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ORIGINAL PAPER

# Lack of genetic variation in the response of a trematode parasite to ocean acidification

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**Abstract** Ocean acidification is already having measurable impacts on marine ecosystems. Intraspecific variation in the responses of marine organisms to ocean acidification can reveal genetic differences in tolerance to low pH conditions and determine the potential for a species to adapt to a changing environment. This study tests for the existence of genetic variation in both the transmission success of the trematode *Maritrema novaezealandense* to its second intermediate amphipod host, *Paracalliope novizealandiae*, and the extent of parasite-induced mortality in that host, in response to decreasing pH. Eight parasite genotypes were tested in a custom-built ocean acidification simulation system, at 8.1 pH (current ocean conditions) and under conditions of 7.4 pH (worst-case scenario future prediction). The parasites had significantly higher infection success in the more acidic treatment, but there was no significant difference among genotypes in how infection success was affected by pH. In contrast, some parasite genotypes induced higher mortality in amphipods than other genotypes, but this genetic effect was also independent of pH. Overall, our results reveal no significant intergenotype variation in how the parasite responds to ocean acidification with respect to two key traits, infection success and parasite-induced host mortality, suggesting limited potential for adaptation in the face of acidifying conditions.

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## Introduction

The impacts of climate change and ocean acidification on marine species will be complex and involve both direct and indirect effects (Kroeker et al. 2013). As with other environmental stressors, the consequences of ocean acidification (OA) are likely to be variable, not only among species but also within species, with some individuals showing greater tolerance to low pH conditions (Doney et al. 2009). Phenotypic plasticity may play an important role for species faced with environmental fluctuations (Hofmann et al. 2010). However, ultimately it is genetic diversity within a species that determines its potential for adaptive evolution when subjected to altered selective pressures (Merila and Hendry 2014). If the ability to cope with low pH conditions is heritable, selection for tolerant genotypes can allow species to adapt to their changing environment (Schlegel et al. 2012).

Currently, the relative roles of genetic variation and phenotypic plasticity in response to climate change are not well understood (Merila and Hendry 2014). Recent studies have found intraspecific variation in response to OA (Gazeau et al. 2010; Caldwell et al. 2011; Schlegel et al. 2012; Reusch 2014); however, many seemingly adaptive responses to the environment are likely to be environmentally induced phenotypic plasticity (Dupont and Thorndyke 2009). Inherent adaptive flexibility may be critical in an organism's ability to cope with environmental change (Hofmann et al. 2010), yet rapid change may test the limits of a species' potential to persist (Donnelly et al. 2012). With a rapid change in oceanic pH, the pressure for adaptation will intensify (Hofmann et al. 2010; Schlegel et al. 2012; Doney et al. 2012; Hoegh-Guldberg and Bruno 2010). As a first step, quantifying intraspecific genetic variability is vital to

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assess population adaptability and resilience in the face of OA (Dupont and Thorndyke 2009).

Although the effects of OA are receiving increasing attention, their specific influence on host–parasite interactions has not received much scrutiny (Koprivnikar et al. 2010; MacLeod and Poulin 2012). In coastal ecosystems, trematodes are often the most diverse and abundant parasites, affecting the physiology and behaviour of individual hosts, the dynamics of host populations, and the structure and diversity of whole communities (Mouritsen and Poulin 2002). The influence of OA on trematode infections is therefore potentially very important. Trematodes are also good models for investigations of genetic variation in the responses of parasites to OA. Indeed, they multiply asexually within their first intermediate hosts, producing large numbers of genetically identical clones that go on to infect the next host in the life cycle (Galaktionov and Dobrovolskij 2003). This allows for multiple replicates of each genotype to be used in controlled experiments. Intraspecific variation in responses to rising temperatures has already been shown to be frequent among trematode species, suggesting that they may be capable of rapid adaptation to global warming (Studer and Poulin 2014). It remains to be determined whether the same applies to their responses to OA.

