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

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ABSTRACT

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Yhteenveto: Lämpötilan, toukkatuotannon, virulenssin ja maantieteellisen levinneisyden väliset yhteydet pikkujärvisimpukan *Rhipidocotyle*-loisilla.
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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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.


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 extinct.

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, Bogán 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 fluviatilis (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. rutilus, 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).

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 snorkeling, 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 *Rhipidocotyle* 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 data 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 χ²-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 cercarial 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 χ²-tests or Fisher’s exact tests. The relationship between latitude and the mean prevalence or abundance of the *Rhipidocotyle* 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 novaeezealandensis* 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 intermediate 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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Lämpötilan, toukkatuotannon, virulenssin ja maantieteellisen levinneisyden väliset yhteydet pikkujärvisimpukan *Rhipidocotyle*-loisilla


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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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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), acclimated 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.
fenica cercaria with peak emergence during the warmest summer. In addition, by increasing temperature, release of R. fenica cercaria could be induced even during winter, outside the natural shedding period [20]. However, the response of R. fenica 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. fenica 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. fenica includes three host species. The parasite matures in the definitive host, the esocid fish Esox lucius [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. fenica, 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 ever 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 × 80 × 100 cm³ 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 ($\chi^2$-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 ($\chi^2$-test, $df = 2$, $\chi^2 = 1.470$, $P = .414$; 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 ($\chi^2$-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”: $F_{2,34} = 5.515$, $P = .009$). The change in cercarial release was the highest when the clams were transferred from 17 °C to 20 °C, with
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 (F₁,₃₁ = 0.370, P = 0.547), 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, nobserved = 7), 17°C (5.8 ± 0.7 y, nobserved = 6), and 20°C (6.1 ± 0.4 y, nobserved = 8) (one-way ANOVA, F₀,₄ = 0.276, P = 0.762).

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 clams 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...
Therefore, in the future the response of main factors affecting host-parasite relationships \[13,33\]. 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 \textit{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 (months, years) changes in temperature affect cercarial production by \textit{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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sediment. For example, a mean crawling track length of about 2 m was evident among \textit{A. anatina} clams of Lake Saravesi, Finland \[31\]—a lake with 30% prevalence of \textit{R. fennica} infection in \textit{A. anatina} \[5\]. In theory, moving would enable infected \textit{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, \textit{Planorbarius corneus}, and explained as a defense response against the parasites \[32\]. Therefore, \textit{A. anodonta} infected by \textit{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 \textit{R. fennica}. Prevalences of infection are found to be significantly lower in deeper water than in the littoral zone \[5\]. If infection by \textit{R. fennica} has an impact on the vertical movements and thermal preference of \textit{A. anatina}, could the parasite, \textit{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.
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