

Non-linear effects of ocean acidification on the transmission of a marine intertidal parasite

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ABSTRACT: High levels of atmospheric carbon dioxide are driving the acidification of the world's oceans, with considerable and generally negative impacts on the physiology, performance and survival of marine organisms. The differential and often idiosyncratic responses shown by different taxa suggest that interspecific interactions may be drastically affected by ocean acidification. Here, we quantified the transmission success of the trematode *Maritrema novaezealandense* to its intertidal amphipod intermediate host *Paracalliope novizealandiae*, as well as the host's survival, under acidified conditions. We used a custom-built system to simulate ocean acidification with 3 different seawater treatments: 8.1 pH, corresponding to current average ocean surface waters, as well as 7.6 and 7.4 pH, the levels predicted for the years 2100 and 2300, respectively. In 2 separate experiments, parasite transmission success tended to peak in the most acidified conditions (7.4 pH), although this was only statistically significant when a wide range of infection doses was used. Because the survival of the parasite's transmission stages decreases with decreasing pH, this pattern suggests that host susceptibility remains unaffected at 7.6 pH and is only compromised with further acidification. Amphipod mortality was not affected by pH levels, though it tended to be lowest at 7.6 pH, where the longevity of parasite transmission stages was reduced but host susceptibility was unaffected. These results suggest that ocean acidification could change the dynamics of parasite transmission with possible consequences for intertidal community structure, and emphasise the need to consider the transmission and severity of marine parasites and diseases in ocean acidification research.

KEY WORDS: Host survival · Infection success · Intertidal zone · Ocean acidification · Parasite transmission · *Maritrema novaezealandense* · Trematodes

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INTRODUCTION

Ocean acidification (OA) and decreased carbonate ion concentration are a direct result of elevated atmospheric carbon dioxide, and together represent a major threat to the present structure and function of marine ecosystems (Raven et al. 2005, Fabry et al. 2008, Doney et al. 2009). At the individual level, one of the main physiological impacts of OA on marine organisms is the increased metabolic cost associated with maintaining internal acid/base homeostasis (Pörtner et al. 2004), which is particularly challenging for some invertebrate taxa (Pörtner 2008). As a

consequence, many marine organisms experience reduced growth, fecundity and/or survival under simulated OA conditions (Kroeker et al. 2010). These negative effects are not universal, however, and are often contingent upon the species investigated or other experimental conditions (Kroeker et al. 2013). The differential responses of various taxa to acidified water suggest that OA might alter the dynamics and outcome of interspecific interactions. For example, reduced seawater pH can change predator–prey interactions by affecting predator performance, impairing prey defences, or both (Bibby et al. 2007, Allan et al. 2013, Kroeker et al. 2013).

Surprisingly, host–parasite interactions have received very little attention in the context of OA, despite several lines of evidence suggesting that OA may affect either the abundance of parasites or their impacts on hosts (MacLeod & Poulin 2012, 2015). First, changes to parasite assemblages and infection levels in fish have previously been linked to the effect of acid precipitation on freshwater ecosystems (Cone et al. 1993, Marcogliese & Cone 1996); second, the free-swimming transmission stages of many aquatic parasites are generally very sensitive to abiotic conditions such as pH (Pietroock & Marcogliese 2003); and third, OA can cause immunosuppression in some marine host organisms (Bibby et al. 2008). Given the diversity and ubiquity of parasites in marine ecosystems (Mouritsen & Poulin 2002) and the important ways in which abiotic stressors can modify the effects of parasitism (Lafferty & Kuris 1999), we need empirical tests of the effects of OA on host–parasite interactions for a fuller understanding of the impacts of acidification on marine communities.

Here, we used the trematode *Maritrema novaezealandense* (formerly *M. novaezealandensis*) as a model species to quantify the influence of acidified seawater on both transmission success and impacts of infection on host survival. This trematode is widespread in New Zealand coastal ecosystems, where it has measurable impacts on the populations of its invertebrate hosts (Fredensborg et al. 2004, 2005). It has also been previously used as a model parasite to investigate the influence of other abiotic variables related to climate change on host–parasite interactions (Studer et al. 2010, 2012, Studer & Poulin 2012). Like many other trematodes, *M. novaezealandense* has a 3-host life cycle. Adult worms live in the gut of shorebirds such as gulls (*Larus* spp.), where the eggs they produce are released in host feces. Eggs hatch after accidental ingestion by their first intermediate host, the mudsnail *Zeacumantus subcarinatus*, a common grazer in New Zealand intertidal habitats. Asexual multiplication within the snail host over months and years leads to the production of large numbers of free-swimming infective stages, known as cercariae, which emerge from the snail to seek the second intermediate host in the life cycle. Our study focused on the amphipod *Paracalliope novizealandiae*, which is numerically the most abundant of the many small crustacean species that are suitable as second intermediate hosts for *M. novaezealandense* at our study site (Koehler & Poulin 2010). After penetrating a crustacean host, the cercariae encyst and await eventual ingestion by a suitable avian host.