Here, we quantify genetic variation in the trematode *Maritrema novaezealandense* Martorelli, Fredensborg, Mouritsen, and Poulin, with respect to infection success of the second intermediate host and parasite-induced host mortality, in response to low pH conditions. Widespread in intertidal areas of southern New Zealand, *M. novaezealandense* has the typical three-host trematode life cycle (Martorelli et al. 2004). Adult worms live in the gut of birds, from where they release eggs in host faeces. The intertidal snail *Zeacumantus subcarinatus* Sowerby serves as first intermediate host, becoming infected after accidentally ingesting eggs. Following asexual multiplication within the snail host, free-swimming infective stages, or cercariae, emerge from snails in large numbers during low tide and seek their second intermediate host, which can be any small crustacean, including the amphipod *Paracalliope novizealandiae* Dana. Cercariae penetrate the crustacean host, encyst within its body cavity, and await ingestion by a suitable shorebird, in which they develop into adult worms. Previous studies provide evidence that distinct *M. novaezealandense* genotypes differ for a range of phenotypic traits. Koehler et al. (2011) found substantial differences in the morphology, phototactic behaviour, and survivorship of cercariae from different genotypes. More importantly, Berkhout et al. (2014) uncovered significant differences among *M. novaezealandense* genotypes in how within-snail cercarial production and post-emergence cercarial activity responded to elevated temperature, suggesting the existence of genetic variation in responses to environmental change.

Earlier, we found that OA can affect *M. novaezealandense* and how it interacts with its hosts: the survival of its cercariae decreases with decreasing seawater pH (MacLeod and Poulin 2015), although transmission success to the second intermediate host peaked under low pH conditions, possibly because acidification increases host susceptibility (Harland et al. 2015). These are general species-level responses; here, we examine intraspecific variation among genotypes. At our study site, the majority of infected *Z. subcarinatus* snails harbour only one *M. novaezealandense* genotype (Keeney et al. 2007; Berkhout et al. 2014). Therefore, cercariae issued from one snail are likely to represent clones of a single-parasite genotype. However, even if a snail harbours more than one parasite genotype, each parasite–snail combination still represents a unique genetic complex, because a parasite genotype can only be associated with a single snail. Whether part of the interindividual variation is ascribed to the snail host instead of being all attributed to parasite genes does not diminish its significance for evolutionary responses to OA. Hereafter, we refer to each parasite–snail combination as a separate parasite genotype. We compared eight *M. novaezealandense* genotypes by testing the performance of each genotype at two different pH levels representing control seawater and low pH seawater, measuring both cercarial infection success and parasite-induced mortality of the second intermediate host, the amphipod *P. novizealandiae*. Our findings provide the first insight into genetic variation and the adaptive potential of parasites in the face of increasing OA.

## Methods

### Snail and amphipod collections

Two sites on the Otago Peninsula, South Island, New Zealand, separated by 35 km of coastline, were chosen as sources of experimental animals. Amphipods (*Paracalliope novizealandiae*) were collected from Hoopers Inlet using a dipnet (mesh size 250 μm) dragged along the bottom in shallow water, on four occasions between March and July 2014, to obtain fresh amphipods prior to each of the four infection trials. Multiple earlier samples from this semi-closed inlet have demonstrated that amphipods at that site are never parasitised by *M. novaezealandense* nor by any other metazoan parasite (see Bryan-Walker et al. 2007; Harland et al. 2015), and dissection of a subsample ($\gg$100 amphipods) confirmed that this applied to those used in the present experiment. Amphipods were placed in seawater from the collection site and transported immediately to the laboratory, where they were divided equally into two groups and placed in chambers in each of the two experimental tanks (see 'Experimental apparatus'). After 2 days

of acclimatisation, amphipods were selected from each pH for use in infection trials (see 'Experimental procedures'). Selection was without regard to sex, but only medium-sized amphipods between 1.5 and 2.5 mm were used. This procedure excluded young amphipods and unusually large ones, on the assumption that their ability to cope with infection may be different.

