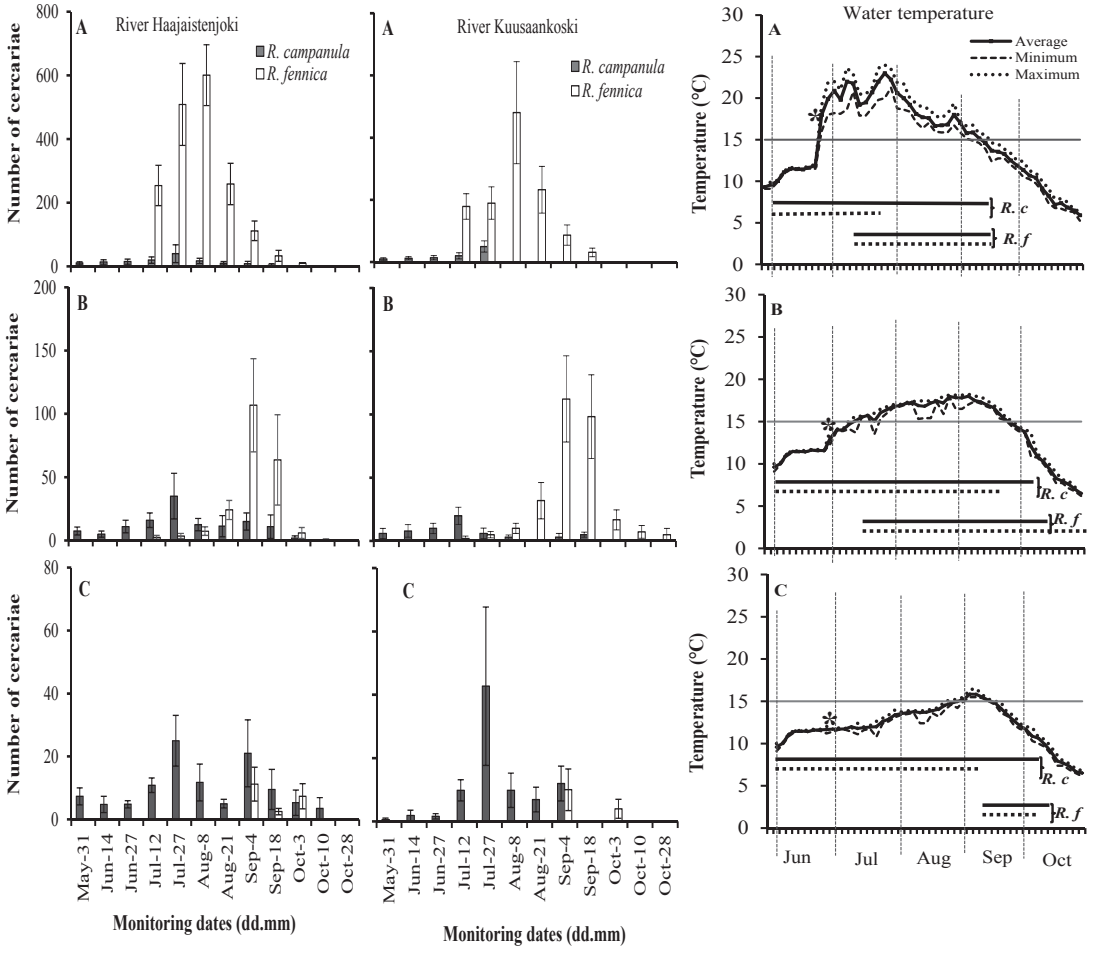

FIGURE 1 The daily (mean ± SE) cercarial release of *Rhipidocotyle campanula* (*R. c*) and *R. fennica* (*R. f*), water temperature profile at 3-day intervals and the total duration of cercarial release by mussels from the River Haajaistenjoki (straight line) and by mussels from the River Kuusaankoski (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.

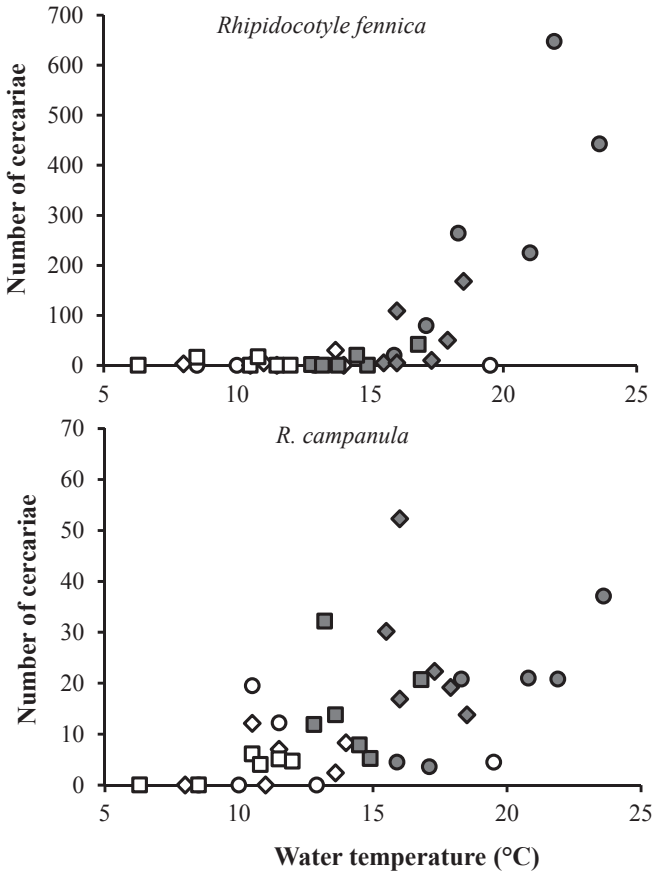

FIGURE 2 Mean number of cercariae released by *Rhipidocotyle fennica* and *R. campanula* in relation to water temperature at each of the twelve cercarial release monitoring sessions from mussels assigned to high (circles, n = 12), intermediate (diamonds, n = 12) and low temperature (squares, n = 12).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

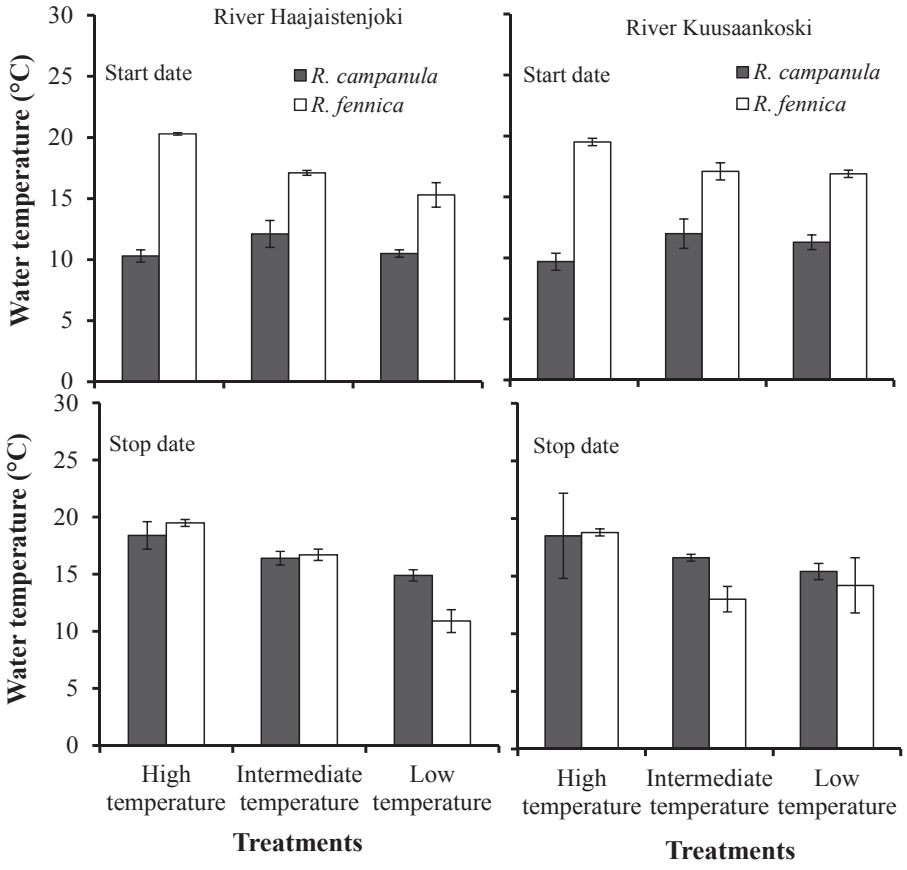

FIGURE 3 Water temperatures (mean ± S.E.) in the three temperature treatments on the dates of the first and last observation of emerged cercariae.

