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Differential effects of temperature variability on the transmission of a marine parasite

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Abstract Temperature variability is particularly pronounced in intertidal systems. The importance of considering this variability has been increasingly recognised, especially in the context of climate change and disease dynamics. Here, we investigated the effects of temperature variability on the transmission of the intertidal trematode *Maritrema novaezealandensis*. The experimental treatments were 15 °C (control), 15 + 5 °C daily, 15 + 10 °C every second day, 15 + 15 °C every third day (overall equal thermal loading), and a heat wave treatment (15 + 10 °C daily). Daily 6 h incubations were carried out corresponding to daytime low tides over a 12-day period. Effects on output of transmission stages (cercariae) from infected *Zeacumantus subcarinatus* snail hosts and transmission success of cercariae to *Paracalliope novizealandiae* amphipod hosts were quantified, as well as the survival of amphipods. Results showed differential effects on output and transmission success. The number of cercariae emerging was similar for treatments with equal thermal loading, but was substantially increased in the heat wave treatment. Transmission success was highest and comparable for the treatments with regular daily temperature increases (i.e. 15 + 5 °C and heat wave), compared to other treatments. Amphipod survival was not affected by temperature treatment directly, but by the number of parasites infecting an amphipod, as well as amphipod sex. These results demonstrate that cercarial output depends mostly on total thermal loading, whereas successful

infection of amphipods is determined by total time above 15 °C. Repeated exposure to ∼25 °C, as expected under a heat wave scenario, therefore increases both transmission pressure and success, and hence, the risk of parasite-induced mortality in amphipods.

Introduction

As climate and in particular temperature are important modulators of disease dynamics, some effects of climate change on the distribution and intensity of diseases can be expected (Cattadori et al. 2005; Ostfeld 2009; Lafferty 2009). One main concern is increasing diseases with increasing temperatures as a consequence of global warming, due to increasing transmission rates and extended seasonal windows for parasite development (Kutz et al. 2005; Poulin and Mouritsen 2006). However, not only does this complex topic remain somewhat controversial, there is also an issue with the fact that most laboratory-based assessments of temperature effects on disease transmission, on which many predictions are based, have been conducted at constant temperatures. The pertinence of using constant temperatures to estimate the influence of fluctuating temperatures on biological responses and ecological processes has thus been debated (Fischer et al. 2011; Smith 2011; Niehaus et al. 2012; Thompson et al. 2013), and calls for more event-oriented, rather than trend-oriented experiments have been made (Jentsch et al. 2007; Thompson et al. 2013).

Temperature change can be manifested as a change in mean intensity (average over a given period), amplitude (variance around mean) and/or temporal variance (Benedetti-Cecchi 2003). There is mounting evidence for the importance of considering this variability for biological

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and ecological phenomena (Denny et al. 2009; Benedetti-Cecchi 2003; Benedetti-Cecchi et al. 2006; Wethey et al. 2011; Pincebourde et al. 2012), including for disease epidemiology and host demography (Saunders et al. 2002; Paaijmans et al. 2010; Duncan et al. 2011; Lambrechts et al. 2011; Hernandez et al. 2013). Considering environmental variability is crucial for a more accurate understanding of natural systems and hence, an increased ability to make predictions in the context of climate change. Firstly, because many habitats and ecosystems, for example, intertidal ecosystems, are characterised by highly fluctuating environmental conditions affecting individual organisms as well as biotic interactions (e.g. Pincebourde et al. 2012). And secondly, because global climate change does not only affect climate averages, but also its variability, including the frequency and intensity of extreme events such as heat waves (Easterling et al. 2000; IPCC 2007).

Anticipating and predicting the potential impacts of climate change on the dynamics of host–parasite systems are difficult due to the intrinsic complexity of multi-species interactions and the numerous ways in which the environment (biotic and abiotic) can influence these dynamics (Molnar et al. 2013). Despite the fact that the importance of assessing the effects of variable temperatures for host–parasite interactions have long been recognised (e.g. Pflüger 1981; Al-Habbib and Grainger 1983), its urgency has only been emphasised recently (e.g. Paaijmans et al. 2009). For example, malaria transmission has been shown to depend on daily temperature variation, with differential responses for temperature fluctuations around a low mean compared to a high mean (Paaijmans et al. 2010). This study highlights the need to consider diurnal variability, as it is, for example, crucial for accurately estimating risks from a disease in cooler versus warmer environments (Pascual et al. 2009).

One attempt to add ecological realisms to assessments of host–parasite interactions in the context of climate change has been through experimental heat wave studies (Roth et al. 2010; Seppälä and Jokela 2011; Landis et al. 2012). However, the inherently high temperature variability affecting host–parasite interactions in intertidal habitats, coupled with the unusual conditions that would be encountered during extreme events such as a heat wave (e.g. Mislan et al. 2009) has, to our knowledge, not been studied to date. The omnipresence of trematode parasites in intertidal ecosystems (Lauckner 1984; Mouritsen and Poulin 2002), their important ecological roles (Mouritsen and Poulin 2005, 2010; Wood et al. 2007), as well as their temperature sensitivity (e.g. Mouritsen 2002; Thieltges and Rick 2006), makes them potentially ideal model systems to assess the effects of temperature variability on the interaction between parasites and their hosts—especially on

their ectothermic intermediate hosts. As almost all temperature assessments on trematodes have been conducted at constant temperatures, actual transmission dynamics under natural conditions characterised by substantial differences in frequency and amplitude of thermal variability (e.g. Studer and Poulin 2012b) remain elusive (but see Fingerut et al. 2003).