Snails (*Zeacumantus subcarinatus*) were collected by hand in November 2013, prior to the start of the study, from the mudflat at Lower Portobello Bay, a locality known for its very high prevalence of *M. novaezealandense* infections in both snails (Fredensborg et al. 2006) and amphipods (Bates et al. 2010). Weekly water monitoring over 1 year at Lower Portobello Bay showed that the average local seawater pH (8.083 ± 0.122 SD) was very similar to the global average of pH 8.1 (MacLeod 2015). However, seawater conditions within tidal pools fluctuate widely over both short and longer time scales, ranging from pH 9.1 to 7.6 (MacLeod 2015).

Snails were transported back to the laboratory and maintained in 2-L plastic containers with seaweed *Ulva* sp. (collected from Lower Portobello Bay) as a food source. After a few days, infected snails were identified by inducing cercariae to emerge from their snail hosts. This is achieved by incubating snails individually in a few ml of seawater for 2 h at 25 °C (Fredensborg et al. 2005). A subset of eight infected snails of similar sizes (average 14.7 mm shell length, ±1.17 SD) was chosen for use in the experiment. These eight infected snails provided the eight parasite genotypes tested in the following experiment. They were individually numbered with a small plastic tag glued to their shell and randomly divided into two groups of four snails, with one group placed in the pH 7.4 tank and the other group of four placed in the pH 8.1 tank (see 'Experimental apparatus'). A period of 2 weeks was allowed for acclimatisation before the experiment began.

## Experimental apparatus

The experimental system was custom-built to simulate OA conditions (see MacLeod et al. 2015 for full description). It consisted of two 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 to either current average ocean surface waters (8.1 pH) or the more extreme conditions (7.4 pH) predicted for the year 2300 by the IPCC (Field et al. 2014; see also Orr et al. 2005; Raven 2005; Doney et al. 2009; Feely et al. 2009). Seawater (salinity 35) came 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 and then back into each tank. Food grade $CO_2$ was pumped in 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 $CO_2$ inflow to maintain the target seawater pH. The pH meters were calibrated regularly using two saltwater buffers to maintain electrode accuracy. The seawater in each tank was partially (20 L each time) changed twice a week, completely changed once a month, and kept well oxygenated (>95 % saturation throughout all experiments) with Aqua One 9500 air pumps. Conditions were identical in both tanks, except for pH, and previous experiments had ruled out the possibility of tank effects through a temporal series of replicates (Macleod and Poulin 2015). At the start of the experiment, pH levels were reassigned 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) remained constant and reflected the carbonate chemistry expected of control and low pH seawater (see MacLeod et al. 2015, MacLeod and Poulin 2015).

Each tank housed a 2-L plastic chamber for acclimatising amphipods and another identical chamber for acclimatising snails. The chambers had mesh sides (mesh size 250 µm) to allow seawater to flow through. In addition, there were two 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.

## Experimental procedures

After the 2-week acclimatisation at either 8.1 or 7.4 pH, the eight snails were individually placed in Petri dishes (8.6 cm diameter) and covered with the seawater to which they had acclimatised and incubated at 25 °C under light for two hours to induce parasite shedding. After incubation, the snails were removed from the Petri dishes and returned to their respective tanks. Forty-eight acclimatised amphipods from each of the 7.4 pH and 8.1 pH tanks were placed in separate wells of tissue culture plates in 75 µl of their own tank water. Each snail was used as a source of cercariae to infect 12 amphipods. The cercariae-laden water from each Petri dish was stirred gently before a pipette was used to take 20 µl doses that were then added to a well containing an amphipod. For every two doses given to amphipods, one dose was put aside to be counted later to calculate the average dose for each particular snail and infection trial.

After 2 h of exposure to cercariae at room temperature, the amphipods were then placed in individual floating chambers in the tank they came from to allow for parasite establishment. Two days later, amphipods were dissected under an Olympus SZ30 dissecting microscope and the parasites they harboured were counted. Numbers of parasites per amphipod were used as a measure of infection success. Amphipods that died prior to dissection were recorded, but no parasite count was possible because dead amphipods decomposed very rapidly.

After a further 2 weeks to allow for parasite multiplication within snails, another infection trial was carried out exactly as described above. After this the four snails from the 7.4 pH tank were moved to the 8.1 pH tank, and the four snails from the 8.1 pH tank were moved to the 7.4 pH tank. Two weeks were then allowed for acclimatisation of snails, and parasite multiplication within snails, at the new pH level. The full experiment was then repeated, with two further infection trials performed exactly as described above.