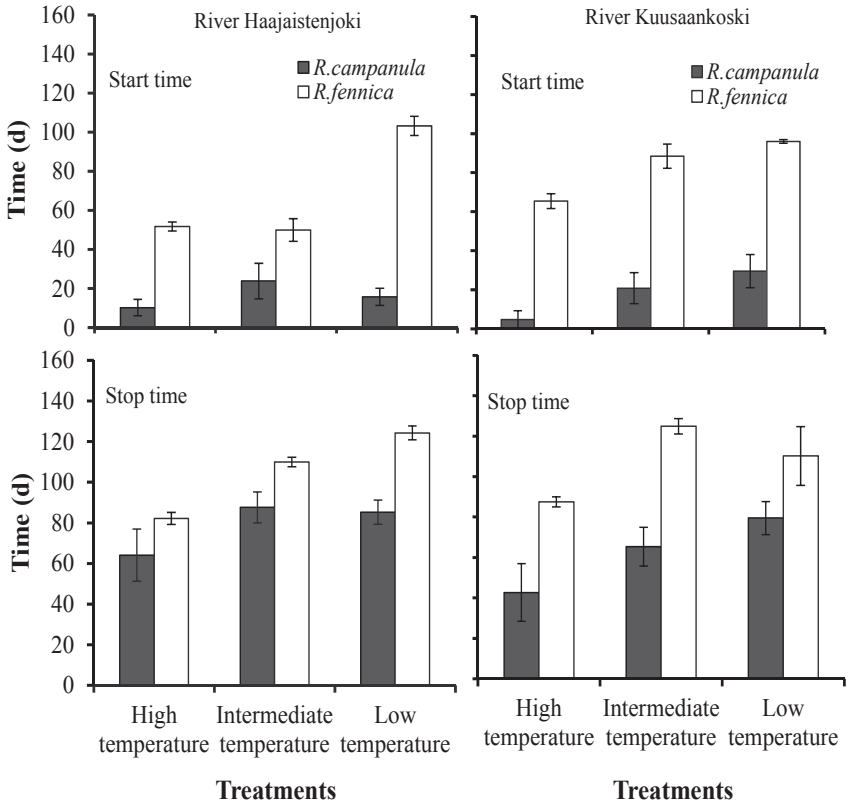

FIGURE 4 Time (days; mean ± S.E.) from the beginning of the experiment (May 31) to the start and stop of cercarial shedding in the different temperature treatments.

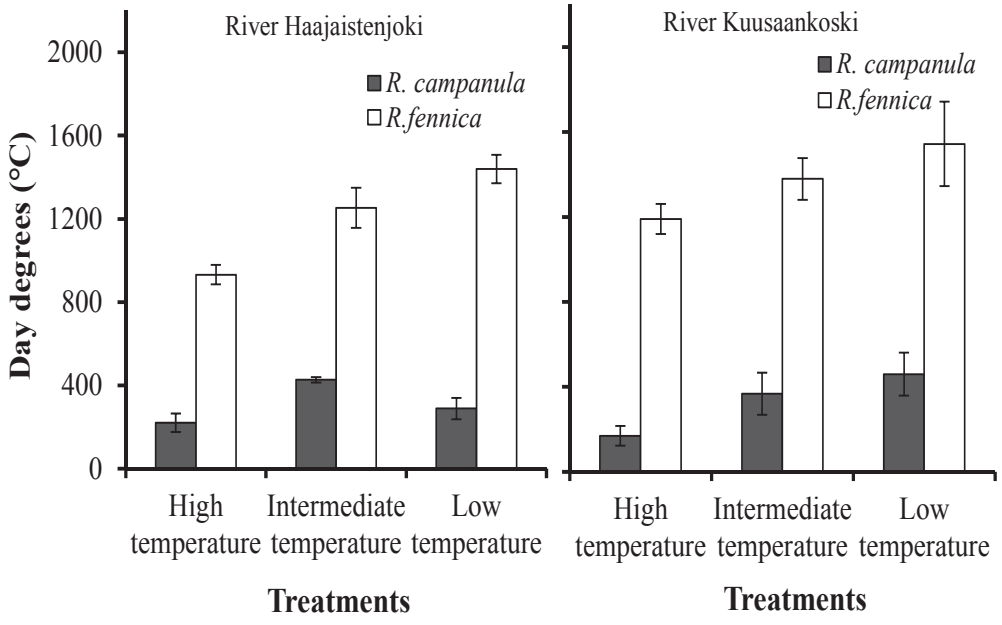

FIGURE 5 Mean (± S.E.) sum of day-degrees (°C) from May 17 to the start of cercarial release in the different temperature treatments.

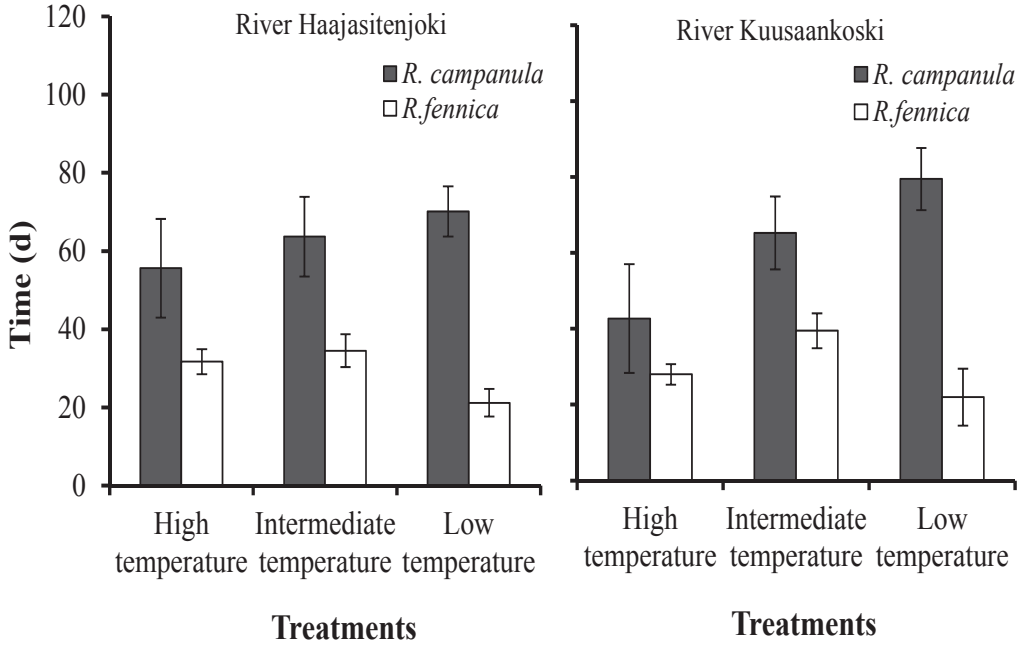

FIGURE 6 Duration of cercarial shedding (mean ± S.E.) of *R. fennica* and *R. campanula* in the different temperature treatments.

