Variation among genotypes in responses to increasing temperature in a marine parasite: evolutionary potential in the face of global warming?

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ABSTRACT

Climates are changing worldwide, and populations are under selection to adapt to these changes. Changing temperature, in particular, can directly impact ectotherms and their parasites, with potential consequences for whole ecosystems. The potential of parasite populations to adapt to climate change largely depends on the amount of genetic variation they possess in their responses to environmental fluctuations. This study is, to our knowledge, the first to look at differences among parasite genotypes in response to temperature, with the goal of quantifying the extent of variation among conspecifics in their responses to increasing temperature. Snails infected with single genotypes of the trematode *Maritrema novaezealandensis* were sequentially acclimatised to two different temperatures, 'current' (15 °C) and 'elevated' (20 °C), over long periods. These temperatures are based on current average field conditions in the natural habitat and those predicted to occur during the next few decades. The output and activity of cercariae (free-swimming infective stages emerging from snails) were assessed for each genotype at each temperature. The results indicate that, on average, both cercarial output and activity are higher at the elevated acclimation temperature. More importantly, the output and activity of cercariae are strongly influenced by a genotype-by-temperature interaction, such that different genotypes show different responses to increasing temperature. Both the magnitude and direction (increase or decrease) of responses to temperature varied widely among genotypes. Therefore, there is much potential for natural selection to act on this variation, and predicting how the trematode *M. novaezealandensis* will respond to the climate changes predicted for the next century will prove challenging.

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1. Introduction

With rising temperatures globally over the past decades (Levitus et al., 2000) and consequently changes in local climates (Karl and Trenberth, 2003), an increasing number of species continue to be affected by climatic changes (Vittoz et al., 2013). Within a given ecosystem, climate change is likely to affect all species, directly or indirectly, but not all of them to the same extent (Menendez et al., 2006). There are different ways in which populations can respond to climatic changes: (i) habitat shifts, (ii) plastic phenotypic responses, or (iii) genetic change (Gienapp et al., 2008; Visser, 2008). Habitat shifts are possible when similar habitats with milder climates are available, but their accessibility may be constrained by barriers and competing species (Kokko and López-Sepulcre, 2006). Phenotypic plasticity consists of changes in behaviour, physiology, morphology or development in response to changes in the environment (Donnelly et al., 2012). However, these are constrained by the organism's plastic limits (Gienapp et al., 2008) that may often be exceeded with rapid, large scale climatic changes (Donnelly et al., 2012). Furthermore, plastic responses are not long-term adaptations (Visser, 2008; Donnelly et al., 2012) and species might already be operating at their plastic limits. Only changes in genetic composition can help populations cope with long-term climatic changes through selection. Populations can adapt through selection for genotypes that are either more resistant to, or better able to benefit from, higher temperatures and other related habitat changes (e.g. longer periods of drought, increased salinity, decrease in pH levels; see Marcogliese, 2001 for a review). However, current changes in climate happen at a rapid rate (Karl and Trenberth, 2003); as a consequence not all

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species will be able to adapt (Bell and Collins, 2008) and some are already becoming extinct (Cahill et al., 2013). This is the case not only for free-living, but also parasitic, species.

Small-bodied ectothermic species such as parasites may be particularly sensitive to climatic changes, but their responses have not been well studied (Marcogliese, 2001; Lafferty, 2009). Parasites play a great variety of roles in ecosystems; they can reduce host numbers (Robar et al., 2010), affect population dynamics (Hudson et al., 1998; Krkosek et al., 2013), and play key roles in shaping communities of free-living species (Poulin, 1999; Mouritsen and Poulin, 2002; Fenton and Brockhurst, 2008). At a population level, parasites can affect host numbers in a variety of ways. They can reduce overall health or increase mortality (Koop et al., 2011), increase susceptibility to secondary infections (Cornet et al., 2009) or increase predation risk (Poulin, 2010). All of these can change species interactions and consequently alter the organisation of ecosystems (Mouritsen and Poulin, 2002; Fenton and Brockhurst, 2008). Therefore, understanding how parasites will respond to climate change is essential to forecast changes in communities and ecosystems.

In many parasites of aquatic ectotherm hosts, the rate at which transmission stages are produced may be sensitive to ambient temperatures. This is particularly true for the cercariae of trematodes, i.e. the free-swimming infective stages produced in the first intermediate host (a mollusc) that generally emerge to infect the second intermediate host of the life cycle. Their output generally increases with rising temperature (Poulin, 2006), at least up to some optimal temperature, above which the output of cercariae declines with further temperature rises (Morley and Lewis, 2013). As a consequence, with modest increases in environmental temperatures the number of individual parasites in ecosystems might be expected to rise (Robar et al., 2010; Studer et al., 2010). Yet, the quality of parasite infective stages produced at higher temperatures may not necessarily match their quantity. For example, while the output of cercariae increases with increasing temperature up to an optimum level, their maximum survival time typically decreases linearly (Studer et al., 2010). However, although there can be substantial intraspecific variation in some trematode responses to rising temperature (see Studer and Poulin, 2014), we currently know nothing of the underlying genetic variation in these responses. It may be that the marked average responses of some trematode species to rising temperatures reflect the high sensitivity of a few genotypes, while many genotypes may show no response. To gain deeper insights into the ability of parasite populations to adapt to future climatic changes, it is essential to quantify the genetic variation in their responses to temperature. The present study therefore investigates the genetically determined variation in the output (quantity) and activity levels (quality) of cercariae in an intertidal trematode species in response to temperature.