Thus, our experimental design resulted in each snail spending the same amount of time in each tank, controlling for any possible tank effect. Half of the snails were first exposed to the low pH level, and half were first exposed to the control pH level, ruling out any influence of treatment sequence. At the conclusion of the experiment, cercariae had been induced to emerge from each snail four times, twice at 7.4 pH, and twice at 8.1 pH, and a total of 48 amphipods had been exposed to cercariae from each snail, with 384 amphipods used in total. Finally, each parasite genotype, i.e. each infected snail, served as its own control in the experiment, as infection success (or parasite-induced host mortality) at one pH is contrasted with infection success (or host mortality) at the other pH for the same genotype.

### Statistical analyses

R version 3.1.0 (R Core Team 2014) was used for statistical analysis. We first tested for differences in average doses among the 8 parasite genotypes, i.e. the 8 infected snails, using a one-way ANOVA. There were 4 estimated average dose values per snail, one from each of the 4 infection trials; there was no significant difference in dose average among the 8 parasite genotypes ($F_{7,24} = 1.99$, $P = 0.10$).

The effect of pH and individual snail ID (= parasite genotype) on infection levels was analysed by a generalised linear mixed model with negative binomial error structure (glmm ADMB package). Snail ID, pH, and average dose were fixed effects in this model, with number of parasites per individual amphipod being the response variable; replicate trial (two per pH level) was treated as a random factor. The impact of pH and individual snail ID on amphipod mortality was analysed with a generalised linear mixed model with binomial error structure. In this model, snail ID, pH, and average dose were again fixed effects, mortality was a response variable (binary: dead or alive), and replicate was again treated as a random factor. We performed likelihood ratio tests to compare models with and without the interaction term between snail ID and pH treatment and identify the model that best explained the data. Raw parasite numbers were used in the analysis, though relative infection success (number of parasites per amphipod divided by average dose) is used for illustrative purposes.

## Results

Of the 384 amphipods exposed to cercariae, 310 (80.7 %) were successfully infected, 38 (9.9 %) were not, and 36 (9.4 %) died. The average cercarial doses for each particular snail and infection trial showed some variation, but overall were very similar between both pH treatments, with an overall average of 24.9 (range 5.0–38.5) for 7.4 pH and 22.4 (range 5.3–41.3) for 8.1 pH.

Out of the 310 amphipods successfully infected by parasites, 158 were within the 7.4 pH treatment and 152 within the 8.1 pH treatment. Numbers of parasites per amphipod ranged from 0 to 95, with an overall average of 6.96. There was a significant effect of pH on numbers of parasites per amphipod, as well as a significant effect of average cercarial dose used (Table 1). Increasing dose level was positively correlated with an increase in numbers of parasites per amphipod (Fig. 1). Accounting for the dose, relative infection success was higher in the 7.4 pH treatment than in the 8.1 pH treatment (overall averages 0.337 versus 0.240) (Fig. 2). However, accounting for the other fixed factors, there was no significant effect of snail ID on numbers of parasites acquired per amphipod (Table 1), and crucially, the interaction between snail ID and pH treatment was not included in the best model, indicating no effect of parasite genotype on infection performance under low pH conditions.

**Table 1** Results of a generalised linear mixed model showing the effect of pH level, average cercarial dose used, and parasite genotype (snail ID) on numbers of parasites acquired by amphipods

| | Estimate | SE | *Z* value | *P* value |
|---|---|---|---|---|
| Intercept (pH 7.4) | 0.38213 | 0.22371 | 1.71 | 0.08761 |
| pH 8.1 | −0.37952 | 0.10891 | −3.48 | **0.00049** |
| Snail ID | −0.00978 | 0.02262 | −0.43 | 0.66559 |
| Dose average | 0.06687 | 0.00641 | 10.44 | **<2e–16** |