In terms of size, cercariae are somewhat similar to the lecithotrophic larvae of marine invertebrates. The latter are generally very sensitive to reduced pH, which typically impairs their development and decreases their survival (e.g. Dupont et al. 2008, Clark et al. 2009, Brennand et al. 2010, Byrne 2011). However, unlike lecithotrophic larvae, trematode cercariae are very short-lived and do not undergo development; thus, we may not necessarily expect similar effects of OA on cercariae. Laboratory exposure of cercariae to acidified seawater typically results in shorter lifespan, though to various extent depending on the species (Shostak 1993, Koprivnikar et al. 2010, MacLeod & Poulin 2015). Of 4 trematode species tested by MacLeod & Poulin (2015), *M. novaezealandense* showed the greatest reduction in survival when exposed to pH values predicted to occur in surface coastal waters over the next several decades. However, although it is widely used to quantify parasite fitness in the face of abiotic stressors (Pietroock & Marcogliese 2003), cercarial survival may be a poor proxy for transmission success. It is only one component in a complex process, and may not represent the eventual outcome. For example, shorter cercarial survival under stressful conditions may be compensated by greater swimming activity or infectivity to the target host, with unchanged or even increased transmission success as a net result. A fuller understanding of the effect of OA on host–parasite interactions requires an experimental approach that allows completion of the infection process and the subsequent assessment of fitness impacts on the host.

The objectives of the present study were to test the effects of acidified seawater on the transmission success of the trematode *M. novaezealandense* to its amphipod host, and the subsequent fitness consequences for the host, measured as survival. We used a custom-designed OA simulation system to create treatment conditions corresponding to current average ocean surface waters (8.1 pH), and those predicted by the Intergovernmental Panel on Climate Change (IPCC 2014) for the years 2100 (7.6 pH) and 2300 (7.4 pH). At the intertidal site where our study organisms were collected, pH values experienced by amphipods in tide pools regularly drop to 7.6 (MacLeod 2015). Therefore, amphipods may be adapted to tolerate moderate reductions in pH by increasing metabolic activity to meet the costs of maintaining internal acid/base homeostasis, but they may enter metabolic depression at extreme pH values (e.g. 7.4 pH), which are beyond the natural range encountered (MacLeod 2015). With cercarial lifespan

decreasing gradually with decreasing pH (MacLeod & Poulin 2015) and host susceptibility likely to follow a more complex pattern, we predicted a non-linear response of the 2 variables investigated here—transmission success and host survival—as a function of increasing acidification.

MATERIALS AND METHODS

Field collections and general procedures

Amphipods *Paracalliope novizealandiae* and snails *Zeacumantus subcarinatus* were collected between November 2013 and July 2014, depending on whether they were used in Expts 1 or 2 (see below). Amphipods were collected from Hoopers Inlet, on the Otago Peninsula (South Island, New Zealand), using a hand net (mesh size 250 μm) at low tide when the bay consisted of a mixture of both exposed mud and areas of shallow surface water. Previous research has demonstrated that the Hoopers Inlet amphipod population harbours no metazoan parasites (Fredensborg et al. 2004, Bryan-Walker et al. 2007, Studer et al. 2010); this was confirmed by dissections of a subsample ($n > 100$) of amphipods collected for the present study. Amphipods were placed in seawater from the collection site and transported to the laboratory where they were divided equally into 3 groups of approximately 100 individuals. Each group was housed in a chamber immersed in a separate tank containing seawater at 7.4, 7.6 or 8.1 pH (see 'OA simulation system' below). After 2 d, amphipods were selected from each tank for use in the experiments. Only medium-sized amphipods (1 to 2.5 mm) of both sexes were used, in order to exclude young or senescent individuals that may have a lower ability to cope with infection. Fresh groups of amphipods were collected in the field for each infection series.

Snails (1 to 2 cm shell length) were collected from Lower Portobello Bay in Otago Harbour (South Island, New Zealand) at low tide by hand dredging the muddy surface layer. Lower Portobello Bay is a known site of high (>50%) prevalence of *Maritrema novaezealandense* infections (Fredensborg et al. 2005). In the laboratory, snails were placed individually in wells of tissue culture plates, covered in seawater (salinity: 35 ppt), and infected individuals were identified by inducing cercariae to emerge during a 2 h incubation at 25°C. *M. novaezealandense* infected snails were divided into 3 groups of 30 individuals and transferred into housing chambers in

each of the 3 tanks at either 7.4, 7.6 or 8.1 pH. Snails were fed ad libitum with the seaweed *Ulva lactuca* (collected from Lower Portobello Bay) while they were acclimated to their given pH for 2 wk before experimental use. A haphazardly chosen subset of 10 infected snails was used in each infection series (see below) as a source of cercariae.