We used the intertidal microphallid trematode, *Maritrema novaezealandensis*, and its first intermediate gastropod host, *Zeacumantus subcarinatus*, as a model system. *Maritrema novaezealandensis* has a typical complex life cycle comprising three hosts. Adults of *M. novaezealandensis* live in the intestine of red-billed gulls (*Chroicocephalus scopulinus*) and possibly other shore birds (Martorelli et al., 2004). Within the bird host, adult worms reproduce sexually and release eggs via the bird's excrement (Fredensborg and Poulin, 2005). *Zeacumantus subcarinatus* (Batillariidae: Prosobranchia) snails are the first intermediate host of *M. novaezealandensis* (Fredensborg and Poulin, 2005). After a snail ingests an egg, the parasites reproduce asexually within the snail while slowly replacing the host's reproductive tissue. Still, infected snails can live for several years (Fredensborg et al., 2005), and during that time hundreds of sporocysts (i.e. the parasite stage within a snail host), which may originate from a single egg, can,

depending on the environmental temperature, produce up to several hundred cercariae per week (Studer et al., 2010). The cercariae emerge from infected snails when the tide is low and snails are exposed to warmer temperatures in tidal pools. After emergence, the free-swimming cercariae locate and infect second intermediate crustacean hosts (Fredensborg and Poulin, 2005; Koehler and Poulin, 2010), in which they develop into metacercariae, grow and encyst (Fredensborg et al., 2004; Martorelli et al., 2004). To complete their life cycle, the encysted parasites then await ingestion of their crustacean host by a shorebird (Fredensborg and Poulin, 2005). The life cycle of *M. novaezealandensis* offers a great opportunity to study differences in response to climatic changes between genotypes. Not only has cercarial sensitivity to temperature been demonstrated in this trematode (Fredensborg and Poulin, 2005; Studer et al., 2010), but also infection levels in snail populations can be high (up to 80%; Martorelli et al., 2004) with a large portion harbouring single genotype infections (>50%; Keeney et al., 2007). Together with the high number of cercariae produced on a weekly basis, infected snails thus supply a steady flow of genetically identical cercariae that can be tested under different conditions.

The two traits investigated here, i.e. number of cercariae produced and cercarial activity, are clearly traits of the parasite and thus under the influence of the parasite's genotype. Nevertheless, they may also be affected by the snail host in which the parasites develop, since individual snails vary in terms of the resources they provide and their immune reactions to infection. However, any parasite-snail combination still represents a unique genetic complex because a parasite genotype can only be associated with a single snail. In the context of responses to climate change, what matters to the severity of infections in the other hosts of the trematode's life cycle is the extent of inter-individual variation in factors such as cercarial output and activity on which selection can act. Whether part of this variation is ascribed to the snail host instead of all of it being attributed to parasite genes does not diminish its potential relevance for evolutionary responses to warmer climates.

The specific objectives of this study were to (i) test for an interaction between temperature and parasite genotype in two responses, i.e. output and activity of *M. novaezealandensis* cercariae in a long term experiment involving a temperature increase, and (ii) test for any trade-off among genotypes between these two responses, allowing an assessment of relative investment in the quantity (output) or quality (activity levels) of infective stages. The first objective quantifies the genetic variation in how this parasite species responds to rising temperature, whereas the second determines whether the two responses considered here are independent of one another. This is the first known study to look at genetically determined differences between individuals of the same species in response to a highly crucial environmental factor, namely temperature.

2. Materials and methods

2.1. Host and parasite collection

Zeacumantus subcarinatus snails were haphazardly collected from the Lower Portobello Bay mudflat (Otago Harbour, South Island, New Zealand, 45° 50′ S, 170° 40′ E) on 26 November 2012 (early Austral summer) at low tide. Snails were acclimatised to laboratory conditions for 1 week before they were screened for *M. novaezealandensis* infections by inducing cercarial emergence. This is achieved by incubating snails for 24 h at 25 °C (see Section 2.2). Subsequently, 30 infected snails with a shell measuring between 12.0 and 14.0 mm from apex to the base of the aperture were

selected and screened again a week later to confirm their infection status. These snails were individually tagged with numbered plastic tags (Bee Works, Orillia, Ontario, Canada) and cyanoacrylate glue to allow repeated measures from the same individuals. Snails were then haphazardly distributed into three different aquaria (5 L, half full of natural, filtered seawater, salinity 35 ppt) ($n = 10$ per aquarium), ensuring that there were no differences in mean snail shell length between the tanks before the start of the experiments (General Linear Model (GLM), $t = -1.047$, $P = 0.304$). During the entire experimental period snails were fed sea lettuce (*Ulva* spp.) ad libitum and water was changed weekly. Because changing the water may induce cercarial shedding, this was done just after the experimental procedures (see Section 2.2).