In the present study, we used the intertidal trematode *Maritrema novaezealandensis* (Martorelli et al. 2004) as a model system. The complex, three-host life cycle of this parasite involves birds as definitive hosts (adult worms reproducing sexually), the mud snail *Zeacumantus subcarinatus* as a first intermediate host, and crustaceans such as the amphipod *Paracalliope novizealandiae* as second intermediate hosts (Martorelli et al. 2004; Koehler and Poulin 2010). Within snails, the parasite replaces the host's gonadal tissue with sporocysts in which cercariae, the free-swimming larval transmission stages, are produced asexually. The cercariae emerge from infected snails under optimal conditions for transmission to infect a second intermediate host, in which the parasite encyst (metacercariae) and awaits trophic transmission to a definitive host. Optimal conditions for transmission, especially for transmission from snails to crustaceans, are expected to prevail during low tides when the water temperature in tide pools exceeds a threshold of about 15 °C (Fredensborg et al. 2004; Studer and Poulin 2012a, b). As a consequence, transmission is seasonal with highest infection levels, at least in amphipod hosts, occurring in summer (Studer and Poulin 2012b). Moreover, the effect of *M. novaezealandensis* on amphipod hosts is dependent on infection-intensity, with higher mortality associated with increasing number of parasites per host (Fredensborg et al. 2004; Bates et al. 2010). The known temperature dependence of the transmission and the intensity-dependent mortality of the amphipod hosts imply that highest transmission pressure in this study system exists during summer low tides, and that amphipods are probably most at risk of parasite- and temperature-induced mortality under heat wave conditions. This is relevant for amphipod host population dynamics including the potential collapse of a population. Therefore, investigating the transmission of *M. novaezealandensis* under more realistic conditions should further increase our understanding of the interaction between this parasite and its hosts under natural conditions and allow more accurate predictions to be made in the context of climate change.

Here, we test whether scenarios creating the same thermal loading (degree-days above a 15 °C threshold) but at different frequencies have different effects on parasite transmission compared to each other, and compared to a scenario with regular pulses of high temperature exposing organisms to double the thermal energy above the 15 °C

threshold (heat wave scenario). Our aim was to simulate summer conditions encountered during daytime low tides on intertidal mudflats more closely in laboratory settings, and hence to conduct a more realistic assessment of parasite transmission dynamics (i.e. transmission pressure and success) under conditions associated with an increased mortality risk for amphipod hosts. The main objectives were to investigate the effects of different temperature variability scenarios on (1) the output of cercariae of the trematode *M. novaezealandensis* from infected first intermediate *Z. subcarinatus* snail hosts and (2) the transmission success of this parasite to second intermediate amphipod hosts (*P. novizealandiae*), including amphipod survival. This approach allows teasing apart the effects of exposure to a certain amount of thermal energy, as opposed to temporal patterns of temperature fluctuations.

Materials and methods

General procedures

The two separate experiments on output of cercariae and their transmission to amphipods included five temperature treatments, corresponding to daytime temperature increases of different frequency and magnitude. The baseline temperature was 15 °C. Temperatures were chosen in accordance with temperature data collected in the actual microhabitat of this parasite, and represent the overall average (~ 15 °C), as well as the average (~ 25 °C) and absolute maxima (~ 30 °C) during a warm summer week at low tide on a temperate mudflat (Studer and Poulin 2012b). The treatments were as follows: (a) 15 °C (control; daily incubation at 15 °C); (b) 15 + 5 °C daily (i.e. daily incubation at 20 °C); (c) 15 + 10 °C every 2nd day (i.e. daily incubations alternating between 15 and 25 °C); (d) 15 + 15 °C every 3rd day (i.e. daily incubations at 30 °C every 3rd day and at 15 °C on other days); and (e) a heat wave (hw) treatment with 15 + 10 °C daily (i.e. daily incubations at 25 °C) (see also Fig. 1). The 15 + 5 °C daily, 15 + 10 °C every 2nd day and the 15 + 15 °C every 3rd day were treatments with overall equal accumulated thermal energy (degree-days) above the 15 °C threshold, whereas the 15 + 10 °C daily was the heat wave treatment with double the thermal energy above the 15 °C threshold. Both experiments were run over a 12-day period to mirror the duration of a heat wave and an associated daytime low tide series. Daytime temperature increases consisted of incubations lasting for 6 h in accordance with the duration of a low tide and were carried out under constant illumination. Incubations were conducted using insulated water bath incubators (53 L, half-filled) covered with glass lids. To increase the degree of replication, both

experiments were replicated across two controlled temperature rooms (constant 15 °C baseline temperature; 12:12 h light–dark cycle).