OA simulation system

The experimental system was custom-built to simulate OA conditions (see MacLeod et al. 2015 for full description). It consisted of 3 identical and independent tank systems, each containing 80 l of seawater maintained at 12°C and under a 12 h light:12 h dark photoperiod. Each tank was set at a different pH corresponding either to current average ocean surface waters (8.1 pH), that predicted for the year 2100 (7.6 pH) or that predicted for the year 2300 (7.4 pH) (IPCC 2014). Seawater (salinity: 35 ppt) was obtained from the Portobello Marine Research Station situated in Otago Harbour and was filtered through sand at high pressure before use. Aqua One Canister Filter (Aquis 700) pumps were used to filter the seawater and circulate it through a Hailea chiller (model HC-150 A) at 400 l h⁻¹ and then back into each tank. Food-grade CO₂ was pumped into the water of each tank through a perforated plastic tube to allow for equal dispersal throughout the tank. A TUNZE glass electrode was immersed in each tank, and connected to a TUNZE pH control system which regulated CO₂ inflow to maintain the target seawater pH. The pH meters were calibrated regularly using 2 saltwater buffers to maintain electrode accuracy. The seawater in each tank was changed regularly, and kept well oxygenated (>95% saturation throughout all experiments) with Aqua One 9500 air pumps. Conditions were identical in all tanks, except for pH, and previous experiments had ruled out the possibility of tank effects other than pH differences by switching pH levels within tanks and running a temporal series of replicates (MacLeod & Poulin 2015). In addition, at the start of each of our 2 experiments, pH levels were re-assigned randomly among tanks.

Earlier studies using this OA simulation system with similar snail densities have validated its ability to maintain appropriate seawater chemical properties. In those studies, regular chemical analysis of seawater samples from the tanks confirmed that tank conditions (salinity, total alkalinity, dissolved inorganic carbon, etc.) remained constant and reflected the carbonate chemistry expected of nor-

mal and acidified seawater (see MacLeod et al. 2015, MacLeod & Poulin 2015).

Each tank housed a 2 l plastic chamber for acclimating amphipods, and another identical chamber for acclimating snails. The chambers had mesh sides (mesh size 250 μm) to allow seawater to flow through. In addition, there were 2 floating platforms per tank, each platform containing 16 wells (3.53 cm^3) with mesh bottoms (mesh size 250 μm) that housed individual amphipods after exposure to parasites.

Expt 1

To determine the effect of pH on parasite transmission and host survival, 10 infected snails from each of the 3 pH treatments were haphazardly selected as a source of parasites. Each group was placed in a Petri dish (8.6 cm diameter) and covered with the seawater to which they had acclimated. To induce the emergence of cercariae, Petri dishes were placed in an incubator at 25°C under full light for 2 h; preliminary tests showed that such brief increases of temperature did not affect seawater pH in the Petri dishes. Each snail may release several hundred cercariae under those conditions. Snails were then removed and returned to their housing chamber, and water in each dish was allowed to quickly return to room temperature (approx. 20°C). Water in each dish was gently stirred to mix the cercariae before each dose was taken by pipette.

Amphipods were housed individually in wells within a 96-well plate in 75 μl of seawater at the pH to which they had been acclimated, and left to reach room temperature (approx. 20°C). A parasite dose of 25 μl was taken by pipette and added to each well containing an amphipod. For each of the 3 pH treatments, 32 amphipods were used in a given infection series; this was repeated 10 times over a few weeks, for a total of 320 amphipods infected in each pH treatment. In each infection series, for every 2 doses taken from each Petri dish, a single dose was put aside for its cercariae to be counted later so that an average dose level of cercariae could be calculated for each infection series at each of the 3 pH treatments.

After addition of the cercariae, each well plate was covered and a 2 h infection period was allowed. Here too, earlier tests showed that seawater pH did not change during this brief infection period. Amphipods were then removed from their wells and housed in individual floating wells on each of the platforms (see description of the OA simulation system above) in the

pH tank from which they had been taken. After allowing 2 d for cercarial growth, amphipods were dissected under an Olympus SZ30 dissecting microscope. Amphipods that died during the 2 d were not dissected (as they decomposed too rapidly to allow dissection and parasite recovery); however, the number of dead individuals was recorded.

Expt 2

To determine the effect of pH on parasite transmission and host survival using a broader range of parasite doses, we performed a second experiment identical to the one above except for the dose. In this second experiment, we used 2 doses: a low dose consisting of 25 μl of the cercarial mixture and a high dose consisting of 50 μl . In each infection series, half of the 96 amphipods (16 per pH level) were given the low dose and the other half was given the high dose. Again, this was repeated 10 times over a few weeks, for a total of 160 amphipods infected with a low dose and 160 with a high dose in each pH treatment. As in Expt 1, some doses were put aside for cercarial counts, to calculate an average dose level of cercariae for each infection series in each of the 3 pH treatments.