Significant *P* values are shown in bold type

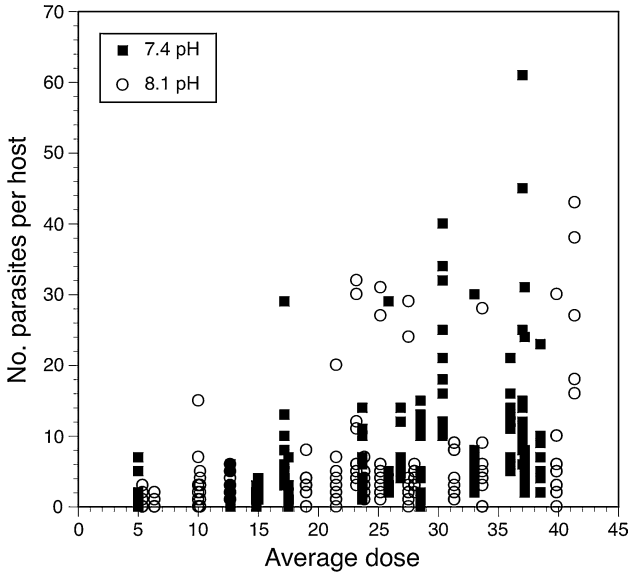


**Fig. 1** Relationship between the number of *Maritrema novaezealandense* cercariae acquired per amphipod and the dose given (average number of parasites per dose in a given infection trial). Different *symbols* are used for the two pH treatments; data shown are for a total of 348 amphipods dissected at the end of the experiment

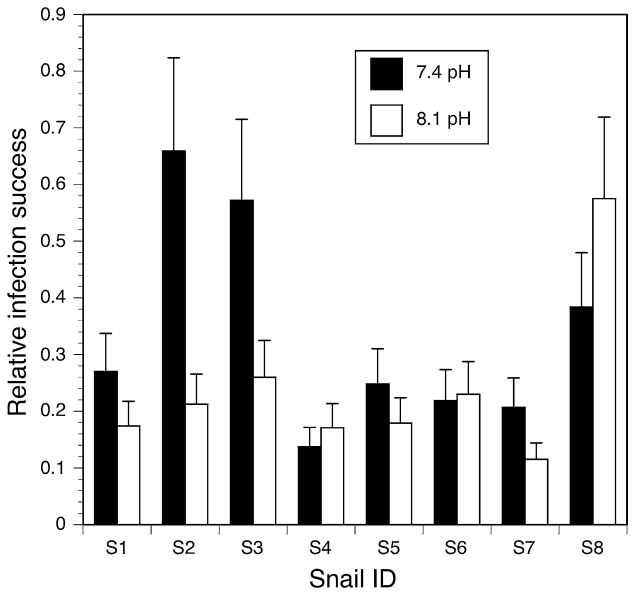


**Fig. 2** Average (±SE) relative infection success (i.e. number of parasites per amphipod divided by average dose given) of *Maritrema novaezealandense* cercariae in the two pH treatments for each of the eight parasite genotypes (snail ID) used in this experiment. Numbers of amphipods dissected in each case ranged from 16 to 24

Out of the 384 amphipods used in the experiment, a total of 21 died in the 7.4 pH treatment and 15 in the 8.1 pH treatment. Amphipod mortality did not differ significantly between pH treatments, but it increased with the cercarial dose to which the amphipods were exposed (Table 2). Snail ID had a significant effect on amphipod mortality, with deaths being more frequent among amphipods infected by cercariae from certain snails than from others (Fig. 3). However, again the interaction between snail ID and pH treatment was excluded from the best model, indicating no difference among parasite genotypes in parasite-induced mortality under low pH conditions.

**Table 2** Results of a binomial generalised linear mixed model showing the effect of pH level, average cercarial dose used, and parasite genotype (snail ID) on amphipod mortality

| | Estimate | SE | *Z* value | *P* value |
|---|---|---|---|---|
| Intercept (pH 7.4) | −4.54270 | 0.78733 | −5.770 | **7.94 e–09** |
| pH 8.1 | −0.47400 | 0.38640 | −1.227 | 0.2199 |
| Snail ID | 0.20119 | 0.08193 | 2.456 | **0.0141** |
| Dose average | 0.05316 | 0.02247 | 2.366 | **0.0180** |