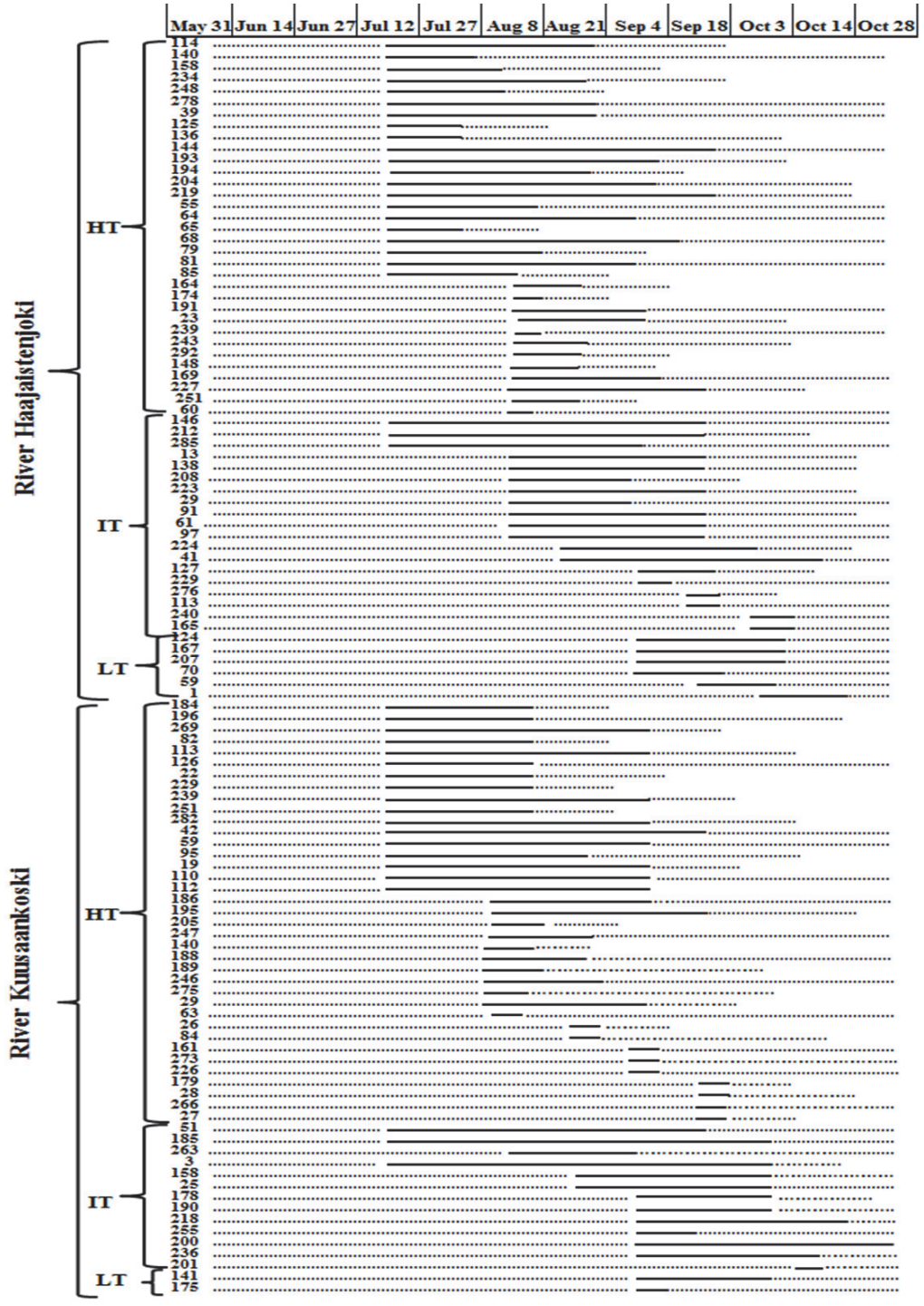

FIGURE S1 The patterns of individual *A. anatina* mussels shedding *R. fennica* at high temperature (HT), intermediate temperature (IT) and low temperature (LT). Solid line indicates the cercarial shedding period, dotted line represents the non-shedding period and the solid line ends when the mussel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

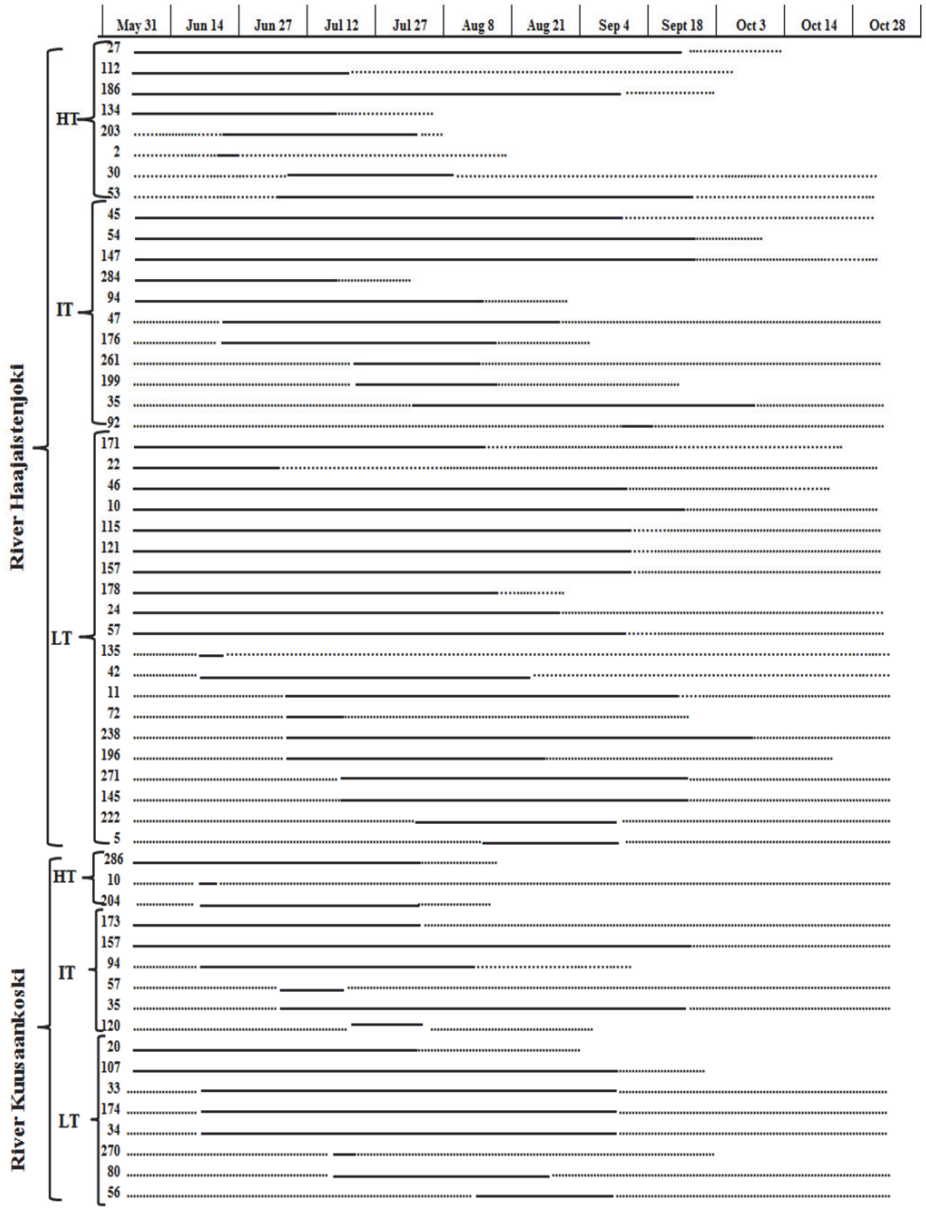

FIGURE S2 The patterns of individual *A. anatina* mussels shedding *R. campanula* at high temperature (HT), intermediate temperature (IT) and low temperature (LT). Solid line indicates the cercarial shedding period, dotted line represents the non-shedding period and the solid line ends when the mussel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

IV

LATITUDINAL DISTRIBUTION AND ABUNDANCE OF THE TREMATODE PARASITES *RHIPIDOCOTYLE FENNICA* AND *R. CAMPANULA*

by

Jouni Taskinen, Hanna M. Mäkelä, Raine Kortet & Jocelyn M. Choo 2015

Manuscript

Latitudinal distribution and abundance of the trematode parasites *Rhipidoctyle fennica* and *R. campanula*

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Running head: Latitudinal distribution of *Rhipidocotyle* parasites

Aim
We studied two trematode parasites, *Rhipidoctyle fennica* and *R. campanula*, and their duck mussel (*Anodonta anatina*) host, at high latitudes, close to the northern limit of their occurrence, in order to evaluate the roles of host availability and transmission factors in determining the distribution and abundance of the parasites. A previous study showed that *R. campanula* started cercarial larvae production immediately after the water temperature had increased to 15 °C, but *R. fennica* only 35-45 days later. Thus, we hypothesized that the abundance of *R. fennica* relative to that of *R. campanula* should decrease with latitude due to the constrained transmission of the former at northern, cold, short summer habitats (transmission hypothesis).