Statistical analysis

R v.3.1.0 (R Development Core Team 2014) was used for all statistical analyses. A generalised linear mixed effects model with a negative binomial error structure ('glmm ADMB' package; Fournier et al. 2012) was used to test the impact of pH on infection success in both experiments. In this model, the number of parasites found in each amphipod was the response variable; the fixed effects were pH and dose average (for each infection series), while infection series was included as a random factor. For the analyses of the effect of pH on amphipod mortality, a generalised linear mixed model with a binomial error structure was used for both experiments ('lme4' package; Bates et al. 2014). In this model, whether or not an amphipod survived for 2 d was the (binary) response variable; the fixed effects were again pH and dose average, with infection series included as a random factor. In each case, we considered 2 models: one including the main effects only (pH and dose average), and one with the interaction between these main effects also included. Model comparison for all analyses used Akaike's information criterion (AIC) to

identify the model of best fit (Akaike 1974), which lead to the omission of the interaction term from all models ($\Delta AIC \geq 4$ in all cases). Although raw numbers of parasites per amphipod were used within models, for illustrative purposes relative infection success is used. Relative infection success was calculated by dividing the number of parasites per amphipod by the average dose used for a particular infection series and pH level.

RESULTS

Overall, both experiments showed the same trend of increased parasite transmission in the most acidified conditions (7.4 pH), although this was only statistically significant in Expt 2 where a wider range of doses was used. In contrast, amphipod mortality was not affected by pH in either experiment.

Expt 1

Of the 960 amphipods used in this experiment, 277 were successfully infected, 447 were not infected and 236 died before they could be dissected. Average cercarial doses to which the amphipods were exposed varied among infection series because of the unpredictable nature of cercarial output from snails, but were fairly similar across pH treatments (Table 1). Within infection series, doses were fairly consistent; among those set aside for counting, the coefficient of variation (standard deviation divided by the mean) in the number of cercariae per dose averaged 0.62, and was less than 0.8 in 85% of series.

Among surviving amphipods, the number of parasites per amphipod ranged from 0 to 36 (overall average: ~ 1 amphipod⁻¹), with the vast majority of infected individuals harbouring 1 to 3 parasites. Relative to the cercarial dose used in a particular infec-

Table 1. Mean (range) number of *Maritrema novaezealandense* cercariae per dose in the 2 infection experiments. Estimates come from 10 infection series per pH level per experiment, though in Expt 2 both low and high dose volumes were used

Expt	pH treatment level		
	7.4	7.6	8.1
1	7.3 (0.75–21.31)	6.3 (1.06–16.00)	6.8 (0.94–17.89)
2 (low dose)	13.3 (1.12–28.00)	11.9 (0.62–21.50)	15.0 (0.75–25.12)
2 (high dose)	21.1 (3.00–39.12)	20.2 (1.50–36.87)	21.1 (2.75–34.87)
2 (overall)	17.1 (1.12–39.12)	16.1 (0.62–36.87)	18.1 (0.75–34.87)

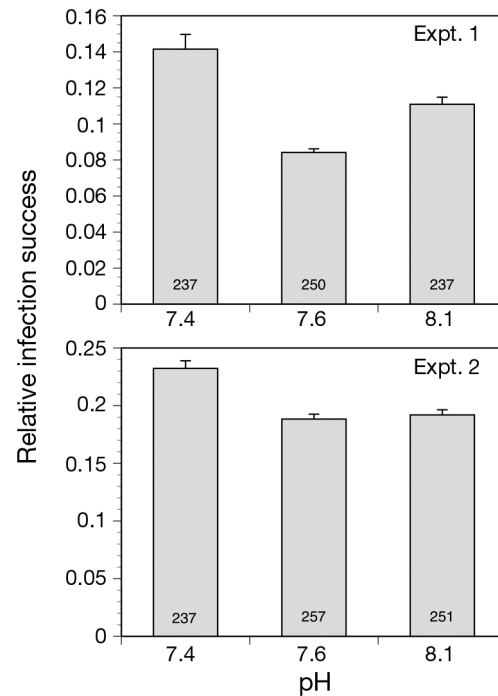


Fig. 1. Average (\pm SE) relative infection success (i.e. no. parasites amphipod⁻¹/average dose given) of *Maritrema novaezealandense* cercariae for each of the 3 pH treatments in both experiments. Numbers of amphipods dissected in each treatment are shown at the base of each bar

tion series, the parasites tended to achieve higher infection success at 7.4 pH (Fig. 1). However, there was no significant effect of pH treatment on the number of parasites acquired per amphipod (Table 2). In contrast, the average cercarial dose used had a significant positive impact on infection (Table 2). Infection series (random factor) accounted for 16% of the variance unexplained by the fixed effects.

Roughly similar numbers of amphipods died per infection series prior to the end of the experiment in all treatments (Fig. 2). The generalised linear mixed model confirmed that there were no significant effects of pH treatments or dose level on amphipod mortality (Table 3).