2.2. Experimental design and procedures

Experiments assessing cercarial output and activity were conducted sequentially at two different temperatures; 'current' (15 °C) (i.e. approximately the average summer temperature in the field; Studer and Poulin, 2012) and 'elevated' (20 °C) (i.e. a temperature above average in the field during summer months, but likely to resemble the future average (Intergovernmental Panel on Climate Change, 2007). Infected snails were first acclimatised to the 15 °C temperature treatment for 4 weeks. Experiments were then conducted over three consecutive days each week, one for each aquarium ($n = 10$ snails per day) and the same snails were tested on the same day each week for another 4 weeks, so that each snail was tested four times during its maintenance at 15 °C. Eight days before the start of the cercarial output experiments, snails were incubated for 24 h at 25 °C to rid them of previously developed cercariae to ensure counts in the first week reflected cercarial production of that week only (7–8 days is the estimated maturation time of cercariae in this species). During experimental weeks, snails were incubated once each week, on the same day and at approximately the same time of day, for 2 h at 25 °C while in individual wells (approximate diameter 22 mm) on 12-well plates (hereafter referred to as shedding plates) with approximately 1.5 ml of filtered seawater, under constant illumination. The choice of 25 °C to induce cercarial emergence is based on the fact that the snails currently experience frequent spikes in temperature on sunny days at low tide in shallow pools, with water temperatures often reaching 30 °C or more (Studer and Poulin, 2012). It is during these brief spikes in temperature that most cercariae emerge and seek their next host; cercarial emergence outside of a temperature spike is minimal (<5% of that during a spike). Although possibly associated with a moderate thermal shock, these conditions mimic natural ones quite closely. After this 2 h incubation, emerging cercariae were counted (see below) to assess total cercarial output and used to quantify cercarial activity levels.

Each experimental week, to assess cercarial activity, eight samples of 50 µl, containing approximately 15 to 50 *M. novaezealandensis* cercariae each, were taken from each well on the shedding plates. The variable number of cercariae per sample should have no density-dependent influence on their activity, as the water volume used is 2 million times greater than the body volume of a single cercaria. These aliquots were placed into separate wells of two 96-well round-bottomed plates in haphazardly selected rows. Plates containing cercariae were then incubated at 25 °C under constant illumination, to simulate conditions in a shallow tidal pool on a summer day. The same temperature of 25 °C was used here for both the sequential temperatures ('current' = 15 °C and 'elevated' = 20 °C) at which the snails were kept long-term, to allow a direct comparison of cercarial activity under identical conditions. Over a total of 8 h, after every full hour of incubation, functionality of the cercariae was assessed based on their activity. Cercariae were rated as being either fully active (category of

relevance for successful infection) or not (i.e. sluggishly motile, immotile or dead and hence unable to infect a host). Once all cercariae had died, the total number of cercariae in each well was counted to calculate the percentage of fully active cercariae at each time point for each well.

After removal of samples for the assessment of cercarial activity, the remainder of the water in the wells of the shedding plates was fixed with ethanol. The numbers of cercariae in these samples were counted before being used for genetic analysis. Together with the total numbers of cercariae in the activity experiment, these numbers gave the total induced output of cercariae per snail per week. Subsequently, acclimatisation and experimental procedures were repeated exactly as above for the same snails at the elevated temperature (i.e. 4 weeks acclimatisation at 20 °C, followed by weekly experimental assessments over the following 4 weeks; total duration of experiment: 4 months).

2.3. Single versus multiple infections determination

Cercarial samples were used to determine whether snails harboured a single or multiple *M. novaezealandensis* genotype infections using microsatellite markers. A method using microsatellite markers similar to that reported in Koehler and Poulin (2012) was used. Briefly, cercariae from each infected snail were pooled from four separate shedding events (from the 15° C experimental period). DNA was extracted using Chelex beads (Walsh et al., 1991) and the concentration determined by NanoDrop (ND-1000 spectrophotometer, Thermo Fisher Scientific, USA). Fourteen previously reported microsatellite markers (Keeney et al., 2006; Abercrombie et al., 2009) were used according to Koehler and Poulin (2012). For each of these primer pairs, PCR was performed on DNA extracted from cercariae of each of the 30 snails infected with *M. novaezealandensis* and used in the above experiments. Each PCR product was diluted 1:20 with distilled water and combined in tubes to be multiplexed according to product size and fluorescent dye. The size of each product was determined using a 3730XL DNA analyser (Applied Biosystems, USA). Results were interpreted using the program GeneMarker (Softgenetics, LLC, State College, PA, USA).

2.4. Statistical analysis

All data was analysed using R version 3.0.2 (http://www.r-project.org/; 2013-09-25). General linear mixed effect models (GLMM) or, in case no random effects were included, GLM, were fitted using the package *lme4* (http://cran.r-project.org/web/packages/lme4/index.html, version: 1.0-6). The main response variables were (i) cercarial output, fitted with a Poisson error structure, and (ii) proportion of cercariae active 8 h post-emergence, fitted with a binomial error structure. For model selection Akaike information criterion (AIC) values were used (Johnson and Omland, 2004) to assess model fit. Initial models had a poor fit (high AIC values), due to overdispersion. To account for this, an additive overdispersion parameter (sensu Nakagawa and Schielzeth, 2010), with as many unique levels as observations in the response variable, was fitted as a random effect. This dramatically decreased AIC values (i.e. increased model fit) for output and activity models. In the initial models for both output and activity, temperature (15 or 20 °C treatments) and tank identity were included as fixed effects; snail shell length was also included as a fixed effect but only for analysis of cercarial output. Because each snail (i.e. each parasite genotype) was used in both temperature treatments, our design allows for each genotype to also serve as its own control. However, genotype had to be included as a random effect in the initial models due to the high number of levels ($n = 16$ for cercarial output and $n = 15$ for survival; see Section 3); week of

observation (4 weeks per temperature treatment) was also included as a random effect. In subsequent models, as an alternative approach to determine the importance of the potential 'temperature-genotype' interaction, genotype was instead included as a random-slopes effect of temperature, which allows for a different slope and intercept to be fitted to each genotype's response to temperature. Models with and without this 'interaction' were compared with each other using log-likelihood ratio tests (statistics package in R) to assess their relative fit to the data. We used $P = 0.05$ as the significance threshold for all fixed effects.