Cercarial output

In this experiment, the number of cercariae emerging from infected *Z. subcarinatus* snails exposed to the different temperature variability treatments was assessed. Five replicate infected snails from Lower Portobello Bay (Otago
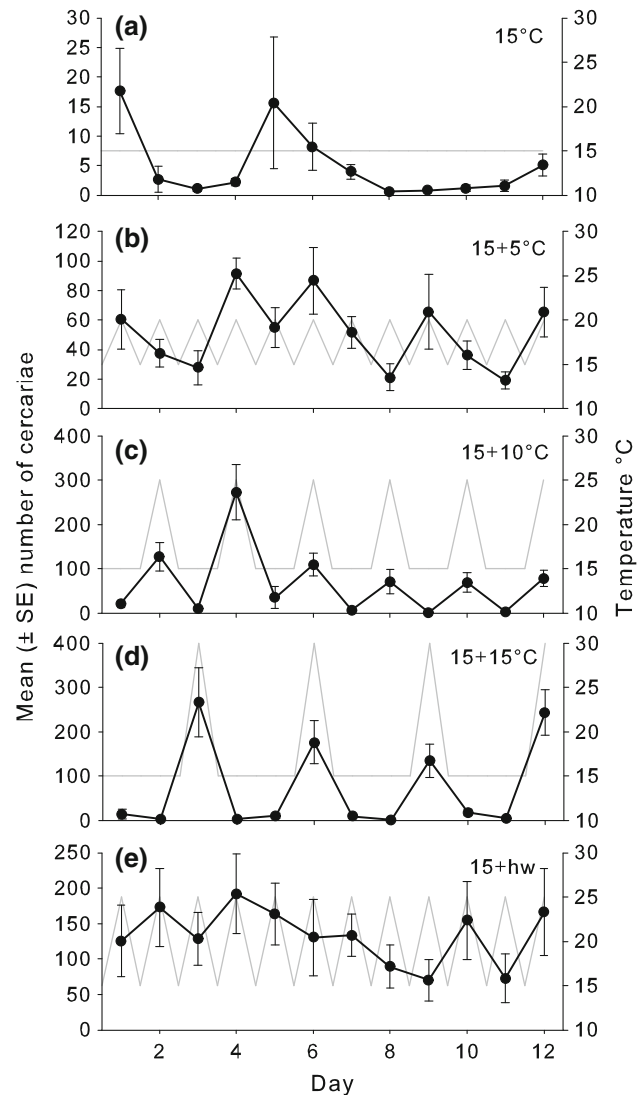
Fig. 1 Cercarial output. Temporal patterns of the mean (± standard error (SE)) number of cercariae of *M. novaezealandensis* emerging form infected *Z. subcarinatus* snail hosts under different temperature variability treatments (**a** 15 °C, **b** 15 + 5 °C daily, **c** 15 + 10 °C every 2nd day, **d** 15 + 15 °C every 3rd day, and **e** 15 + heat wave (hw), i.e. 15 + 10 °C daily) over the 12-day experiment (*n* = 10 per treatment) (*black line*). Note the different scales on the different graphs for output. The *grey line* illustrates the respective temperature profiles of each of the experimental treatments

Harbour, New Zealand; 45°50′S, 170°40′E) were randomly assigned to each treatment in each room (i.e. $n = 10$ snails per treatment) (mean ± standard error (SE) shell length 12.91 ± 0.062 mm; no size difference between treatments, ANOVA: $F_{(4, 45)} = 0.12$, $p = 0.975$). Each snail was individually marked with a plastic tag to allow for repeated counts from the same individuals. Snails were first incubated for 24 h at 25 °C under constant illumination to induce the emergence of fully developed cercariae. Snails were then acclimatised to 15 °C in the controlled temperature rooms for 7 days in aquaria (6.5 L, half-filled, aerated) with food ad libitum (sea lettuce *Ulva* sp.) until the onset of the experiment. During each day of the 12-day experiment, snails were individually transferred into 1.5 mL tubes filled with 1 mL of seawater and incubated at their respective temperature level for a particular day (i.e. 15, 20, 25 or 30 °C). After incubation, snails were removed and placed back into the aquaria, which were filled daily with new, aerated seawater to simulate the incoming tide after exposure of snails in tide pools during low tide. After removal of the snails, 0.5 mL of 99 % ethanol was added to each tube to fix the cercariae. Subsequently, 1 mL of the water–ethanol mixture was removed, checked for the presence of dead cercariae before being discarded, and then replaced with 99 % ethanol to preserve the samples for later counts under a dissecting microscope. The number of cercariae emerging per snail was analysed with a Generalised linear mixed effect model (GLMM) using the package glmmADMB (Skaug et al. 2012) and a negative binomial error structure in R 2.15.1 (R Development Core Team; www.R-project.org). Day was included as a random factor, and temperature variability treatment, room and their interactions as fixed factors.

Transmission to amphipods

Transmission success of cercariae to second intermediate *P. novizealandiae* amphipods hosts, in terms of parasite abundance acquired by each host, was assessed under exposure to the temperature variability treatments specified above (see "General procedures"). Amphipods were collected from low shore on the Harwood mudflat (Otago Harbour, New Zealand; 45°49′S, 170°39′E). Amphipods at this locality and shore height have relatively low natural infection levels with *M. novaezealandensis* (Bates et al. 2010). Of the amphipods used in this experiment, 6.2 % were naturally infected with on average 1.4 (range 1–3) metacercariae per amphipod (i.e. pre-existing infections), as quantified during dissections based on developmental differences between experimental and non-experimental metacercariae. Amphipods were collected 5 days before the start of the experiment to allow for acclimatisation to the conditions in the controlled temperature rooms. For