Expt 2

In Expt 2, 631 amphipods were successfully infected, 114 were not infected and 215 died before the end of the experiment. Cercarial numbers overlapped between the high (50 μ l) and low (25 μ l) doses to which the amphipods were exposed. Nevertheless, the use of high

Table 2. Results of generalised linear mixed models with negative binomial error structure showing the effect of pH treatment and dose on infection level (no. *Maritrema novaezealandense* cercariae amphipod⁻¹) in the 2 infection experiments. Significant effects are shown in **bold**

	Estimate	SE	Z-value	p-value
Expt 1				
Intercept (incl. 7.4 pH)	-1.483	0.248	-5.98	<0.0001
7.6 pH	-0.268	0.152	-1.76	0.078
8.1 pH	-0.181	0.151	-1.20	0.231
Dose average	0.160	0.024	6.69	<0.0001
Expt 2				
Intercept (incl. 7.4 pH)	0.135	0.157	0.86	0.389
7.6 pH	-0.205	0.081	-2.55	0.0109
8.1 pH	-0.300	0.082	-3.67	0.0002
Dose average	0.075	0.006	12.95	<0.0001

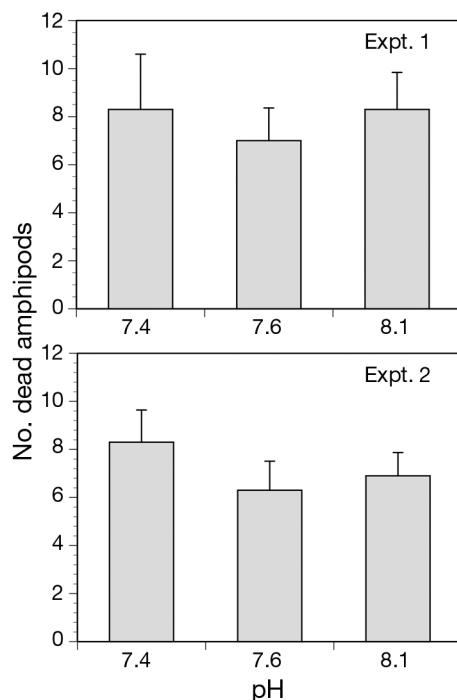


Fig. 2. Average (\pm SE) number of amphipods per infection series that died following exposure to *Maritrema novaezealandense* cercariae in each of the 3 pH treatments in both experiments. All treatments involved 10 series each including 32 amphipods

and low doses resulted in a much greater range of doses than in Expt 1; these were used as a fixed factor in the analysis. As in Expt 1, average cercarial doses varied among infection series but were fairly similar across pH treatments (Table 1). Also as in Expt 1, doses were consistent within infection series; using doses set aside for counting, the coefficient of variation in the number of cercariae per dose aver-

Table 3. Results of binomial generalised linear mixed models showing the effect of pH treatment and dose on amphipod mortality in the 2 infection experiments. Significant effects are shown in **bold**

	Estimate	SE	Z-value	p-value
Expt 1				
Intercept (incl. 7.4 pH)	-1.364	0.424	-3.217	0.0013
7.6 pH	-0.248	0.199	-1.247	0.2126
8.1 pH	-0.005	0.193	-0.025	0.9802
Dose average	0.012	0.036	0.326	0.7443
Expt 2				
Intercept (incl. 7.4 pH)	-1.826	0.410	-4.448	<0.0001
7.6 pH	-0.335	0.195	-1.722	0.0851
8.1 pH	-0.338	0.193	-1.748	0.0804
Dose average	0.041	0.018	2.333	0.0196

aged only 0.35, and was less than 0.7 in 90% of series.

The number of parasites per surviving amphipod ranged from 0 to 37, but the average across both low and high doses was higher than in the first experiment (~ 4.4 amphipod⁻¹). As in Expt 1, relative to the cercarial dose used in a particular infection series, the parasites achieved higher infection success at 7.4 pH than in other treatments (Fig. 1). In this experiment, though, the effect of pH treatment on the number of parasites acquired per amphipod was significant, with infection success at 7.4 pH significantly higher than in the other 2 treatments (Table 2). The average cercarial dose used also had a significant impact on infection (Table 2); this was a strong and unsurprising effect, with higher doses resulting in higher numbers of parasites acquired by amphipods (Fig. 3). Infection series (random factor) accounted for 22% of the variance unexplained by the fixed effects.

As in Expt 1, similar numbers of amphipods died per infection series in all treatments (Fig. 2), and again pH had no significant effect on mortality (Table 3). In contrast, dose level had a significant effect on mortality, with amphipods exposed to higher doses incurring greater mortality (Table 3).