Significant *P* values are shown in bold type

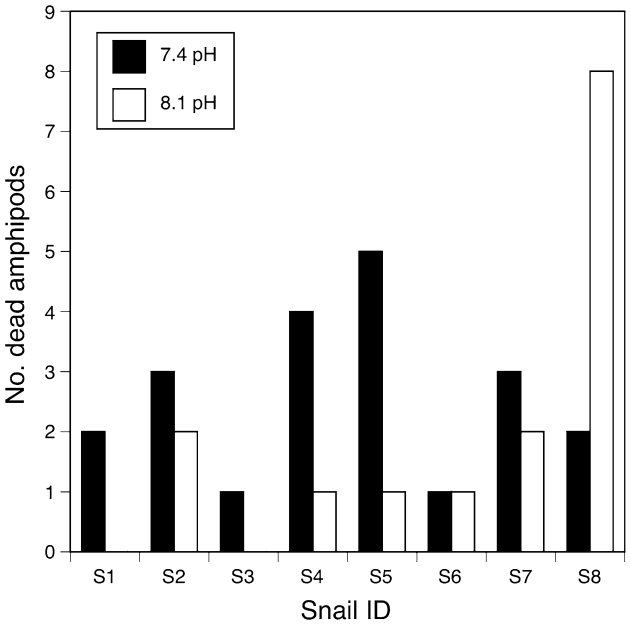


**Fig. 3** Number of amphipods that died (out of 24) following exposure to *Maritrema novaezealandense* cercariae for both pH treatments (7.4 and 8.1) and for each of the eight parasite genotypes (snail ID)

## Discussion

Ocean acidification is one of the major threats to the diversity and functioning of marine ecosystems (Hofmann et al. 2010; Kroeker et al. 2013; Doney et al. 2012). The ability of marine organisms to adapt to acidifying water depends on their phenotypic plasticity, but also on their genetic

variability (Schlegel et al. 2012; Reusch 2014; Merila and Hendry 2014). Here, we quantified genetic variation in infection success and parasite-induced host mortality, in response to low pH conditions, in the trematode *Maritrema novaezealandense*. Overall, our results suggest that there are no significant differences among parasite genotypes, at least at the cercarial transmission stage, in how they respond to acidification.

Our experiment revealed that pH had a significant effect on infection success: regardless of genotypes, infection success was greater at 7.4 pH than at the control 8.1 pH. This finding provides an independent confirmation of the earlier result of Harland et al. (2015), using a slightly modified experimental design (additional pH level, pooled parasite genotypes, etc.) on the same host and parasite species. Thus, in spite of cercarial mortality in the trematode *M. novaezealandense* being greater under low pH conditions (MacLeod and Poulin 2015), the susceptibility of amphipods increases sufficiently at low pH to overcome the reduced lifespan of infective cercariae. It remains to be seen whether this pattern applies to other host–parasite combinations and therefore how widely increased infection levels will occur in a low pH ocean.

However, parasite genotype had no significant impact on infection success and did not interact with pH treatment, indicating that all genotypes experienced roughly similar increases in transmission performance at lower pH. Although Fig. 2 suggests different responses among genotypes, the analysis accounting for other variables reveals no significant interaction between genotype and pH. In contrast, Berkhout et al. (2014) demonstrated that cercarial output and activity in the trematode *M. novaezealandense* show genetic variation in the face of warming, as different genotypes had distinct responses to increased temperature. This parasite also displays genetic variation in morphological and behavioural traits, which clearly differ among genotypes (Koehler et al. 2011). The lack of variation among genotypes in response to pH seen here could simply be the consequence of the limited number of genotypes tested in our experiment; perhaps using a greater number would reveal greater variation. As suggested by Berkhout et al. (2014), a few highly sensitive genotypes may account for observed variation, with most others being equally affected by environmental change. It may also be that intergenotypic variation is subtle and only becomes detectable over longer-term exposure. Alternatively, the finding of our experiment may indicate that this trematode population lacks the genetic variation to adapt in the face of increased OA, with all individual parasite genotypes responding roughly similarly to decreased pH.