In additional analyses, to test for a trade-off in quantity versus quality of cercariae produced, output was related to the proportion of cercariae active 8 h after emergence across the different genotypes (i.e., across infected snails), for each of the two experimental temperatures separately, using linear models (LM).

3. Results

There was no mortality among snails during the experiment. Sixteen (53%) of the 30 snails used for the experiments carried a single *M. novaezealandensis* genotype infection. The other snails harboured two or more genotypes of *M. novaezealandensis*. Although snails with multiple-genotype infections generally released more cercariae, there was no significant difference in overall output between single and multiple infections (GLM, $t = 1.647$, $P = 0.101$). There was also no difference in activity between the two types of infections at either temperature (15 °C, GLMM, $z = 0.212$, $P = 0.832$; 20 °C, GLMM, $z = -0.777$, $P = 0.437$).

Because our focus is on differences among genotypes in response to temperature, hereafter we focus exclusively on the snails with single genotype infections.

Analysing weekly cercarial output for these 16 single genotype infections using a simple model with only temperature as a fixed factor and no other fixed or random factors, indicated that the output at the elevated temperature (20° C) was significantly higher than at the 'current', low temperature (15 °C) (GLM, $t = 3.323$, $P = 0.001$) (Fig. 1). After inclusion of the other fixed (snail shell length, tank identity) and random (genotype) factors into the model, no significant effect of temperature on the total number of cercariae produced was found (GLMM, $z = 0.556$, $P = 0.578$). Lengths of snail hosts prior to the experiment also did not influence the number of cercariae that emerged from them ($P = 0.585$). Adding the fit of different slopes to each genotype's response to temperature resulted in a significantly better model fit (log-likelihood ratio test, $\chi^2 = 27.19$, df = 2, $P < 0.001$). This indicates that differences between genotypes explain an important part of the observed variation in how they respond to higher temperature (Fig. 1). Indeed although for most genotypes mean cercarial output values obtained at 20 °C exceeded those at 15 °C, the difference was only substantial in one case (genotype 1), and the opposite was observed for a few genotypes (Fig. 1).