each treatment and room, 20 amphipods were distributed into four replicate 2 L plastic containers filled with 1 L of aerated seawater, and provided with a strip of sea lettuce for food and shelter (i.e. a total of eight containers and 160 amphipods per treatment). Each day, amphipods were transferred into smaller plastic jars filled with 250 mL of seawater and incubated at the respective temperature for the particular day (i.e. 15, 20, 25 or 30 °C). During the initial period of this incubation, infected *Z. subcarinatus* snails from Lower Portobello Bay were also incubated for 1 h at 25 °C under constant illumination (five snails in each of nine replicate Petri dishes filled with 6 mL of seawater), which triggers the emergence of fully developed cercariae. Cercariae from all dishes were pooled for a genetic mixture of infective stages to be used in the experiment. One mL of this cercarial mixture was then added to all jars containing amphipods (average age of cercariae 30 min; mean of $\sim$180 cercariae mL^{-1}). The jars were then left in the incubators for the remainder of the 6 h period (i.e. $\sim$4.5 h exposure of amphipods to cercariae), after which amphipods were transferred back into containers filled with 1 L of new, aerated seawater. Dead amphipods were collected twice a day, sexed, measured (body length size classes in mm), and dissected under a dissecting microscope to assess the number of parasites present. At the end of the 12-day period, all remaining amphipods were dissected. On days 4, 6, 8, 10 and 12, direct head counts were carried out to assess the number of amphipods remaining in the experiment. Metacercariae abundance in amphipods was analysed with a GLMM (glmmADMB package; negative binomial error structure), with replicate and day of dissection as random factors. Temperature variability treatment, room, interactions between temperature treatment and room, sex and size class of amphipods were fixed factors. The same model was run for prevalence (infected or not) using a binomial error structure. The survival of amphipods was analysed in Statistica with a Cox proportional hazard model and treatment, replicate, sex, size and number of parasites infecting an amphipod as predictor variables and grouping by room. The amphipod counts were used to illustrate amphipod survival and mortality rates in each treatment.

Results

Cercarial output

Over the 12 days of the experiment, temporal patterns in cercarial emergence closely followed the incubation temperatures for the 15 + 10 °C and 15 + 15 °C treatments (Fig. 1c and d); the higher the temperature the snails were exposed to on a given day, the larger the cercarial

emergence peaks. Emergence patterns for snails exposed daily to the same temperature increase (15 + 5 °C, 15 + hw) fluctuated over time but with no obvious pattern (Fig. 1b and e). The number of cercariae emerging from snails exposed to 15 °C was more-or-less consistently low (Fig. 1a). Temperature treatment had a significant effect on the number of cercariae emerging from infected snail hosts, whereas room or the interactions between temperature treatments and rooms did not (Table 1; Fig. 2) (mean ± standard error (SE) number of cercariae; room 1: 59.80 ± 14.593, room 2: 72.10 ± 12.844). In particular,

Table 1 Results of the generalised linear mixed effect model (negative binomial error structure) analysing the number of *M. novaezealandensis* cercariae emerging from infected *Z. subcarinatus* snail hosts under the different temperature variability treatments

	Estimate	SE	z	p
Intercept	**3.84**	**0.260**	**14.78**	**<0.001**
Treatment (15 + 15 °C)	−0.38	0.323	−1.18	0.236
Treatment (15 + 5 °C)	−0.36	0.287	−1.25	0.211
Treatment (15 + hw)	**1.08**	**0.287**	**3.75**	**<0.001**
Treatment (15 °C)	**−2.53**	**0.304**	**−7.43**	**<0.001**
Room (room 2)	0.39	0.289	1.36	0.175
15 + 15 °C × room 2	0.53	0.413	1.29	0.198
15 + 5 °C × room 2	0.30	0.398	0.76	0.447
15 + hw × room 2	−0.38	0.398	−0.96	0.339
15 °C × room 2	−0.58	0.415	−1.40	0.162

Table showing parameter estimates, standard errors (SE), z and p values. The model included day as a random factor, and temperature treatment × room as fixed factors. Significant predictors are in bold. The 15 + 10 °C treatment is included in the intercept
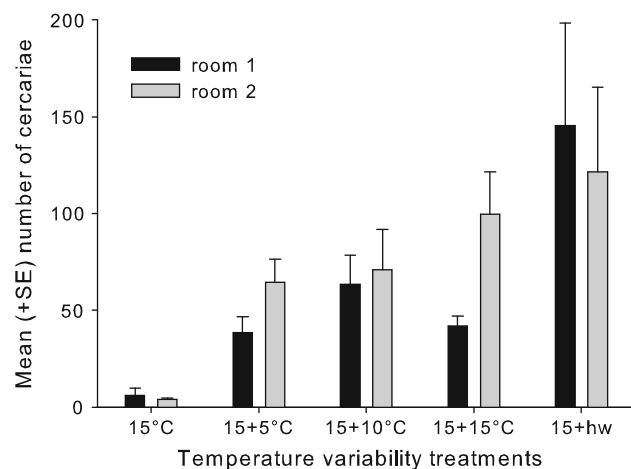
Fig. 2 Cercarial output. Overall mean (+ standard error (SE)) number of cercariae of *M. novaezealandensis* emerging from infected *Z. subcarinatus* snail hosts under different temperature variability treatments (15 °C, 15 + 5 °C daily, 15 + 10 °C every 2nd day, 15 + 15 °C every 3rd day, 15 + heat wave (hw), i.e. 15 + 10 °C daily) across two replicate controlled temperature rooms (n = 5 per treatment and room)