Location
Finland with focus on three regions along a latitudinal gradient; south (61-64 °N), low north (65-66 °N) and high north (67-69 °N).

Methods
Parasite and host data were collected from 37 southern, 13 low northern and 7 high northern water bodies, and temperature data from throughout the country. Frequency of occurrence of the duck mussel was used as a measure of host availability, and the number of days with the mean air temperature ≥ 15 °C (15C-days) was used as a measure of transmission potential.

Results
Number of 15C-days was mostly 41-60 in the south, 21-40 in the low north and ≤ 20 in the high north region. *R. fennica* declined from south to low north, but no difference between south and low north was observed in the frequency of occurrence of *R. campanula* or the duck mussel host. However, both *Rhipidocotyle* parasites and their duck mussel host were absent from the high north region.

Main conclusions
Transmission constraint due to the short summer probably limits the northern range of *R. fennica*. Lack of the duck mussel host probably determines the northern range border of *R. campanula*.

Key words: *Anodonta*, Arctic, climate change, distribution, host availability, latitude, parasite, temperature, transmission

INTRODUCTION

Most free-living animal taxa decrease in diversity with increasing latitude (e.g., MacArthur, 1972; Rosenzweig, 1995; Willig *et al.,* 2003). Parasite species richness has also been found to decrease with increasing latitude in several host groups including fish (Rohde, 1982; Rohde & Heap, 1998; Choudhury & Dick, 2000; Poulin, 2001), crustaceans (Thieltges *et al.,* 2009), primates (Nunn *et al.*, 2005) and humans (Guernier *et al.,* 2004; Cashdan, 2014). However, some studies show an opposite pattern: trematodes of marine gastropods, helminths of primates and parasites of carnivores increased in diversity with increasing latitude (Poulin & Mouritsen 2003; Nunn *et al.,* 2005; Lindefors *et al.* 2007). On the other hand, no dependence between latitude and trematode species richness was found in the European fresh water fish (Thieltges *et al.,* 2011). A recent meta-analysis by Kamiya *et al.* (2014) indicated that the relationship between parasite species richness and latitude is weak, but mainly positive, with richness increasing with latitude. Thus, the latitudinal gradients in parasite diversity may differ from those of free-living taxa, and more research especially on the factors influencing latitude dependence of parasite species occurrence is required (Kamiya *et al.,* 2014).

It has been shown that climate factors, most importantly temperature, contribute to fish species diversity, with decline in richness towards higher latitudes (Griffiths *et al.*, 2014). Therefore, the diversity of fish parasites should also, in theory, decline with latitude since parasite diversity depends on host diversity (Watters, 1992, Hechinger & Lafferty, 2005, Krasnov *et al.,* 2007). The possible decline in parasite species richness at high latitudes could partly result from colonization history or development/transmission constraints. In the northern hemisphere, parasite species or their hosts may not have had enough time to recolonize the high northern areas after the last glaciation. Alternatively, the climatic conditions at high latitudes may be unfavourable for the development and transmission of the parasite or for the development of the host(s). The roles of host availability and transmission factors in determining the geographic patterns of parasite species distribution and abundance are not well known. Global climate warming will inevitably affect distribution and abundance of both hosts and parasites in the future (Marcogliese, 2001; Harvell, *et al.*, 2002; Lafferty, 2009). Therefore, better knowledge of the factors that contribute to the biogeography of parasites will be essential in order to predict the anticipated outcome of climate warming for different parasite species.

Taskinen *et al.*, (1991) and Gibson *et al.,* (1992) described life cycles of the two bucephalid trematodes, *Rhipidocotyle fennica* and *R. campanula*. In northernmost Finland the hosts of these *Rhipidocotyle* species are close to the limit of their geographic distribution. The first intermediate host for both *Rhipidodocotyle* parasites, the bivalve mollusc *Anodonta anatina* and the common second intermediate host fish, roach) *Rutilus rutilus*), have been found up to latitude 68°N (Oulasvirta *et al.,* 2008; Hayden *et al.*, 2013). The definitive hosts for *R. fennica* and *R. campanula*, northern pike (*Esox lucius*) and European perch

(*Perca fluviatilis*) respectively, occur throughout Finland up to 70°N, although more sporadically and in low numbers at the highest latitudes (Hayden *et al*,. 2013; 2014). Later Taskinen *et al*. (1991) reported that the cercarial shedding by *R. campanula* responds quickly to increasing temperature, but that by *R. fennica* is much slower. Consequently, under field conditions *R. campanula* starts seasonal cercarial emission 3-4 weeks earlier (early June vs. late July) and at a lower temperature than *R. fennica* (Taskinen *et al.,* 1994, Taskinen 1998a). Cercarial release by *R. campanula* started almost immediately as water temperature reached 15 °C, whereas emergence of *R. fennica* cercariae only started 30 to 45 days later (Taskinen *et al.,* 1994). These results were experimentally confirmed in a long-term temperature manipulation study (Choo and Taskinen, unpublished). Thus, transmission of *R. fennica* should be more severely temperature-constrained in high latitude, cold, short-summer habitats, than that of *R. campanula*.

We studied the latitudinal distribution and abundance of the *Rhipidoctyle* parasites and their first intermediate bivalve host (*Anodonta antina*) at their northern boundary of distribution. Frequency of occurrence of *A. anatina* was used as a measure of host availability, and the number of days with mean air temperature ≥ 15 °C (15C-days) was used as a measure of transmission potential. As the critical length of the warm (≥ 15 °C) period for cercarial production by *R. fennica* is 30-45 days (Taskinen *et al*. 1994), our hypothesis was that (i) the northernmost occurrence of *R. fennica* would be in the zone of 30-45 15C-days. In addition, we hypothesised that (ii) the abundance of *R. fennica* relative to that of *R. campanula* should decrease with latitude due to the constrained transmission at northern, cold, short-summer habitats. We further hypothesised that (iii) transmission factors should constrain the occurrence of *R. campanula* only if the number of 15C-days is very low. On the other hand, lack of the obligatory host (and the parasites) from a given region would indicate host availability as a decisive factor contributing to the biogeography of the parasites.

MATERIALS AND METHODS

We mapped the occurrence, prevalence and the abundance of *R. fennica* and *R. campanula* in their first and second intermediate hosts, the duck mussel (Unionidae) and roach (Cyprinidae), respectively, in three regions along a latitudinal gradient: south (61-64 °N), low north (65-66 °N) and high north (67-69 °N) (Fig. 1). In addition, we investigated the occurrence of the duck mussel by examining roach and perch for presence of parasitic glochidium larvae of the mussel, as these fish are suitable hosts for *A. anatina* glochidia (Jokela *et al.,* 1991). The northernmost site of the low north region, the River Kemijoki (Table 1), is located on the Arctic Circle. All the high north sites can be regarded as

belonging to the Arctic, whereas the low north and the south regions belong to the Northern temperate zone.