For the activity analyses, one of the 16 single genotype infection snails had to be excluded from the data set because, after trimming of the data (i.e. removal of data points based on fewer than 10 cercariae per well), it only had four data points left which were all in the low temperature treatment. The proportion of cercariae active at the 'current' (15 °C) and 'elevated' (20 °C) temperature
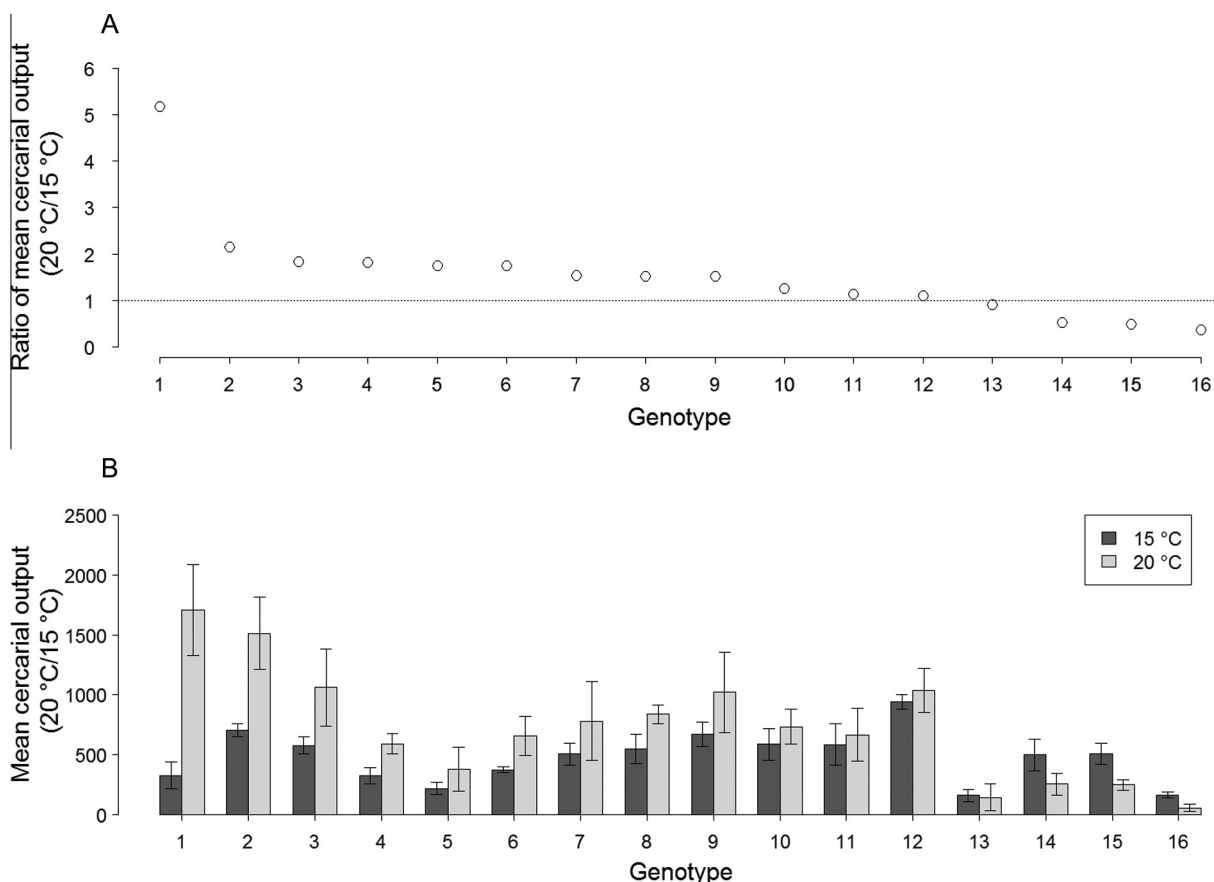
Fig. 1. Responses of *Maritrema novaezealandensis* to rising temperatures. (A) Ratio between the mean number of cercariae released per snail per week at 20 °C and that at 15 °C, for each of 16 single genotype infections. Dashed line represents ratio of 1, indicating no difference in output between temperatures; points above the line indicate higher output at 20 °C and those below the line indicate higher output at 15 °C. (B) Mean (±S.D.) cercarial output for each genotype at both temperatures. Note that genotypes are arbitrarily, but consistently, numbered for all figures.
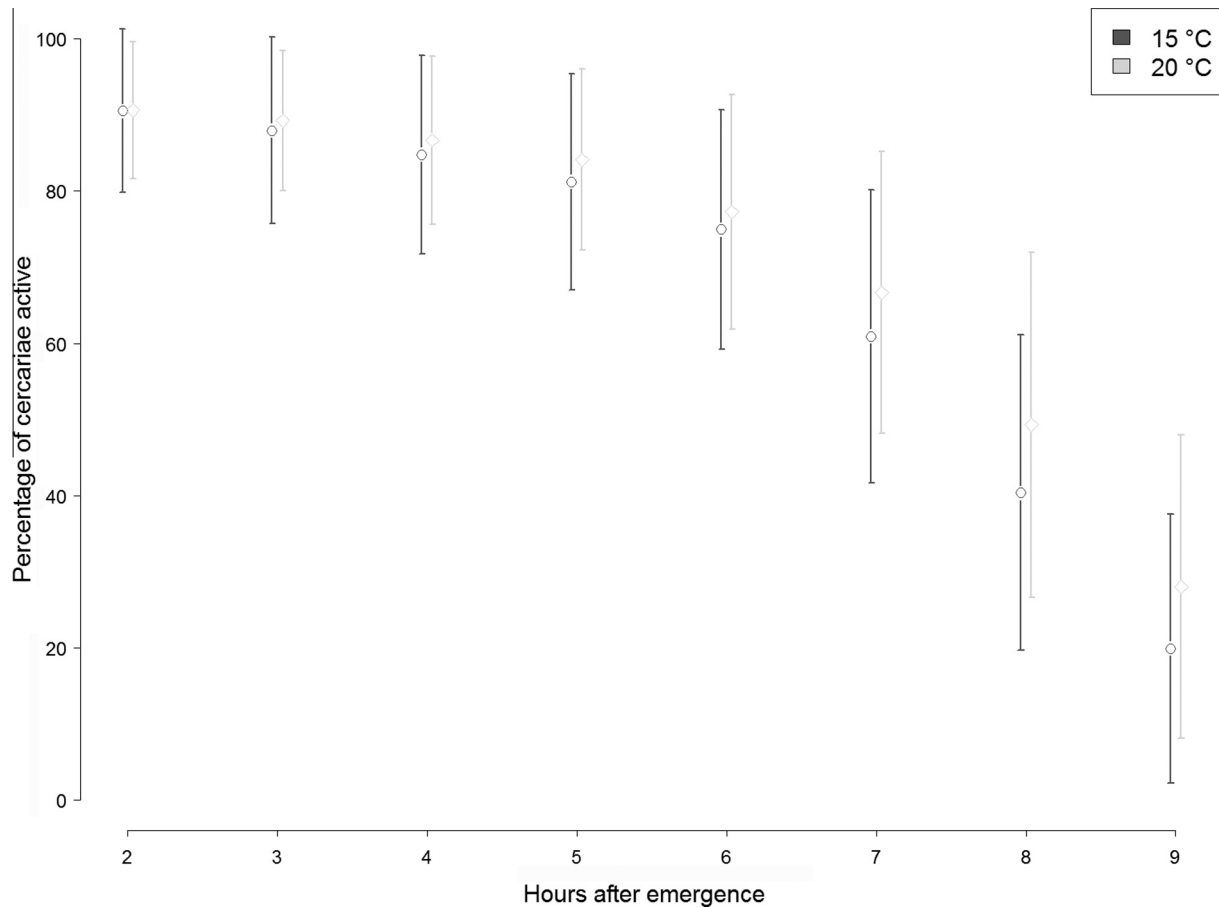
Fig. 2. Mean percentage (±S.D.) of *Maritrema novaezealandensis* cercariae active as a function of time after emergence from the snail, at each acclimation temperature, averaged over genotypes and weeks.

was initially the same, but diverged with increasing time after emergence from the snail (Fig. 2). Indeed, a larger proportion of cercariae remained active among those that emerged from snails at the elevated temperature, although variation was high (Fig. 2). Analysis of overall activity, using only temperature treatment as a fixed factor, showed that activity after 8 h was significantly higher at the elevated temperature (GLM, t = 7.731, P < 0.001). However, after including other fixed (tank identity) and random (genotype) factors, no significant effect of temperature was apparent (GLMM, z = 0.250, P = 0.802). Again, fitting different slopes to each genotype's response to temperature resulted in a significantly better model fit overall (log-likelihood ratio test, χ^2 = 201.44, df = 1, P < 0.001). This is a strong indicator that variability in cercarial activity levels has a genetic basis, i.e. specific genotypes respond differently in terms of activity to changes in temperature (Fig. 3).