Our study also revealed that parasite-induced host mortality was unaffected by pH but highly dependent on infection dose. Earlier studies on the same trematode–amphipod system also indicated that host mortality is greatly dependent on the number of parasites to which a host is exposed (Fredensborg et al. 2004; Bates et al. 2010). There was also a significant effect of snail identity, i.e. parasite genotype, on parasite-induced host mortality, indicating pH-independent variation in virulence within the trematode population. The fact that some parasite genotypes are more virulent than others may be the consequence of each genotype achieving a particular resolution to the trade-off between virulence and other traits, such as within-host growth. More importantly, for parasite-induced host mortality too, we observed no significant interaction between parasite genotype and pH, indicating no genetic variation in how different parasite genotypes change their impact on hosts under low pH conditions.

The lack of variation among parasite genotypes in their responses to decreased pH, measured through infection success and parasite-induced host mortality, may indicate that this parasite population has limited genetic variation and will be constrained in how quickly it can adapt to OA. Physiological limits may prevent variability in how these parasites react to low pH. Alternative explanations are possible, of course. First, we only tested eight genotypes, and perhaps variation would be detected across a larger sample. Snail selection may also have created bias towards certain genotypes, as the snails we selected from our initial pool of infected snails were the ones that released high numbers of cercariae. As we stated in the 'Introduction', it is not possible to disentangle effects of parasite genotype from those of snail genotype, as an individual trematode genotype is inextricably linked with a single snail host. It is therefore conceivable that genetic variation among parasites was masked by strong responses of certain snail hosts to low pH. Second, we did not establish that each snail harboured only one genotype, and the occurrence of mixed-genotype infections could have affected the results, in particular the finding of differences among trematode genotypes in parasite-induced amphipod mortality. However, not only are mixed-genotype infections relatively rare in this system (Keeney et al. 2007; Berkhout et al. 2014), but mixed-genotype infections have also been shown not to cause greater amphipod mortality than single-genotype infections (Keeney et al. 2009). Third, our experimental exposures to different pH lasted several weeks, but longer periods may be necessary to detect subtle differences among genotypes. Much of our present understanding of the responses of marine organisms to OA is based primarily on short-term experiments (Lohbeck et al. 2012). Longer-term studies spanning multiple generations may therefore be necessary to uncover the adaptive potential of organisms to OA. Fourth, we have not considered here variation among amphipod genotypes in how they cope with parasites under low pH conditions. When investigating interactions between parasite and host populations,

host genotype is also important when it comes to infection success (Minchella 1985). Other crustacean hosts of *M. novaezealandense*, such as the crab *Macrophthalmus hirtipes* Jacquinot, are known to vary in their immune response to trematode cercariae (Koehler and Poulin 2012). Variation among hosts may have masked existing variation among parasite genotypes in our experiments.

In conclusion, although we cannot rule out the existence of intergenotypic variation in responses to OA in the trematode *M. novaezealandense*, our experiment did not reveal any. Yet significant variation in other traits is clearly discernible within the same *M. novaezealandense* population, using similar procedures and similar sample sizes (see Koehler et al. 2011; Berkhout et al. 2014). Environmental change can influence the evolution of host–parasite interactions (Wolinska and King 2009). Ocean acidification is clearly an important new selective force acting on marine organisms, both free-living and parasitic (Hofmann et al. 2010; Kroeker et al. 2013; Doney et al. 2012). Genetic variation will be one of the key elements determining the potential of marine populations to adapt to low pH conditions. Our data suggest limited potential for rapid adaptation to OA in the trematode *M. novaezealandense*. Although logistically very challenging, multi-generational selection experiments will be necessary to fully assess how this parasite evolves in the face of an acidifying environment.