Materials were collected from 57 water bodies; 37 south, 13 low north and 7 high north lakes and rivers, belonging to 10 catchments (Table 1). All the sites are in Finland except for the River Patajoki, Sweden (site 55, high north). Eight of the catchments (53 sites) drain into the Baltic Sea. Two catchments drain into the White Sea (catchments 7 and 8, sites 46-49, Table 1, Fig. 1). The proportion of lakes vs. rivers among the studied water bodies (84, 92 and 86 % in the south, low north and high north, respectively) did not differ between regions (χ^2-test, P = 0.748). Nor did the median (min-max) surface area of the south, low north and high north lakes [3.0 (0.02-1081), 4.0 (0.03-273) and 0.9 (0.27-6.9) km^2, respectively] differ between regions (Kruskall-Wallis test, test statistics = 1.420, P= 0.492). Material was collected between 1989 and 2015, so that the south and low north sites were sampled earlier (1989-2015) than the high north sites (2013-2015) (Table 1).

Duck mussels were collected from 29 of the 57 water bodies (Table 1), and examined for *R. fennica* and *R. campanula*. Roach plus perch were caught from 19 water bodies, while from eight water bodies, only perch were caught (Table 1). Only roach was collected from the River Patojoki. When *R. fennica* or *R. campanula* were found either in the duck mussel or in roach, the parasites were recorded as occurring in that water body. Similarly, if duck mussel glochidia were found from either roach or perch, or both species, duck mussel was recorded as occurring in that site.

Fish were collected by ice-fishing between February and April (except for the River Patojoki, which was sampled in October using a fish trap) because glochidia of duck mussel in fish are found in winter in this area (Jokela *et al*., 1991) and since no marked seasonal changes in the prevalence and the intensity of *Rhipidocotyle* parasitism in roach takes place during the ice-covered winter months (Taskinen *et al*. 1994). When using the fish data to study the geographic occurrence of the duck mussel, the River Patojoki site, sampled in October, was excluded because that is not a suitable time to find the glochidia of duck mussel (Jokela *et al*. 1991). Fishes were euthanized with a sharp blow to the head, stored and transported on ice to the laboratory. Fish were measured for length and the fins of roach were examined for *R. fennica* metacercariae and duck mussel glochidia, and the gills of roach were examined for *R. campanula* metacercariae and mussel glochidia, while fins and gills of perch were examined for duck mussel glochidia (Jokela *et al*., 1991, Taskinen *et al.* 1991). Mean site-specific length of roach differed between the three latitudinal regions (log-transformed length, one-way ANOVA, $F_{2, 21}$ = 7.403, P = 0.004). Tukey's B post hoc test indicated that the south (12 water bodies) and the low north (7 water bodies) areas formed a homogenous subset with no difference in the average fish size (mean ± s.e; 145.8 ± 3.1 and 152.1 ± 3.3 mm, respectively), but roach from the high north region (five water bodies) were significantly larger (177.4 ± 4.0 mm). Mean site-specific length of perch differed significantly between the three regions (one-way ANOVA, $F_{2, 26}$ = 19.312, P < 0.001). Tukey's B post hoc test

indicated that all pair-wise differences between latitudes were significant ($P < 0.05$). Hence the mean (± s.e.) site-specific mean length of perch increased from the south (119.8 ±2.5 mm, 16 water bodies) to the low north (136.2 ± 4.2 mm, seven water bodies) and to the high north (151.8 ± 5.9 mm, six available locations).

Duck mussels were randomly collected by hand picking, snorkelling or SCUBA diving and transported alive to the laboratory. Mussels were measured for length and age (growth rings on the shell), and examined microscopically for *Rhipidocotyle* sporocysts by pressing pieces of the gonad tissue between two large glass plates and viewing with transmitted light (Taskinen *et al*. 1991). The duck mussels were collected during the summer; seasonal changes in the prevalence of *Rhipidocotyle* parasitism in duck mussels are not significant (Taskinen *et al*. 1994). Mean (± s.e.) site-specific age of mussels was lower in the 22 southern water bodies (5.2 ± 0.3 y) than in the six low north sites (8.5 ± 0.4 y) (mussels were not available from the high north region) (One-way ANOVA, $F_{1, 26} = 24.996$, $P < 0.001$). Average (± s.e.) site-specific mean length of mussels was also lower in the south (67.1 ± 2.2 mm) than in the low north (77.1 ± 4.8 mm) materials (One-way ANOVA, $F_{1, 26} = 4.342$, $P < 0.047$).

Using meteorological data from 1961-2014, a map was constructed with the number of days when the daily mean air temperature was ≥ 15 °C (Fig. 1) in order to evaluate the length of the seasonal transmission window of the parasites. Climatological data on the mean daily air temperatures were obtained from the Finnish Meteorological Institute database.

Differences between the three regions in the frequency of water bodies occupied by *R. fennica*, *R. campanula* or duck mussel (glochidia) were analysed using χ^2 test or Fisher's exact test. Differences in the site-specific infection prevalences, infection abundances, ages and lengths between the latitudinal regions were analysed using one-way ANOVA and Tukey's B post hoc tests, using site-specific mean values. If the assumptions of ANOVA were not met, even after log-transformation of the response variable, non-parametric tests were used. Relationship between latitude and the prevalence or mean abundance of *Rhipidocotyle* species was studied using Spearman rank correlation analysis. To account for multiple tests, the Bonferroni correction was applied to p-values.

RESULTS

Geographic variation in air temperatures

The average length of the period when the mean daily air temperature was ≥ 15 °C ranged from > 60 days in southernmost Finland to less than 10 days in the north (Fig. 1). Southern sites belonged mainly to the 41-50 and 51-60 d zones, while one southern site experienced > 60 d of ≥ 15 °C. With one exception, the low north sites belonged to 21-30 and 31-40 d of ≥ 15 °C zones. With one

exception, the high north sites belonged to the 11-20 d of ≥ 15 °C zone (Fig. 1). The average seasonal period when air temperature is ≥ 15 °C was about 20 days longer in the southern sites than in the low north sites, and 10 days longer in the low north than in the high north sites.

Occurrence of R. fennica *and* R. campanula *in the combined mussel and fish data*

Both parasites occurred in the southernmost site of the study, River Kymijoki in Kuusankoski (site 1, 60° 99′ N, Table 1, Fig. 1), where the prevalence of infection in the duck mussel was 46.8 and 3.4 % for *R. fennica* and *R. campanula*, respectively. The most northerly site where *R. fennica* occurred was Lake Siikalampi (site 39, 65° 58′ N, Table 1), with 9.1 % of the duck mussels infected. The northernmost occurrence of *R. campanula* was in the River Kemijoki (site 50, 66° 33′ N, Table 1, Fig. 1), with 2.2 % of the duck mussels infected. Thus, in the northernmost temperature zones where *R. fennica* and *R. campanula* occurred, the mean durations when the air temperature was ≥ 15 °C were 31-40 and 21-30 days, respectively.

The frequency of occurrence of *R. fennica* was significantly higher in the south (25 out of 32 sites, 78%) than in the low north (one out of 12 sites, 8%) (Fisher's exact test, $P < 0.001$) (Fig. 2). *R. fennica* was not found in any of the five available high north water bodies, and the difference between the low and the high north regions was not significant (Fisher's exact test, $P = 1.000$). In the case of *R. campanula*, the frequency of occurrence was almost equal in the south (21 out of 32 water bodies, 66 %) and the low north (8 out of 12, 67 %) region. However, the difference in the frequency of occurrence of *R. campanula* between the low north and the high north, where *R. campanula* did not occur in any of the five sites studied, was statistically significant (Fisher's exact test, $P = 0.029$) (Fig. 2).

No catchment-specific differences were evident within the southern region. *R. fennica* and *R. campanula* occurred in all of the three southern catchments, and there was no difference between the catchments with regards to the frequency of occurrence of *R. fennica* (χ^2 test, $P = 0.212$) and *R. campanula* (χ^2 test, $P = 0.253$). In the low north region, *R. fennica* occurred only in one site, which belongs to the River Iijoki catchment, but *R. campanula* was found in five of the six catchments studied. Moreover, in the low north region, *R. campanula* occurred in all the catchments draining to the Baltic Sea, and in one (River Vienan Kemijoki) of the two catchments that drain into the White Sea. The River Kemijoki catchment was the only one to run through two regions, low north and high north. There *R. campanula* was observed in site 50 (66° 33′ N), low north, but not in the more northerly high north water bodies (sites 52-54, 68° 39-44′ N, Table 1). The catchments from which *Rhipidocotyle* parasites were not found included the River Koutajoki (sites 47 and 48, White Sea drainage) and the River Tornionjoki (sites 55-57, Baltic Sea drainage).