DISCUSSION

With OA having generally negative impacts on many different marine organisms and ecological processes (Kroeker et al. 2010, 2013, Gaylord et al. 2015), it is becoming imperative to understand how these effects will alter interspecific interactions, in particular host–parasite interactions which have to

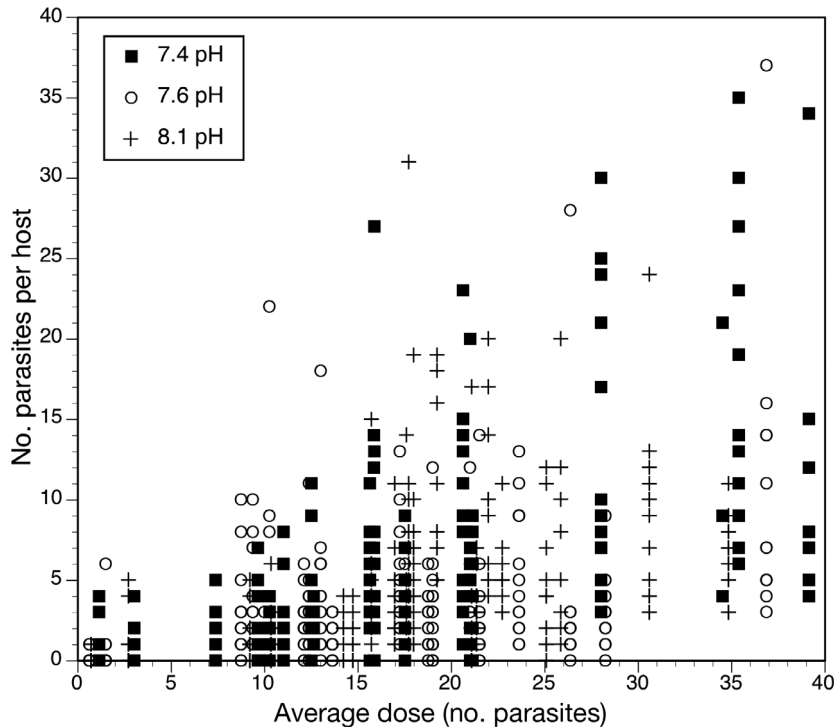


Fig. 3. Relationship between the number of *Maritrema novaezealandense* cercariae acquired per amphipod and the dose given (average no. parasites dose⁻¹ in a given infection series) in Expt 2. Different symbols are used for each of the 3 pH treatments; data shown are for a total of 745 amphipods dissected at the end of the experiment

date been neglected in the context of OA (MacLeod & Poulin 2012). Here, both of our experiments suggest that the transmission success of the trematode *Maritrema novaezealandense* responded non-linearly to decreasing pH, reaching its highest value in the most acidified treatment (7.4 pH), although this was only statistically significant in the experiment using a broader range of doses. The non-significant interaction between pH level and dose was excluded from our models, and the fact that the effect of pH only became significant in Expt 2 may be due to the greater absolute magnitude of the differences in numbers of parasites per amphipods observed when using high and low doses. Regardless, the non-linear response of infection success to decreasing pH hints at differential impacts of acidification on hosts and parasites.

An earlier study demonstrated that the longevity of *M. novaezealandense* cercariae, as well as cercariae of other trematode species, is reduced under acidified conditions (MacLeod & Poulin 2015). Cercarial longevity is not necessarily a good proxy for the ability of cercariae to infect their target hosts; nevertheless, the effect of pH on longevity suggests a gradual decrease of cercarial performance with increasing acidification.

In the natural habitat, pH values experienced by amphipods regularly reach 7.6 (MacLeod 2015). Amphipods may therefore be capable of tolerating moderate reductions in pH, by increasing metabolic activity to meet the costs of maintaining internal acid/base homeostasis (Whiteley 2011). This may explain why at 7.6 pH, with impaired parasites and hosts still performing normally, infection success was overall slightly (but not significantly) lower than under normal 8.1 pH conditions. However, with a further drop in pH to 7.4, below the natural range encountered (MacLeod 2015), amphipods may have entered metabolic depression. Their behavioural and other defences against cercariae may have been compromised, more than compensating for the lower performance of cercariae and allowing the latter to achieve high infection success.

Similar complex, non-linear relationships have been observed between transmission success of *M. novaezealandense* cercariae and other abiotic stressors. For example, Studer et al. (2012) found no apparent impact on transmission success under elevated ultraviolet radiation, even though cercarial survival was reduced. In addition, Studer et al. (2010) observed increased cercarial release from snail hosts and greater transmission success to amphipods, along with increased amphipod mortality, at moderately high temperatures (20 to 25°C), followed by a drop in cercarial transmission success at higher temperatures (>25°C). Finally, Studer & Poulin (2012) found that cercariae were more tolerant of high salinity than their amphipod host, resulting in more complex responses of infection success to increasing salinities than those expected from the individual tolerances of either host or parasite.