the number of cercariae at 15 °C and those in the heat wave treatment were significantly different from the 15 + 5 °C, 15 + 10 °C and 15 + 15 °C treatments (Table 1; Fig. 2). Compared to the 15 + 5 °C daily treatment, exposing snails to double the thermal energy during daytime incubations (i.e. 15 + 10 °C daily (hw)) resulted, on average, in 2.6 times more cercariae emerging, and twice as many emerging compared to 15 + 10 °C every 2nd day (Fig. 2; overall mean ± SE; 15 °C: 5.06 ± 1.78; 15 + 5 °C: 51.48 ± 8.02; 15 + 10 °C: 67.31 ± 12.08; 15 + 15 °C: 73.92 ± 15.51; 15 + hw: 133.38 ± 32.80). The biggest discrepancy between rooms was found in the 15 + 15 °C treatment (Fig. 2); output in room 1 was similar while output in room 2 was higher than in the two other treatments with equal thermal loading (Fig. 2).
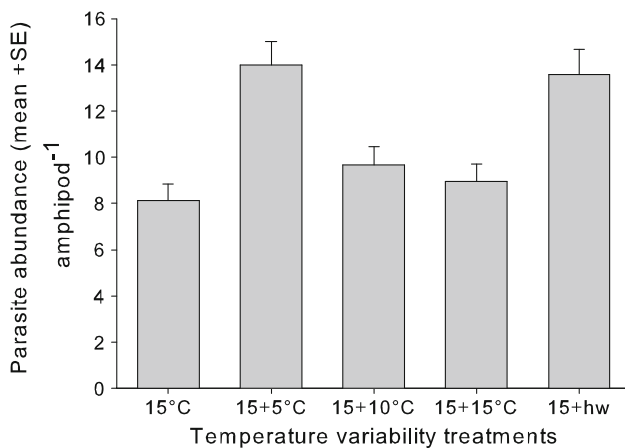
Transmission to amphipods

Parasite abundance in amphipods was significantly affected by the temperature variability treatment and sex of amphipods (Table 2). In particular, amphipods from the treatments daily exposed to a higher temperature level (i.e. 15 + 5 °C and 15 + hw) harboured markedly more parasites compared to those from the other treatments (Fig. 3). Parasite abundance in amphipods from the 15 + 5 °C and the 15 + hw treatments was very similar (15 + 5 °C: 13.99 ± 1.005, 15 + hw: 13.60 ± 1.087). Males harboured on average more parasites than females (males: 11.93 ± 0.608, females: 10.14 ± 0.560), while amphipods from different size classes had comparable numbers of parasites (2.5 mm: 10.69 ± 0.951, 3.0 mm: 11.62 ± 0.736; 3.5 mm: 12.50 ± 0.850, 4.0 mm: 8.06 ± 0.696, 4.5 mm: 9.47 ± 2.638) (Note that largest size classes include only males; maximum size of females is approx. 3.5 mm). Prevalence did not differ between treatments and the model was not better than the respective null model based on Akaike Information Criterion (AIC) values (AIC model: 206.79, AIC null model: 201.46) (prevalence: 94 % (15 °C), 96 % (15 + 5 °C), 96 % (15 + 10 °C), 96 % (15 + 15 °C), 94 % (15 + hw)). A total of 598 amphipods were recovered from this experiment (305 males, 293 females; 15 °C: n = 120, 15 + 5 °C: n = 148, 15 + 10 °C: n = 123, 15 + 15 °C: n = 94, 15 + hw: n = 113).

Over the 12-day period of the experiment, mean mortality rates of amphipods based on direct amphipod counts were 0.93, 1.05, 1.08, 1.26 and 1.41 amphipods day^{-1} in the 15 °C, 15 + 5 °C, 15 + 10 °C, 15 + 15 °C and 15 + hw treatments, respectively. The proportion of amphipods surviving was therefore lowest in amphipods exposed to 30 °C every 3rd day and those exposed to a heat wave, but curves of the proportion of amphipods surviving were overall relatively similar (Fig. 4). Based