Occurrence of the duck mussel host

Duck mussel glochidia were found in eight (57 %) of the 14 southern sites, in six (86 %) of the seven low north sites and in none of the six high north locations (Fig. 2). The difference between the south and low north regions was not significant (Fisher's exact test, $P = 0.660$), but the decline in the frequency of occurrence of duck mussel from the low north to the high north region was statistically significant (Fisher's exact test, $P_{\text{Bonferroni corrected}} = 0.010$). The River Kemijoki, low north (site 50, 66° 33′ N, Table 1, Fig. 1) was the northernmost waterbody where the duck mussel was found.

Mean prevalence of R. fennica *and* R. campanula *in the duck mussel*

Duck mussels were found only from the south and low north regions. Mean prevalence of *R. fennica* infection in the duck mussel was significantly higher in the 23 southern sites than in the six low north sites (Mann-Whitney U = 25.00, $P = 0.016$) (Fig. 3). There was a significant negative correlation between latitude and the prevalence of *R. fennica* infection in the duck mussel over the south and low north regions (Spearman's rho = -0.511, $P = 0.005$, n = 29).

In contrast, the mean prevalence of *R. campanula* infection in the duck mussel did not differ between the south and low north (Mann-Whitney U = 93.00, $P = 0.212$) (Fig. 3). In addition, there was no relationship between latitude and the mean prevalence of infection by *R. campanula* over the south and low north regions (Spearman's rho = -0.204, $P = 0.290$, n = 29).

The mean ± s.e. difference (*R. fennica* minus *R. campanula*) in the site-specific prevalence of the parasites in the duck mussel was +15.5 ± 4.0 % (thus, in favour of *R. fennica*) in the south and -5.1 ± 2.0 % (in favour of *R. campanula*) in the low north. This difference was statistically significant (One-way ANOVA, $F_{2, 17} = 6.800$, $P = 0.015$).

Mean abundance of R. fennica *and* R. campanula *in roach*

The average site-specific mean abundance of *R. fennica* in roach was 58, 0.3 and zero metacercariae fish^{-1} in the 12 available southern, seven low north and five high north water bodies, respectively (Fig. 4). The decrease in the abundance of *R. fennica* from the south to the low north was statistically significant (Mann-Whitney U = 38.00, $P = 0.005$), but the abundances of *R. fennica* in the low north and high north regions did not differ (Mann-Whitney U = 15.900, $P = 0.755$). When the relationship between latitude and *R. fennica* abundance was studied over the whole roach material, there was a significant decrease in infection abundance with latitude (Spearman's rho = -0.717, $P < 0.001$, n= 24).

The average mean ± s.e. abundance of *R. campanula* in roach was equal in the south and low north latitudes (Fig. 4), respectively, but zero in the high north. The mean abundance of *R. campanula* decreased significantly from the low north to high north region (Mann-Whitney U = 5.00, $P = 0.048$). Over the whole geographic area of the study, *R. campanula* abundance decreased significantly with latitude (Spearman's rho = -0.603, $P = 0.002$, n= 24).

The mean ± s.e. difference (*R. fennica* minus *R. campanula*) in the abundance of the parasites in roach was +41.8 ± 15.8 metacercariae fish^{-1} (thus

in favour of *R. fennica*) in the south and -16.1 ± 12.1 metacercariae fish^{-1} (in favour of *R. campanula*) in the low north, the difference being statistically significant (One-way ANOVA, $F_{2, 17} = 6.427$, $P = 0.021$).

DISCUSSION

In our large scale study of 57 sampling sites, the frequency of occurrence, the mean prevalence and the average site-specific mean abundance (in both duck mussel and roach) of *R. fennica* decreased from the south to the low north region, but this pattern was not detected in *R. campanula*. Moreover, both *Rhipidodcotyle* parasites, and their first intermediated host, the duck mussel, were completely absent from the high north region.

How can the transmission hypothesis explain the geographic range of *R. fennica* and *R. campanula*? In a previous study, emergence of *R. fennica* cercariae started only 30 to 45 days after the rise of water temperature to 15 °C (Taskinen *et al.*, 1994). As expected, the zone of 31-40 15C-days was the northernmost temperature zone where *R. fennica* was found (Lake Siikalampi, site 39, low north). It is possible that beyond the zone of 31-40 15C-days the short summer and low temperature will constrain the cercarial production, and hence the transmission, of *R. fennica*. In the high north region, the prevailing temperature conditions should strongly limit the occurrence of *R. fennica*, as there was mostly only 11-20 days with air temperature ≥ 15 °C.

In addition, the general occurrence frequency of *R. fennica* in water bodies, as well as the mean prevalence of *R. fennica* infection in the duck mussel and the average mean abundance of *R. fennica* metacercariae in roach, were lower in the low north than in the south. This could also be explained by the transmission (larval release) hypothesis, i.e. the shorter warm water period in the low north region. The only site where *R. fennica* was found in the low north region, Lake Siikalampi, is a shallow pond, presumably a warmer-than-average habitat.

In a previous study by Taskinen *et al.* (1994), *R. campanula* started cercarial release almost immediately after the water temperature had increased to 15 °C. Thus, if the water temperature corresponds to that of air, it is reasonable to assume that occurrence of *R. campanula* is not constrained by cercarial release in any of the present geographic areas, not even in the high north where mostly more than 20 days with ≥ 15 °C was observed. Consistent with this, there was no difference in the occurrence frequency, in the mean prevalence in duck mussel or in the average mean abundance in roach, of *R. campanula* between the south and the low north. Thus, the transmission hypothesis cannot necessarily explain the lack of *R. campanula* from the high north, although it can explain the geographic occurrence of *R. fennica*.

How might the host availability hypothesis then explain the geographic range of the parasites? The molluscan host of *Rhipidocotyle* parasites, duck mussel, did not occur in any of the high north sites. Therefore, host availability may well explain the lack of *Rhipidocotyle* spp. in the high north region. Duck mussel is the only suitable mussel host available for *Rhipidocotyle* parasites in

these latitudes; other unionids have not been found to serve as a host for *Rhipidocotyle* species here (Taskinen *et al.*, 1991) and the distribution of other unionid mussels in northern Europe is more southerly than that of the duck mussel, *A. anatina* (Lopes-Lima *et al.*, 2015). In the present study, *R. campanula* occurred as far north as its host, the duck mussel, i.e. in the River Kemijoki, low north (at the Arctic Circle, 66° 33' N, site 50, Table 1).

The high north sites were located in two major drainages, the River Kemijoki and the River Tornionjoki (catchments 9 and 10, Table 1). These catchments are both occupied by duck mussel, as shown by this study and Oulasvirta *et al*. (2008), respectively. The southern part of the River Kemijoki catchment, at least in the main channel of the river, is also occupied by *R. campanula*. In northern Finland, higher latitude is generally associated with increase in altitude because of the Scandinavian Mountains. Therefore, in addition to the latitudinal gradient there is also an altitudinal gradient in the present study area, and many of the high north sites are also of higher altitude. The high north sites, located at higher altitudes in the headwaters, are presumably less readily colonized by host fishes of duck mussel and *Rhipidocotyle* species. However, duck mussel has earlier been reported as far north as 68° N, in the headwaters of the River Tornionjoki watershed (Oulasvirta *et al.*, 2008), one of our high north catchments.

There was a temporal mismatch in the collection of materials, as the high north sites were sampled later than the other regions. However, that should have increased, rather than decreased, the probability of occurrence of the parasites in the north since the ongoing climate warming has increased the annual mean temperature and the length of summer in this region (Mikkonen *et al*. 2014).

In the southern latitudes, the average abundance of *R. fennica* in roach was about 40 metacercariae fish^{-1} higher than that of *R. campanula*. This could be explained by the clearly higher cercarial production by *R. fennica* (9.500 larvae day^{-1}) than by *R. campanula* (1.400 cercariae day^{-1}) at 20 °C observed previously in the south region of the present study (Taskinen *et al.*, 1991).