Finally, we tested for a trade-off between the quantity and quality of cercariae produced by different genotypes. Using only the 16 single genotype infections, no correlation was found among genotypes between cercarial activity levels and cercarial output (r_S = -0.311, P = 0.259) (Fig. 4).

4. Discussion

Global climate change has the potential to alter the interactions between parasites and their hosts, and thus the way parasitism influences ecosystem functions (Marcogliese, 2001; Lafferty, 2009). Trematodes appear particularly sensitive to rising temperatures, as the average output of cercariae per infected snail has been shown to increase with rising temperatures in numerous species, at least up to an optimal point above which it declines (see Poulin, 2006; Morley and Lewis, 2013; Studer and Poulin, 2014). Here, differences in cercarial output and activity were found between *M. novaezealandensis* genotypes in response to temperature, providing the first known evidence of genetic variation in a trematode species. This finding has important implications. Although the average response of trematodes, at the species level, to rising temperature may be significant, this may be due to a few highly sensitive genotypes; many other genotypes may be relatively insensitive to changing temperatures. Our results indicate that, at least in *M. novaezealandensis*, there exists substantial intraspecific variation to allow selection in the face of global climate change.

When first intermediate snail hosts infected with single genotypes of *M. novaezealandensis* were kept at the 'elevated' temperature (20 °C), an overall increase in cercarial output was observed compared with when the same snails were kept at the 'current' temperature (15 °C). This confirms earlier observations on the same species by Studer et al. (2010) and for trematodes in general (Poulin, 2006; Morley and Lewis, 2013). When considering also data from snails with multiple clone infections, the pattern remains consistent. However, individual genotypes showed markedly different responses to the temperature treatments. Some genotypes clearly have a higher cercarial output at the elevated temperature (Fig. 1; e.g. genotypes 1, 2), whereas others do not seem to show a change (Fig. 1; e.g. genotypes 10, 11), and yet others have a slightly higher output at the current temperature than at

the higher one (Fig. 1; e.g. genotypes 14, 15). Overall, there is a suite of observed responses to the 'elevated' temperature, from halving the number of cercariae produced to a fivefold increase in output, with most genotypes falling in between those extremes and, on average, showing an increase in cercarial output. The analysis shows that genotypic differences account for much of the variance in the data, and thus confirms the genetic basis of responses to changing temperature. Since each parasite genotype is matched with a single host genotype (i.e. a trematode genotype can only occur in a single snail individual), it is possible that the variation is in part due to snail genetics and not only parasite genetics; nevertheless, the outcome remains one in which there is much intraspecific variation in cercarial output and activity in response to temperature.

Unidirectional shifts in output towards higher values at higher temperatures can, at least partly, be ascribed to an increase in metabolic rate with higher temperatures when the temperatures fall within the normal thermal range encountered by a population (Morley, 2011). However, the observed responses in the present study were diverse, i.e. there was great variation in the number of cercariae produced by individual genotypes (Fig. 1B). This result can thus not be solely explained by increased metabolism. Our

results therefore strongly indicate that genetic variation is an important factor in the variability in responses of *M. novaezealandensis* cercariae to temperature. They are also consistent with previous findings of genotypic differences in cercarial morphology and host compatibility in the same trematode species (Koehler et al., 2011, 2012; Koehler and Poulin, 2012).