Table 2 Results of the generalised linear mixed effect model (negative binomial error structure), analysing parasite abundance in *P. novizealandiae* amphipod hosts from the different temperature variability treatments
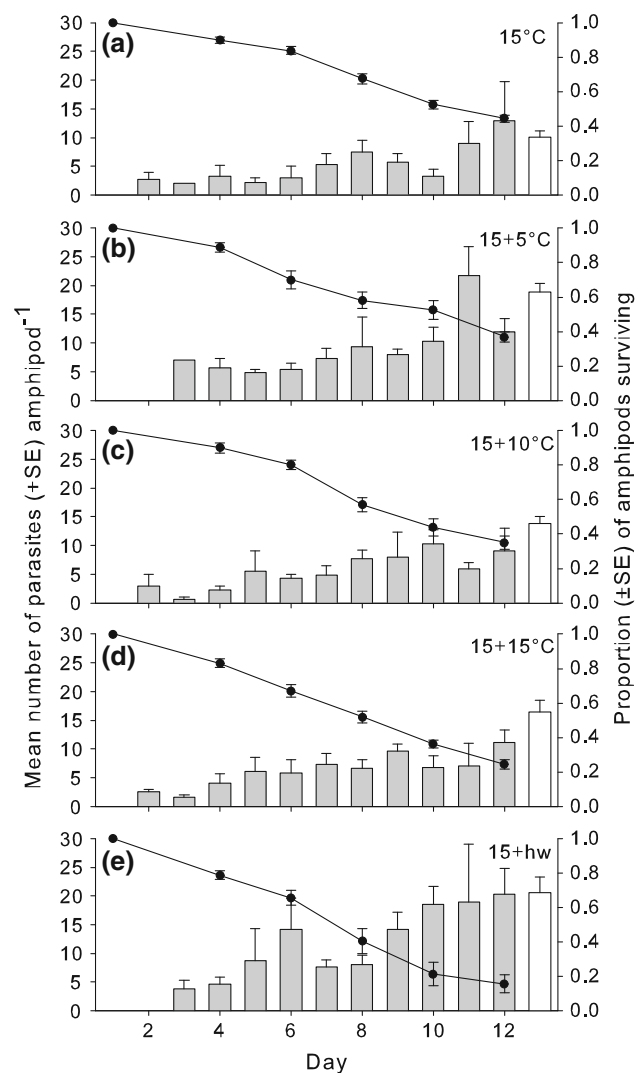
	Estimate	SE	*z*	*p*
Intercept	**1.32**	**0.251**	**5.26**	**<0.001**
Treatment (15 + 15 °C)	0.24	0.14	1.68	0.094
Treatment (15 + 5 °C)	**0.42**	**0.135**	**3.08**	**0.002**
Treatment (15 + hw)	**0.62**	**0.144**	**4.33**	**<0.001**
Treatment (15 °C)	−0.23	0.138	−1.65	0.100
Room (room 2)	0.23	0.136	1.71	0.086
Sex (male)	**0.33**	**0.086**	**3.80**	**<0.001**
Size (3.0)	0.08	0.092	0.82	0.414
Size (3.5)	0.09	0.113	0.78	0.436
Size (4.0)	−0.12	0.138	−0.85	0.394
Size (4.5)	−0.05	0.234	−0.22	0.827
15 + 15 °C × room 2	−0.17	0.211	−0.82	0.410
15 + 5 °C × room 2	−0.22	0.184	−1.20	0.229
15 + hw × room 2	−0.21	0.195	−1.09	0.276
15 °C × room 2	−0.07	0.194	−0.34	0.736

Table showing parameter estimates, standard errors (SE), *z* and *p* values. The model included replicate and day of dissection as random factors, and temperature treatment × room, sex and size class of amphipods as fixed factors. Significant predictors are in bold. The 15 + 10 °C treatment is included in the intercept

Fig. 3 Transmission to amphipods. Mean (+ standard error (SE)) abundance of *M. novaezealandensis* parasites in *P. novizealandiae* amphipods from different temperature variability treatments (15 °C (*n* = 120), 15 + 5 °C daily (*n* = 148), 15 + 10 °C every 2nd day (*n* = 123), 15 + 15 °C every 3rd day (*n* = 94), and 15 + heat wave (hw), i.e. 15 + 10 °C daily; *n* = 113) (from four replicate containers per two replicate rooms)

Fig. 4 Transmission to amphipods and survival under the different temperature variability treatments (**a** 15 °C, **b** 15 + 5 °C daily, **c** 15 + 10 °C every 2nd day, **d** 15 + 15 °C every 3rd day, and **e** 15 + heat wave (hw), i.e. 15 + 10 °C daily). Mean (+ standard error (SE)) number of parasites from dead amphipods dissected throughout the 12-day experiment (*grey bars*), plus from the amphipods dissected afterwards (day 13) (*white bars*). And proportions (± standard error (SE)) of amphipods surviving during the 12-day duration of the experiment (*black filled circle*), as assessed by direct head counts on day 4, 6, 8, 10 and 12

on the survival analysis (including all amphipods recovered during the experiment), the survival of amphipods did indeed not differ significantly between temperature variability treatments. Survival was, however, affected by the size of amphipods, amphipod sex and the number of

parasites infecting an amphipod ($\lambda_2^2 = 131.54$, *df* = 5, *p* < 0.001; Table 3). For those amphipods that died during the experiment (*n* = 356), males (*n* = 202) died on average slightly faster than females (*n* = 154) (mean days until death, males: 7.23 ± 0.173; females: 7.76 ± 0.216), and larger size classes (4.0 and 4.5 mm) faster than smaller size classes (2.5, 3.0 and 3.5 mm) (*n* = 37, 103, 117, 86, 13 for the 2.5, 3.0, 3.5, 4.0 and 4.5 mm size classes).

Table 3 Results of the Cox proportional hazard model assessing the survival of *P. novizealandiae* amphipods

	β	SE	*t*	Wald	*p*
Temperature treatment	0.044	0.042	1.03	1.06	0.304
Replicate	−0.005	0.005	−1.11	1.23	0.267
Amphipod sex	**0.507**	**0.113**	**4.48**	**20.07**	**<0.001**
Amphipod size	**0.108**	**0.047**	**2.30**	**5.28**	**0.022**
Number of parasites	**−0.071**	**0.008**	**−8.91**	**79.42**	**<0.001**

Temperature variability treatment, replicate, sex and size of amphipods and the number of *M. novaezealandensis* parasites infecting an amphipod were predictor variables and the analysis was grouped by room