There was no difference between the southern catchments with respect to occurrence of *R. fennica* and *R. campanula*. Two catchments in the northern areas did not have either of the *Rhipidocotyle* species, the River Tornionjoki (three high north sites, Baltic Sea drainage) and the River Koutajoki (two low north sites, White Sea drainage). In the River Tornionjoki area, this is probably connected to the northern location of the study sites; high north sites were not inhabited by *Rhipidocotyle* spp. regardless of catchment. That only two sites were sampled from the River Koutajoki catchment may have contributed to the apparent absence of *Rhipidocotyle* spp., since it belongs to the low north region which does harbour *Rhipidocotyle* parasites. Nor should belonging to the White Sea drainage rule out the occurrence of *Rhipidocotyle* spp., as *R. campanula* was observed in the River Vienan Kemijoki catchment, also flowing to the White Sea. Hence at least *R. campanula* has colonized the White Sea drainage, although the core distribution range of *Rhipidocotyle* parasites may be the Baltic Sea

drainage area (Taskinen *et al.,* 1991; Müller *et al.,* 2014; Petkevičiūtė *et al.,* 2014; Stunžėnas *et al.,* 2014).

Climate models predict a 2 to 7° C increase in annual temperature in Finland by the 2080s, compared to a 1961-1990 baseline period (Jylhä *et al.*, 2004). Climate warming is predicted to affect the global distribution of parasites, with range expansion or shift towards higher latitudes (Marcogliese, 2001; Harvell, *et al*., 2002; Lafferty, 2009; Laaksonen *et al*. 2010). Thus, climate warming will inevitably also change the northern distribution of the *Rhipidocotyle* parasites. Based on the present results, and the observed need for high temperature and a long warm period for the cercarial release by *R. fennica* (Taskinen *et al.,* 1994), the first change to take place will probably be an increase of *R. fennica* in the low north region due to the longer and warmer summers predicted. The next change can be the expansion of the core distribution area for duck mussel to the high north region, accompanied with colonization of the area by *R. campanula*. Later, the high north region would probably also be colonized by *R. fennica*.

Currently the high north populations of roach are living without *Rhipidocotyle* parasites. As the present results show, the numbers of *R. fennica* and *R. campanula* can be as high as 1024 and 180 metacercariae $fish^{-1}$ with mean abundances of 194 and 63 parasites $fish^{-1}$ $lake^{-1}$. Parasites of the family Bucephalidae, including *Rhipidocotyle*, can severely harm their fish host, and even cause mass mortality in their cyprinid hosts under stressful conditions (Hoffman *et al.,* 1990). Therefore, both the current lack of *Rhipidocotyle* parasites from roach in the northernmost water bodies and the anticipated spread of these parasites to those habitats in the future climate can be expected to have an important influence on roach individuals and populations in northern latitudes. In addition, both *Rhipidocotyle* species decrease the growth, survival and reproduction of the duck mussel (Taskinen & Valtonen, 1995; Taskinen, 1998b; Jokela *et al.,* 2005; Müller *et al.,* 2014). Therefore, the current low frequency of occurrence of *R. fennica* in the low north, and the predicted increase of the species there in the future, should have a major impact on the duck mussel individuals and populations in northern latitudes. As roach and mussels can have important roles in their ecosystems (e.g., Jeppesen *et al*., 2010; Vaughn *et al*., 2008), lack or spread of these organisms from/to the high north region have also potential ecosystem level consequences.

Our results suggest that i) the low occurrence, prevalence and abundance of *R. fennica* in the low north region can be explained by the possible transmission constrain, while ii) the lack of *R. fennica* and *R. campanula* from the high north may be explained by host availability, as the obligatory host, duck mussel, was also missing from the high north sites. Arctic and Subarctic regions may offer good models for studying the impacts of climate change on parasite ecology because they are generally simple systems with few other, confounding anthropogenic factors (Kutz *et al.,* 2009). Thus, our study will provide a baseline for future monitoring of the geographic distribution of these parasites and their hosts at high latitudes in warming climates.

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Table 1. Study lakes (L.) and rivers (R.), study site number (#),catchments (C), lake surface area (km^2), latitude (Lat.), longitude (Long.), numbers of individuals studied and year of collection (n/year), mean age (y) of duck mussels and mean length (mm) of mussels and fishes (roach and perch).

						Duck mussel			Roach		Perch	
	#	C	Area	Lat.	Long.	n/year	Age	L	n/year	L	n/year	L
South												
R. Kuusankoski	1	1		60.90	26.62	177/1996	-	-	-	-	-	-
L. Saraavesi	2	1	10.5	61.51	25.99	85/1996	6.1	64.9	90/1989[a]	-	-	-
L. Särkijärvi	3	1	0.04	61.93	27.71	-	-	-	-	-	10/1996	128.3
L. Päijänne	4	1	1081	61.93	25.54	142/2012	4.0	71.2	15/1996	173.3	15/1996	145.1
L. Huhtalampi	5	1	0.16	62.06	26.27	-	-	-	-	-	15/1996	109.7
L. Pettämä	6	1	9.00	62.06	25.16	100/2014	7.0	68.8	-	-	-	-
L. Valkonen_1	7	1	0.03	62.21	25.51	-			-	-	15/1996	118.8
L. Iso-Kairahta	8	1	0.16	62.22	25.91	42/1996	7.4	70.8	-	-	-	-
L. Valkonen_2	9	1	0.06	62.22	25.58	-	-	-	-	-	15/1996	124.1
L. Leppävesi	10	1	64.0	62.23	25.96	-	-	-	15*/1996	130.9	15*/1996	118.1
L. Jyväsjärvi	11	1	3.00	62.23	25.74	161/1996	6.1	61.1	15/1996	149.2	11/1996	113.6
R. Myllylänjoki	12	1		62.23	24.87	60/2014	6.3	62.5	-	-	-	-
L. Palokkajärvi	13	1	2.58	62.26	25.75	12/1996	4.8	52.1	-	-	-	-
L. Tuomiojärvi	14	1	2.98	62.26	25.74	55/1996	5.8	55.0	-	-	-	-
L. Vuorilampi	15	1	0.02	62.26	25.69	-	-	-	-	-	15/1996	113.1
L. Ala-Kintaus	16	1	7.00	62.28	25.33	-	-	-	15/1996	148.6	15/1996	125.1
L. Alvajärvi	17	1	2.09	62.32	25.73	154/1996	7.0	65.4	-	-	-	-
L. Kuuhankavesi	18	1	19.0	62.38	26.42	-	-	-	15*/1996	148.6	15*/1996	113.3
L. Vuojärvi	19	1	0.73	62.42	25.93	7/1996	4.7	55.0	-	-	-	-
L. Ahveninen	20	1	1.57	62.44	25.99	57/1996	4.2	45.1	-	-	-	-
R. Kuusaankoski	21	1		62.46	25.95	168/1996	4.9	77.7	-	-	-	-
L. Kuusvesi	22	1	22,00	62.47	26.03	-	-	-	15*/1996	134.3	15*/1996	109.5
L. Uurainen	23	1	13,00	62.53	26.07	-	-	-	-	-	15/1996	112.8
R. Pesiäissalmi	24	1		62.57	26.24	54/1996	4.4	60.9	-	-	-	-
L. Konnevesi	25	1	189	62.58	26.45	-			15*/1996	152.5	15*/1996	134.6
R. Siikakoski	26	1		62.62	26.34	37/1996	7.1	64.4	-		15/1996	115.3
L. Kivijärvi	27	1	154	63.03	25.13	-	-	-	15*/1996	146.0	15*/1996	112.6
L. Keitele	28	1	494	63.19	25.60	-	-	-	15*/1996	142.5	15*/1996	122.4
L. Katumajärvi	29	2	3.78	60.99	24.51	41/2013	5.8	74.0	-	-	-	-