The observed genetically determined variation in the response to rising temperatures implies that some genotypes will benefit from elevated temperatures, while others will not. With increasing temperatures, selection for genotypes with increased cercarial output is expected. In other words, genotypes better equipped to proliferate under warmer temperatures should have greater success in completing their life cycle. This may in turn lead to an increase in overall cercarial production and release into the environment as a consequence of climate change, although predictions are difficult as interpretations differ on this issue (Poulin, 2006; Morley and Lewis, 2013). Increased cercarial numbers can have a negative impact on populations of second intermediate hosts (Jensen and Mouritsen, 1992) and consequently on ecosystem functioning (Mouritsen and Poulin, 2002). Thus, even if overall host population health is not negatively affected by changing temperatures, increased parasite-induced mortality is expected through
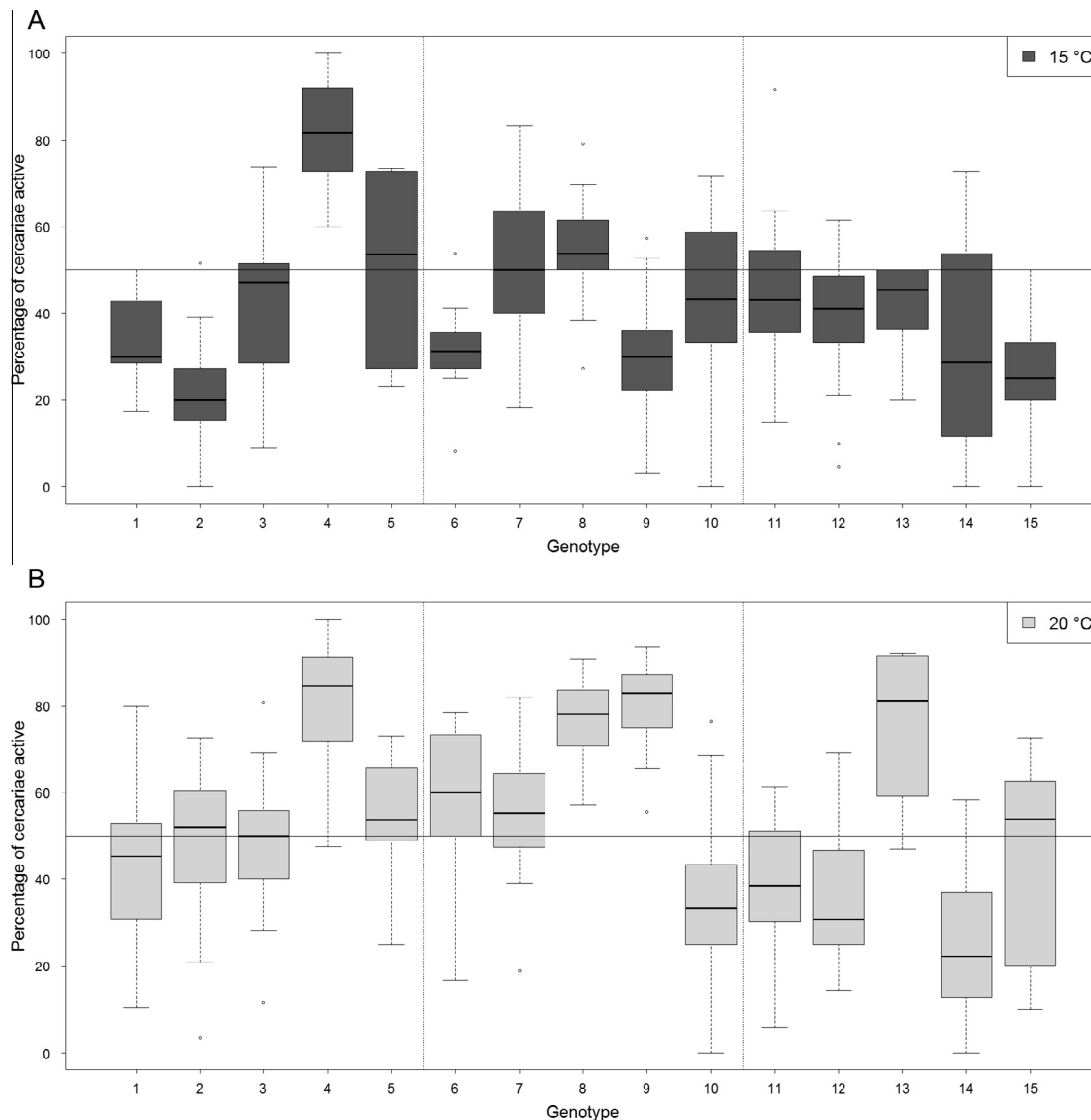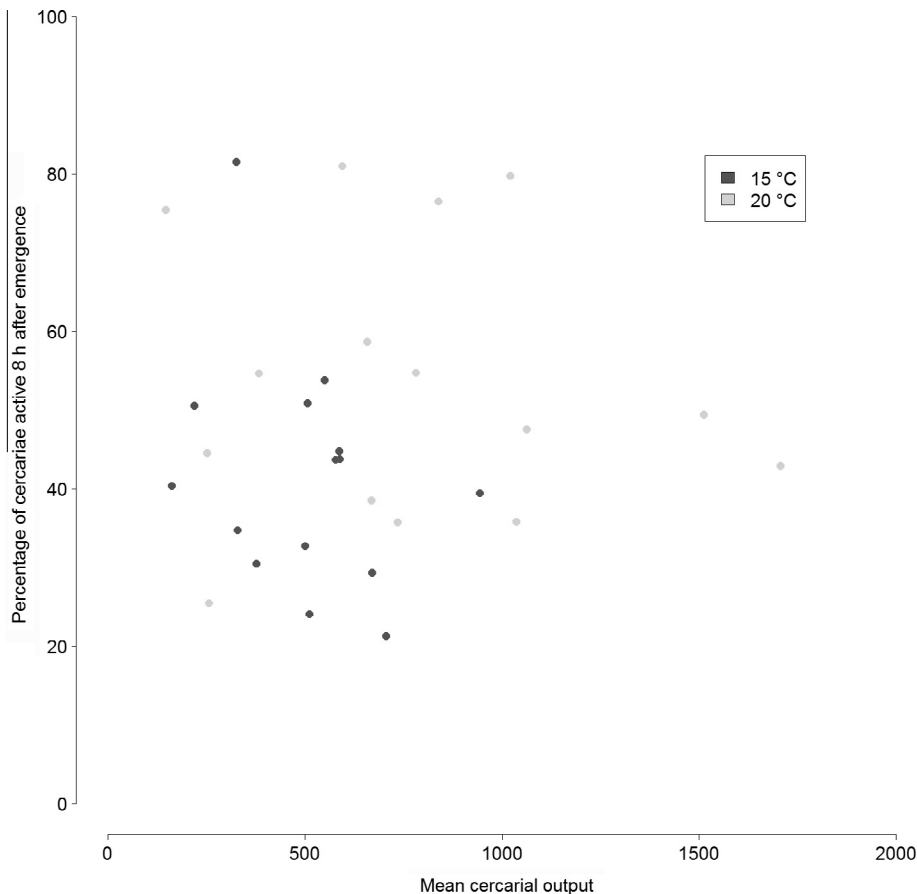

Fig. 3. Boxplots showing the percentage of *Maritrema novaezealandensis* cercariae active 8 h after they emerged from the snails, at 15 °C (A) and at 20 °C (B). Outliers are defined as points that fall outside the 1.5 times interquartile range. Center line indicates 50% for convenient comparison between plots.