Discussion

To better account for the highly fluctuating environmental conditions encountered by intertidal organisms in their current and future natural habitats, especially during summer, the present study assessed the effect of diurnal temperature variation on the output of *M. novaezealandensis* cercariae from infected snail hosts and the transmission success of these cercariae to amphipod hosts. Our results show that output and transmission success are differentially affected. For cercarial output, the number of cercariae emerging was similar among the treatments with overall equal thermal energy accumulated above the 15 °C threshold, but was increased substantially in the heat wave treatment. In contrast, daily exposure to increased temperatures resulted in considerably higher infection levels in amphipods compared to treatments with less frequent (but larger) temperature increases, though there was no difference in transmission success between a moderate daily temperature increase (15 + 5 °C daily) and a more extreme increase (15 + 10 °C daily, heat wave). In addition, while temperature regime affected transmission success, the associated amphipod mortality was mainly determined by the number of parasites infecting an amphipod, rather than temperature treatment directly.

Given the short lifespan of cercariae (generally <24 h), their emergence from infected snail hosts needs to be timed such that the probability of transmission is increased (Combes et al. 1994; Galaktionov and Dobrovolskij 2003). In habitats with frequent changes of environmental conditions, as is the case for intertidal and estuarine systems, pulses of cercarial emergence are common (Galaktionov and Dobrovolskij 2003). This synchronised emergence is controlled by environmental factors such as photoperiod, light intensity and water temperature, with often species-specific temperature thresholds for emergence (Fingerut et al. 2003). Such pulses in response to temperature were obvious for *M. novaezealandensis* in the treatments with occasional large temperature peaks, with the number

emerging corresponding to the respective height of temperature increase. This finding is consistent with previous studies suggesting that such bursts of emergence are frequent for *M. novaezealandensis*, as indicated by a high numbers of parasites of the same developmental stage and/or genotype infecting individual amphipod hosts (Fredensborg et al. 2004; Keeney et al. 2007; Studer and Poulin 2012b). The findings are also in agreement with the patterns expected for other intertidal species, for example, *Maritrema subdolum*, where emergence of cercariae is also thought to take place during daytime low tides in tide pools when water warms up (Mouritsen 2002; Galaktionov and Dobrovolskij 2003).

In general, cercarial output from infected snails increases with increasing temperature up to an optimum temperature range, after which there is a decline (Morley and Lewis 2012). This pattern has also been described for *M. novaezealandensis* under constant temperatures (Studer et al. 2010). In the present assessment on variable temperature regimes, the overall output from the treatments with equal thermal loading (i.e. 15 + 5 °C daily, 15 + 10 °C every 2nd day, 15 + 15 °C every 3rd day) were roughly equivalent, that is, the number of cercariae emerging at moderate daily temperature increases equated to the higher number of cercariae emerging at a corresponding occasional large increase in temperature. This indicates that a regular moderate increase in temperature and hence, thermal energy, overall, has a similar effect on cercarial production and hence output, as that of an occasional, correspondingly larger increase from a baseline temperature of 15 °C. However, the regular exposure to double the thermal energy under a heat wave scenario (i.e. 15 + 10 °C daily) resulted in cercarial output increasing 2.6-fold compared to the 15 + 5 °C daily and twofold compared to the 15 + 10 °C every second day treatments. This is most likely due to the corresponding acceleration in development of cercariae under these conditions. However, there were some differences in output patterns between the two controlled temperature rooms; the biggest discrepancy being between the two rooms in the 15 + 15 °C every third day treatment. In one room, numbers were comparable to the other treatments with equal thermal loading, whereas in the second room, numbers were considerably higher. While small differences between the two rooms or the incubators in the rooms could be responsible for this, the result highlights the need for proper replication of warming and heat wave experiments, and also highlights the need to be cautious about drawing conclusions from experiments conducted in single units (Rohr et al. 2011). Furthermore, it remains unclear to what extent putting snails into 1 mL of seawater in a tube reflects actual output of cercariae under natural conditions, as assessing these parameters in nature is logistically extremely difficult

especially in small species such as *M. novaezealandensis*. Overall, however, these results provide further evidence for the strong and immediate effect of temperature on the emergence of cercariae in this trematode species, which is clearly driven by thermal loading (i.e. the combined degree × time exposure). Studies on other trematode species and strains are now imperative to allow for inter- and intraspecific comparisons of responses in order to determine the uniqueness or generality of the pattern observed for *M. novaezealandensis*.

In contrast to the effects on cercarial output, transmission success of *M. novaezealandensis* was increased under regular exposure to moderate increases compared to occasional large increases, but there was no difference in transmission success between daily increase to 20 °C (i.e. 15 + 5 °C) or to 25 °C (i.e. heat wave). A proximate explanation may simply involve the longer total time above 15 °C in those two treatments during which both parasite and amphipod exhibited higher activity levels leading to enhanced contact rates. A possible explanation for the lack of difference between the 15 + 5 °C daily and the heat wave treatment may be that different mechanisms have different relative importance under each of the treatments. For example, differential effects of temperature on infectivity of cercariae, differences in cercariae avoidance behaviour, overall susceptibility or immune system activity of amphipods may all play a role. As lowered immunocompetence has been shown for another crustacean host exposed to an experimental heat wave (Roth et al. 2010), assessing the effects of the temperature variability on immune system functioning would be necessary to elucidate this component. Furthermore, the current assessment also shows that given a high enough number of cercariae in the system, transmission at 15 °C is still relatively efficient (Figs. 3, 4). However, under natural condition, few cercariae would emerge at this temperature (Figs. 1, 2), which indicates that the temperature threshold for cercarial emergence is higher than that of actual transmission.