L. Vanaja	30	2	0.76	60.99	24.47	50/1996	4.1	80.9	-	-	-	-
R. Moisionjoki	31	2		61.38	23.78	51/1996	6.3	78.9	-	-	-	-
L. Suolijärvi	32	2	2.03	61.44	24.80	19/2013	3.6	69.3	-	-	-	-
L. Keurusselkä	33	2	118	62.22	24.70	-	-	-	15/1996	140.0	-	-
L. Koijärvi	34	3	0.27	61.89	29.20	71/2005	2.7	81.8	-	-	-	-
L. Haukivesi	35	3	560	62.07	28.61	41/2005	7.0	69.1	-	-	-	-
L. Valkeinen	36	3	0.10	62.89	27.67	50/2005	2.5	83.6	-	-	-	-
L. Ala-Haajainen	37	3	1.19	63.63	26.99	43/2006	3.5	64.4	-	-	-	-
Low North												
L. Kuivasjärvi	38	4	0.82	65.07	25.47	107/1996	9.5	93.2	12/1989[a]	150.0	-	-
L. Siikalampi	39	5	0.38	65.58	28.25	72/1996	8.4	82.0	-		-	-
L. Iso-Kero	40	5	61.3	65.68	29.12	-	-	-	15*/2014	164.2	15*/2014	138.5
L. Vähäjärvi	41	5	0.99	65.75	29.14	-	-	-	15*/2014	140.4	15*/2014	134.4
L. Yli-Kuoliojärvi	42	5	1.53	65.82	28.84	-	-	-	20*/2014	145.6	15*/2014	134.7
L. Ranuanjärvi	43	5	4.62	65.92	26.58	72/1996	8.1	62.3	-	-	-	-
L. Aimolampi	44	5	0.03	66.03	27.86	-	-	-	-	-	15/2014	159.7
L. Hyrynjärvi	45	6	18.0	64.72	28.55	140/1996	8.5	85.0	-	-	-	-
L. Posionjärvi	47	7	18.9	66.13	28.13	-	-	-	30*/2014	146.4	15*/2014	127.0
L. Yli-Kitka	48	7	273	66.14	28.64	-	-	-	15*/2014	160.0	15*/2014	126.5
L. Oivanginjärvi	46	8	3.38	66.04	29.05	98/1996	9.7	73.5	-	-	-	-
L. Kuusamojärvi	49	8	47.4	65.93	29.27	-	-	-	15*/2014	157.9	15*/2014	132.5
R. Kemijoki	50	9		66.33	27.67	60/1996	6.8	66.8	-	-	-	-
High North												
L. Ounasjärvi	51	9	6.93	68.38	23.64	-	-	-	17*/2014	208.4	15*/2014	164.7
L. Angelijärvi	52	9	0.33	68.39	24.19	-	-	-	20*/2014	150.8	15*/2014	169.7
L. Venejärvi	53	9	0.86	68.41	24.44	-	-	-	15*/2014	159.1	15*/2014	146.9
L. Vuontisjärvi	54	9	0.92	68.44	24.00	-	-	-	41*/2014	154.1	15*/2014	140.5
R. Patojoki	55	10		67.39	23.40	-	-	-	10/2014	210.4	-	-
L. Leppäjärvi	56	10	0.27	68,52	23.31	-	-	-	-	-	15*/2014	132.6
L. Palojärvi	57	10	3.62	68.59	23.36	-	-	-	-	-	15*/2014	156.6

Catchments: 1, River Kymijoki; 2, River Kokemäenjoki; 3, River Vuoksi/Neva; 4, River Kuivasoja; 5, River River Iijoki; 6, River Oulujoki,; 7, River Koutajoki; 8, River Vienan Kemijoki; 9, River Kemijoki; 10, River Tornionjoki

* ice-fishing competition, tens or hundreds of fishermen, hundreds or thousands of fish collected

[a] from Taskinen *et al*. 1991

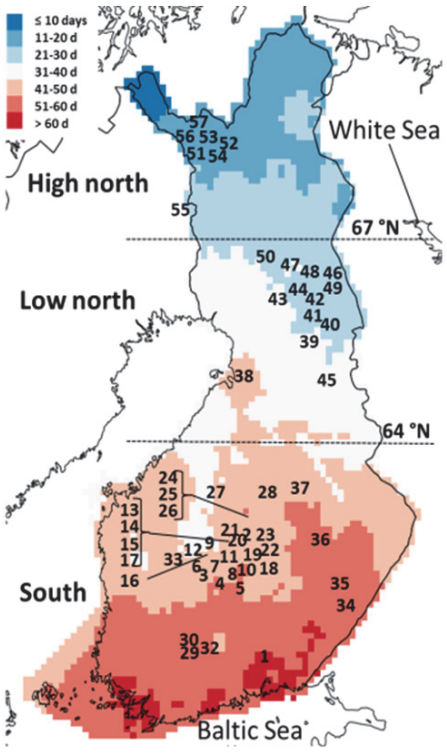

Figure 1. Geographic location of the study sites from 1 to 57 (see Table 1 for details) and the temperature zones (number of days when daily mean temperature ≥ 15 °C) within Finland.

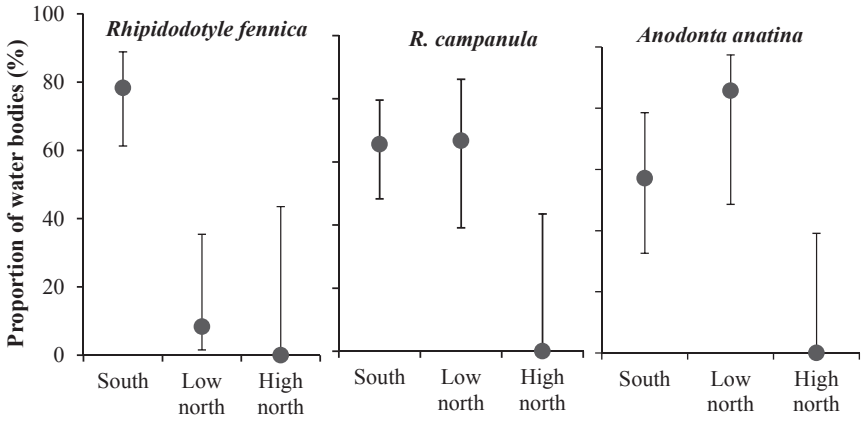

Figure 2. Mean (± 95 % confidence interval) frequency of occurrence of the parasites *Rhipidocotyle fennica* and *R. campanula*, and their first intermediate host, duck mussel (*A. anatina*), in the south, low north and high north regions.

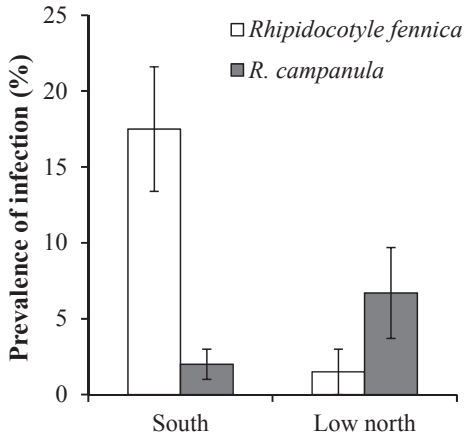

Figure 3. Mean (± s.e.) prevalence of infection of the parasites *Rhipidocotyle fennica* and *R. campanula* in their first intermediate mussel host, duck mussel, in the south and low north regions.

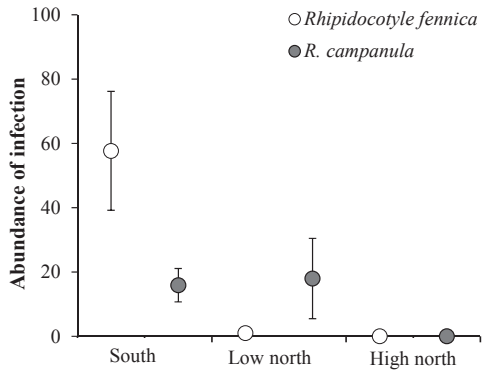

Figure 4. Average (± s.e.) site-specific mean abundance of *Rhipidocotyle fennica* and *R. campanula* infection in their second intermediate host fish, roach, in the south, low north and high north regions.