Fig. 4. Mean percentage of *Maritrema novaezealandensis* cercariae still active 8 h after emergence from the snail host, plotted against the mean cercarial output per snail per week for each genotype temperature separately.

increased exposure to cercariae (Shim et al., 2013) and selection for more resistant host genotypes may ensue. However, the completion of a trematode life cycle depends on a variety of factors which cannot be accounted for based on our results, and our limited understanding of other parts of the transmission process limits our predictive abilities with regards to the potential consequences of climate change on parasites such as trematodes.

Overall, cercariae that emerged from snails kept at the 'elevated' temperature (20 °C) remained active longer when placed under conditions typical of transmission windows in the field (i.e., warm water of shallow pools at low tide in summer; Studer and Poulin, 2012), than cercariae emerging from snails that were kept at the 'current' temperature (15 °C) under the same conditions. The effect of temperature on cercarial survival varies among trematode species (Koprivnikar et al., 2010), and here we found its effect on cercarial activity also varies within species. Some genotypes show clear differences in activity after 8 h between the two treatments (Fig. 3; e.g. genotypes 6, 9 and 13), while for others there appears to be no difference in activity between temperatures (Fig. 3; e.g. genotypes 3, 7 and 11). None of the genotypes showed distinctly higher activity at the lower temperature. The difference between genotypes in their response to temperature was confirmed by our analysis, indicating that the interaction between genotype and temperature is more important than the sole effect of temperature. This also means that some genotypes are more important in accounting for variation in the data between the two temperatures than others. These findings confirm the existence of genetic differences in cercarial activity among conspecific

parasites in their responses to rising temperature. Of course, cercarial activity is only one component of their infectivity to the second intermediate hosts, and higher activity levels do not automatically translate into higher infectivity. Nevertheless, cercarial activity is certainly a key factor, as opportunities for infection of the next host are in large part determined by how long cercariae remain active (loss of active swimming is equivalent to functional death).

Increased activity of cercariae that developed at higher temperatures may shorten their lifespan, as cercariae typically survive longer at lower temperatures (Fried and Ponder, 2003; Studer et al., 2010; Studer and Poulin, 2014). In the present study the same snails were kept at different temperatures (15 and 20 °C) and the cercariae then tested at a single temperature (25 °C). The higher proportion remaining active after 8 h probably indicates that cercariae produced in snails that experienced 20 °C for several weeks coped better with more extreme temperatures than cercariae that emerged from snails that experienced 15 °C for several weeks. Possibly cercariae produced in the elevated thermal regime were primed to remain active for longer, for example through changes in physiology that cause them to rest more frequently and thus to conserve their limited energy stores (Morley, 2011). These contrasting results highlight the need for a better understanding of the effects of climate change on the reproductive output and activity of parasites through long-term studies with suitable experimental acclimation.

With increasing temperatures, and consequently warmer temperatures in intertidal pools, the cercariae of some genotypes of

M. novaezealandensis are likely to be able to remain active longer than others after they emerge from their first intermediate hosts. Climatic changes are predicted not only to increase mean temperature and the occurrence of heat waves, but also the peak temperatures during those heat waves. The effects of increased maximum temperatures during heat waves were not tested in this study, but are likely to increase cercarial mortality (Studer et al., 2010), especially when temperatures rise far above temperatures normally encountered by the species (Morley, 2011). The net effects of climate change on cercarial activity are thus difficult to predict. Nevertheless, our findings reveal genetic variability in cercarial activity and thus the potential for this trait to respond to climate-driven selection. Any such adaptive change in cercarial output or activity patterns could have repercussions on the parasite-induced mortality incurred by populations of second intermediate hosts (Fredensborg et al., 2004).

The absence of any correlation across genotypes between mean cercarial output and the average proportion of cercariae alive 8 h post-emergence indicates that there is no trade-off between the number of cercariae that are produced and their quality in terms of activity. The two traits appear independent of each other: genotypes producing many cercariae do not also produce relatively short- or long-lived cercariae. However, it is possible that there is a trade-off between the number of cercariae produced and some other factor that influences the fitness of the trematode.

Observed differences in cercarial output between snails may be influenced by other factors, including the age of the infection and individual properties of snail hosts. Younger infections are expected to produce fewer cercariae, because less cercariae-producing sporocysts have been formed. Alternatively, regardless of parasite genotype, some snails may be easier to exploit than others, and allow for greater cercarial production. In our system, it is not possible to distinguish between parasite genotype effects and snail genotype effects. Differences in response may thus reflect some degree of interaction between the parasite and snail host genotype. However, as each snail-genotype combination is used in both temperature treatments, each serves as its own control, and the general conclusions of this study remain unchanged. Thus, in the trematode *M. novaezealandensis*, and possibly other trematode species, there is significant genetic variation in responses to rising temperature, measured by both cercarial output and cercarial activity. Some genotypes are very sensitive to temperature, others are not, and the direction of the responses observed (increased or decreased output/activity at a higher temperature) differs among genotypes. These findings indicate the potential for adaptive selection in the face of global warming, and point to the importance of considering genetic variation in any assessment of the possible impact of climate change on host-parasite interactions.

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