Predicting the impact of environmental changes on host–parasite interactions cannot be made solely based on measurements of effects on the parasites. In our system, the amphipod *Paracalliope novizealandiae*, the most abundant host of the trematode *M. novaezealandense*, responds differently to abiotic stressors than its parasite, and often is the more vulnerable of the 2 antagonists. This amphipod is not the only crustacean second intermediate host

of *M. novaezealandense*; several other species of amphipods, isopods, crabs and shrimps are also used by this trematode (Koehler & Poulin 2010). The different life history traits of these various taxa make them variously susceptible to the direct physiological effects of OA (Whiteley 2011). Therefore, the patterns observed here with respect to changes in infection susceptibility as a function of increasing acidification in one amphipod species may not apply to other crustacean hosts.

The other outcome of infection recorded in our experiments was amphipod mortality, which was not significantly impacted by pH level in either experiment. Although the impact of OA on crustacean survival is generally negative (see Dupont & Thorndyke 2009, Kroeker et al. 2010), some crustaceans are capable of controlling their extracellular pH through active ion transport and thus can be quite tolerant of acidification for short periods of time (Whiteley 2011). The only factor that affected amphipod mortality in our study was the cercarial dose used (only in Expt 2). The actual effect may have been stronger than the one detected, since amphipods that died prior to the completion of the experiment and that could not be dissected may have harboured high intensities of infection. Nevertheless, this result supports earlier findings demonstrating intensity-dependent mortality of amphipods infected by *M. novaezealandense* (Fredensborg et al. 2004, Bates et al. 2010). The greater the dose, the more cercariae successfully infected their host. Every separate cercaria penetrating an amphipod can have additive effects on host survivorship. Penetration holes in the host's exoskeleton may lead to loss of haemolymph resulting in both anaemia and osmotic stress, and migration of cercariae within the host body can cause damage to tissues and organs (Fredensborg et al. 2004). These mechanisms can account for host death within 2 d post-infection. However, the greater susceptibility of amphipods to infection at 7.4 pH did not cause significantly reduced survival in that treatment, although penetration of the exoskeleton should allow acidified seawater to enter the host's body, exacerbating internal acidosis.

Our study focused on a simplified version of the interaction between *M. novaezealandense* and its hosts. In nature, the life cycle comprises other host species, either other crustaceans used as second intermediate hosts, or shorebirds used as definitive hosts. We cannot predict how OA would affect the abundance or susceptibility to infection of these other hosts. In addition, cercarial transmission under natural conditions is also affected by the consump-

tion of cercariae by predators. For example, the anemone *Anthopleura aureoradiata* is a known predator of *M. novaezealandense* cercariae (Hopper et al. 2008), and anemones are predicted to benefit from future acidified conditions with increased growth and abundance (Suggett et al. 2012). The differential responses of the many other players involved in the *M. novaezealandense* life cycle make it impossible to predict the net outcome of OA for the local abundance and effects of this parasite in intertidal ecosystems.

Finally, we acknowledge that our experimental design is not ideal, with all individuals in one pH treatment maintained in the same tank. However, we believe tank effects can be ruled out because (1) pH levels were assigned randomly and independently to various tanks at the start of each of our 2 experiments; (2) studies conducted in the same system only months before the present experiments swapped pH levels and relocated experimental subjects twice per trial and found no tank effects (MacLeod & Poulin 2015); and (3) physico-chemical conditions were monitored throughout and found to be identical among tanks. Nevertheless, as previously pointed out (Rohr et al. 2011), the ideal design for this sort of study would involve proper tank-level replication.

In summary, our results indicate that OA is likely to change the dynamics of trematode transmission from first to second intermediate hosts. It is already clear that OA can have a range of direct and indirect effects on the diversity and structure of benthic intertidal communities (Hale et al. 2011). Because the effect of OA on the susceptibility to infection of the different hosts used by trematodes at a particular life stage is likely to be species-specific, we may also expect the parasite-driven structuring of intertidal communities to change under increasing acidification. Our findings add to an earlier call (MacLeod & Poulin 2012) to broaden the current OA research agenda to include its potential impact on the geographic distribution, local prevalence, and severity of marine parasites and diseases.

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LITERATURE CITED

- Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Automat Contr* 19:716–723
Allan BJM, Domenici P, McCormick MI, Watson SA, Mun-