This result highlights the increased transmission efficiency of this parasite with increasing temperatures above 15 °C, especially during warm periods with temperature regularly reaching ≥20 °C. During winter, the mean and amplitude of temperature in tide pools are far below this temperature level, and transmission pressure and infection levels in amphipods are low (Studer and Poulin 2012b). During summer, the amplitude of temperature variability often exceeds 10 °C, with a mean of around 15 °C and maximum peaks around 30 °C with a corresponding maximum in transmission pressure and highest infection levels in amphipods (Studer and Poulin 2012b); the present findings therefore confirm that *M. novaezealandensis* is best adapted for transmission during warmer months and hence periods of increasing water temperatures during low

tide above a threshold temperature of about 20 °C (Fredensborg et al. 2004; Studer et al. 2010; Studer and Poulin 2012b). This further supports the expectation that increasing winter temperatures and prolonging of the seasonal window with ongoing global warming probably benefits the transmission of *M. novaezealandensis*, and hence may increase the risk of parasite-induced mortality of amphipods. While infections in first intermediate snail hosts are long lived and snails themselves have an estimated lifespan of about 5–6 years (Fredensborg et al. 2005), the seasonal peak in transmission associated with optimal thermal conditions in summer is most crucial for the immediate impact of this parasite on second intermediate amphipod populations (Studer et al. 2013).

While there were slight differences in mortality rates of amphipods from different temperature variability treatments with highest rates in the 15 + 15 °C and 15 + hw treatment, the survival did not differ significantly between treatments based on the survival analysis. This result deviates from a previous study showing that temperature strongly affected amphipod survival under constant temperatures (Studer et al. 2010), and therefore highlights the importance of considering realistic variable temperature conditions. The temperature drop in warmed-up tide pools due to the incoming tide should play an important role in the recovery of ectotherms from the heat exposure during low tide (Somero 2002). Moreover, although parasite abundance in amphipods (i.e. parasite abundance in all amphipods recovered) was affected by temperature treatment, being highest in the 15 + 5 °C and the 15 + hw treatments (Table 2; Fig. 3), this did not translate directly into the expected survival patterns with highest mortality in these two treatments (see Fig. 4). Instead, when their respective effects are corrected statistically, the number of parasites infecting an amphipod but not the temperature treatment itself emerged as a significant predictor for the survival of amphipods (Table 3). Hence, actual infection levels may be more important than immediate thermal conditions experienced by the amphipod hosts in intertidal systems, granted these do not exceed extreme levels for prolonged periods of time (Studer et al. 2010).

While our study provides an important step towards increased realism of climate warming experiments assessing aquatic and especially marine host–parasite interactions, further considerations can be envisaged for future studies. Firstly, to further increase the ecological realism of laboratory assessments and their usefulness for predictive frameworks in the context of climate change, the inclusion of even more ecologically relevant conditions (e.g. incorporating night time low tide conditions, adding infected snails as a source of cercariae rather than adding standardised doses of cercariae), stochastic temperature treatments and more extreme variable temperature events

according to predictions (see Thompson et al. 2013) are necessary. For example, the present study could have included a 15 + 15 °C heat wave treatment (i.e. daily exposures to 30 °C). As 30 °C is beyond optimum for long-term cercarial production as well as for amphipod survival (Studer et al. 2010), the inclusion of such a treatment may have revealed a more complete picture of variable temperature effects on transmission. Secondly, changes in other environmental factors besides temperature, such as pH, solar radiation, and salinity have been shown to impose stress on marine and freshwater invertebrates, impact their immunity (Le Moullac and Haffner 2000; Mydlarz et al. 2006), and affect the transmission of parasites (Pietrock and Marcogliese 2003), including the study system used here (Studer and Poulin 2012a; Studer et al. 2012). The inclusion of other abiotic factors is necessary to better account for natural complexity and is required as multifactorial designs to assess interactive effects of multiple stressors (Boyd and Hutchins 2012; Wernberg et al. 2012). This extends to biotic factors (Thieltges et al. 2008; Johnson and Thieltges 2010) and the complex interplay of biodiversity, community-context and disease dynamics (e.g. Keesing et al. 2010; Raffel et al. 2010; Orlofske et al. 2012).

In conclusion, as variability in environmental conditions is the norm and not the exception, especially for biotic interactions in intertidal ecosystems, the present results highlight differential effects of variable temperatures on distinct aspects of trematode transmission and their potential consequences for the future direction of this host–parasite interaction. During more variable conditions (i.e. only occasional, large temperature peaks), the number of *M. novaezealandensis* cercariae emerging reflects the height of the peak with no major impact on transmission success. During periods of regular moderate increases to 20 °C, cercarial emergence fluctuates at a relatively low level, but transmission success is increased. At regular increases to 25 °C, transmission success is not further increased, but the number of cercariae emerging into the system is substantially enhanced. This implies an increased risk of parasite-induced mortality under the heat wave scenario. While the variable temperature conditions may offer amphipods time to recover from heat exposure, the number of parasites infecting amphipods seems to be a main driver of amphipod mortality. This mortality risk is highest under a current, realistic heat wave scenario and is based on both transmission pressure and success resulting from these conditions.

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