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## References

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

Berkhout BW, Lloyd MM, Poulin R, Studer A (2014) Variation among genotypes in responses to increasing temperature in a marine parasite: evolutionary potential in the face of global warming? Int J Parasitol 44:1019–1027

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

Caldwell GS, Fitzer S, Gillespie CS, Pickavance G, Turnbull E, Bentley MG (2011) Ocean acidification takes sperm back in time. Invertebr Reprod Dev 55:217–221

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other $CO_2$ problem. Annu Rev Mar Sci 1:169–192

Doney SC, Ruckelshaus M, Duffy E, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4:11–37

Donnelly A, Caffara A, Kelleher CT, O'Niel BF, Diskin E, Plestsers A, Proctor H, Stirnemann R, O'Halloran J, Penuelas J, Kodkinson TR, Sparks TH (2012) Surviving in a warmer world: environmental and genetic responses. Clim Rese 53:245–262

Dupont S, Thorndyke MC (2009) Impact of $CO_2$-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

Feely RA, Doney SC, Cooley SR (2009) Ocean acidification: present conditions and future changes in a high-$CO_2$ world. Oceanography 22:36–47

Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC (eds) (2014) IPCC 2014: Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of working group ll to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

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 Progr Ser 290:109–117

Fredensborg BL, Mouritsen KN, Poulin R (2006) Relating bird host distribution and spatial heterogeneity in trematode infections in an intertidal snail—from small to large scale. Mar Biol 149:275–283

Galaktionov KV, Dobrovolskij AA (2003) The biology and evolution of trematodes. Kluwer Academic Publishers, Dordrecht

Gazeau FPH, Gattuso JA, Dawber C, Pronker AE, Peene F, Penne J, Heip CHR, Middelburg JJ (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences 7:2051–2060

Harland H, MacLeod CD, Poulin R (2015) Non-linear effects of ocean acidification on the transmission of a marine intertidal parasite. Mar Ecol Prog Series. doi:10.3354/meps11416

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523–1528

Hofmann GE, Barry JP, Edmunds PJ, Gates RD, Hutchins DA, Klinger T, Sewell MA (2010) The effect of ocean acidification on calcifying organisms in marine ecosystems: an organisms-to-ecosystem perspective. Annu Rev Ecol Evol Syst 41:127–147

Keeney DB, Waters JM, Poulin R (2007) Clonal diversity of the marine trematode *Maritrema novaezealandensis* within intermediate hosts: the molecular ecology of parasite life cycles. Mol Ecol 16:431–439

Keeney DB, Bryan-Walker K, Khan N, King TM, Poulin R (2009) The influence of clonal diversity and intensity-dependence on trematode infections in an amphipod. Parasitology 136:339–348

Koehler AV, Poulin R (2012) Clone-specific immune reactions in a trematode-crustacean system. Parasitology 139:128–136

Koehler AV, Springer YP, Keeney DB, Poulin R (2011) Intra- and interclonal phenotypic and genetic variability of the trematode *Maritrema novaezealandensis*. Biol J Linn Soc 101:106–116

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 R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso J-P (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob Change Biol 19:1884–1896

Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nature Geosci 5:346–351

MacLeod CD (2015) The effects of ocean acidification on host-parasite associations. Ph.D. thesis, University of Otago, Dunedin, New Zealand

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 by 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

Martorelli SR, Fredensborg BL, Mouritsen KN, Poulin R (2004) Description and proposed life cycle of *Maritrema novaezealandensis* n. sp. (Microphallidae) parasitic in red-billed gulls, *Larus novaehollandiae scopulinus*, from Otago Harbor, South Island, New Zealand. J Parasitol 90:272–277

Merila J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol Appl 7:1–14

Minchella DJ (1985) Host life-history variation in response to parasitism. Parasitology 90:205–216

Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124:S101–S117

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

R Core Team (2014) R: A language and environment for statistical computing. Vienna, Austria. R Foundation for Statistical Computing. http://www.R-project.org/

Raven J (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy Document 12/05. The Royal Society, London

Reusch TB (2014) Climate change in the oceans: evolutionary versus phenotypically plastic responses of marine animals and plants. Evol Appl 7:104–122

Schlegel P, Havenhand JN, Gillings MR, Williamson JE (2012) Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. PLoS ONE 7:e53118

Studer A, Poulin R (2014) Analysis of trait mean and variability versus temperature in trematode cercariae: is there scope for adaptation to global warming? Int J Parasitol 44:403–413

Wolinska J, King KC (2009) Environment can alter selection in host-parasite interactions. Trends Parasitol 25:236–244