- day PL (2013) Elevated CO₂ affects predator-prey interactions through altered performance. *PLoS ONE* 8: e58520
- Bates AE, Poulin R, Lamare MD (2010) Spatial variation in parasite-induced mortality in an amphipod: shore height versus exposure history. *Oecologia* 163:651–659
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7 <http://CRAN.R-project.org/package=lme4>
- Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol Lett* 3: 699–701
- Bibby R, Widdicombe S, Parry H, Spicer J, Pipe R (2008) Effects of ocean acidification on the immune response of the blue mussel *Mytilus edulis*. *Aquat Biol* 2:67–74
- Brennand HS, Soars N, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *PLoS ONE* 5:e11372
- Bryan-Walker K, Leung TLF, Poulin R (2007) Local adaptation of immunity against a trematode parasite in marine amphipod populations. *Mar Biol* 152:687–695
- Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanogr Mar Biol Annu Rev* 49:1–42
- Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. *Mar Biol* 156:1125–1137
- Cone DK, Marcogliese DJ, Watt WD (1993) Metazoan parasite communities of yellow eels (*Anguilla rostrata*) in acidic and limed rivers of Nova Scotia. *Can J Zool* 71: 177–184
- Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO₂ problem. *Annu Rev Mar Sci* 1:169–192
- Dupont S, Thorndyke MC (2009) Impact of CO₂-driven ocean acidification on invertebrates early life-history — what we know, what we need to know and what we can do. *Biogeosci Discuss* 6:3109–3131
- Dupont S, Havenhand J, Thorndyke W, Peck LS, Thorndyke M (2008) Near-future level of CO₂-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar Ecol Prog Ser* 373:285–294
- Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J Mar Sci* 65:414–432
- Fournier DA, Skaug HJ, Ancheta J, Ianelli J and others (2012) AD model builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim Methods Softw* 27: 233–249
- Fredensborg BL, Mouritsen KN, Poulin R (2004) Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental infections and field observations. *J Exp Mar Biol Ecol* 311:253–265
- Fredensborg BL, Mouritsen KN, Poulin R (2005) Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Mar Ecol Prog Ser* 290:109–117
- Gaylord B, Kroeker KJ, Sunday JM, Anderson KM and others (2015) Ocean acidification through the lens of ecological theory. *Ecology* 96:3–15
- Hale R, Calosi P, McNeill L, Mieszowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos* 120: 661–674
- Hopper JV, Poulin R, Thieltges DW (2008) Buffering role of the intertidal anemone *Anthopleura aureoradiata* in cercarial transmission from snails to crabs. *J Exp Mar Biol Ecol* 367:153–156
- IPCC (Intergovernmental Panel on Climate Change) (2014) Climate change 2014: impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. In: Field CB, Barros VR, Dokken DJ, Mach KJ and others (eds) Contribution of Working Group II to the 5th assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
- Koehler AV, Poulin R (2010) Host partitioning by parasites in an intertidal crustacean community. *J Parasitol* 96: 862–868
- Koprivnikar J, Lim D, Fu C, Brack SHM (2010) Effects of temperature, salinity, and pH on the survival and activity of marine cercariae. *Parasitol Res* 106:1167–1177
- Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol Lett* 13: 1419–1434
- Kroeker KJ, Kordas RL, Crim R, Hendriks IE and others (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob Change Biol* 19:1884–1896
- Lafferty KD, Kuris AM (1999) How environmental stress affects the impacts of parasites. *Limnol Oceanogr* 44: 925–931
- MacLeod CD (2015) The effects of ocean acidification on host-parasite associations. PhD thesis, University of Otago, Dunedin
- MacLeod CD, Poulin R (2012) Host-parasite interactions: a litmus test for ocean acidification? *Trends Parasitol* 28: 365–369
- MacLeod CD, Poulin R (2015) Differential tolerances to ocean acidification in parasites that share the same host. *Int J Parasitol* 45:485–493
- MacLeod CD, Doyle HL, Currie KI (2015) Maximising accuracy and minimising cost of a potentiometrically regulated ocean acidification simulation system. *Biogeosciences* 12:713–721
- Marcogliese DJ, Cone DK (1996) On the distribution and abundance of eel parasites in Nova Scotia: influence of pH. *J Parasitol* 82:389–399
- Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124:S101–S117
- Pietroccelli M, Marcogliese DJ (2003) Free-living endo-helminth stages: at the mercy of environmental conditions. *Trends Parasitol* 19:293–299
- Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar Ecol Prog Ser* 373:203–217
- Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO₂ concentrations: lessons from animal physiology and earth history. *J Oceanogr* 60: 705–718

- R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Royal Society Policy Doc 12/05, The Royal Society, London
- Rohr JR, Dobson AP, Johnson PTJ, Kilpatrick AM and others (2011) Frontiers in climate change-disease research. *Trends Ecol Evol* 26:270–277
- Shostak AW (1993) Survival of *Petasiger nitidus* (Digenea: Echinostomatidae) cercariae in relation to temperature, pH, and salinity. *Can J Zool* 71:431–434
- Studer A, Poulin R (2012) Effects of salinity on an intertidal host-parasite system: Is the parasite more sensitive than its host? *J Exp Mar Biol Ecol* 412:110–116
- Studer A, Thielges DW, Poulin R (2010) Parasites and global warming: net effects of temperature on an intertidal host-parasite system. *Mar Ecol Prog Ser* 415:11–22
- Studer A, Lamare MD, Poulin R (2012) Effects of ultraviolet radiation on the transmission process of an intertidal trematode parasite. *Parasitology* 139:537–546
- Suggett DJ, Hall-Spencer JM, Rodolfo-Metalpa R, Boatman TG and others (2012) Sea anemones may thrive in a high CO₂ world. *Glob Change Biol* 18:3015–3025
- Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. *Mar Ecol Prog Ser* 430:257–271

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