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## Research

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## Marine biology

# Parasitic infection: a buffer against ocean acidification?

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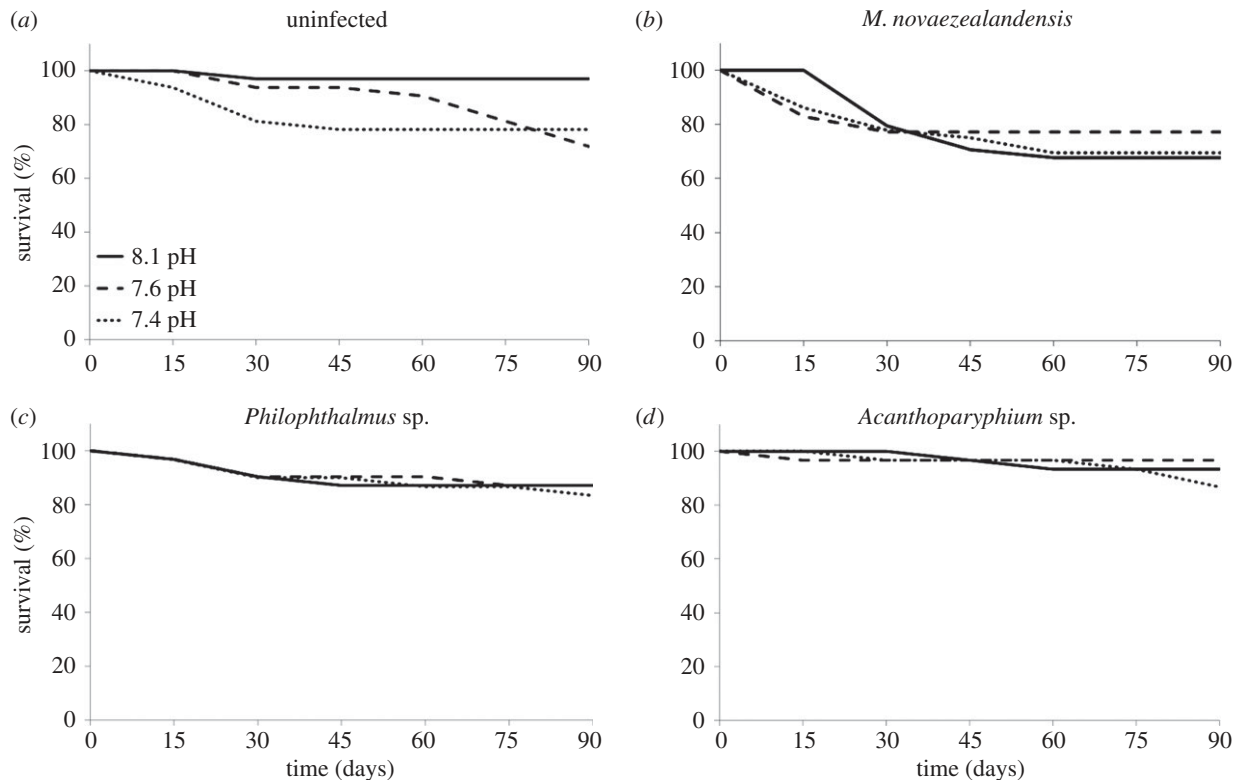
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Recently, there has been a concerted research effort by marine scientists to quantify the sensitivity of marine organisms to ocean acidification (OA). Empirical data generated by this research have been used to predict changes to marine ecosystem health, biodiversity and productivity that will be caused by continued acidification. These studies have also found that the effects of OA on marine organisms can be significantly modified by additional abiotic stressors (e.g. temperature or oxygen) and biotic interactions (e.g. competition or predation). To date, however, the effects of parasitic infection on the sensitivity of marine organisms to OA have been largely ignored. We show that parasitic infection significantly altered the response of a marine gastropod to simulated OA conditions by reducing the mortality of infected individuals relative to uninfected conspecifics. Without the inclusion of infection data, our analysis would not have detected the significant effect of pH on host mortality. These results strongly suggest that parasitic infection may be an important confounding factor in OA research and must be taken into consideration when assessing the response of marine species to OA.

## 1. Introduction

A central objective of ocean acidification (OA) research is to quantify the response of ecologically and commercially important species exposed to acidified seawater, and predict future changes to marine ecosystem health, biodiversity and productivity [1]. This objective has been pursued using increasingly sophisticated apparatus to simulate OA and introduce additional abiotic stressors, such as elevated temperature and hypoxia [2]. Some research has also investigated the effects of OA on biological interactions (see review in [3]), which shows that differential tolerances between predator and prey or between competing species can exacerbate the effects of OA [4]. The interaction between parasite and host, however, is only now being incorporated into OA research, despite evidence that shows that infection can modify the response of host organisms to changing abiotic conditions [5] and alter the outcomes of competitive or predator–prey interactions [6].

The gastropod–trematode association is an ideal model system to evaluate the interactive effects of OA and parasitic infection. Marine gastropods act as an intermediate host for most trematode species, which are ubiquitous in coastal ecosystems, and, as calcifying organisms, shelled gastropods are affected by the major physiological challenges caused by OA, i.e. altered acid–base balance [7] and reduced availability of carbonate ions [8]. Here, we present mortality data gathered as part of a long-term project that quantified the response of infected and uninfected marine gastropods (*Zeacumantus subcarinatus*) to simulated OA [9]. In this study, mortality is interpreted as a meta-parameter, as it can be affected by a wide range of biological factors that are altered by infection and/or exposure to acidified seawater, e.g. foraging ability, nutrient uptake, respiration and energy consumption. *Z. subcarinatus* is well suited for this study, as the effects of trematode infection on this species have been extensively



**Figure 1.** Cumulative mortality of snails from each infection category maintained in acidified or unmodified seawater for 90 days.

documented, e.g. altered heat tolerance [10], modified shell morphology [11], impaired predator evasion [12] and reduced reproductive output and survival [13]. Given the diverse effects of trematode infection on *Z. subcarinatus*, we predicted that exposure to acidified seawater (7.6 and 7.4 pH) would alter the survival of infected individuals relative to uninfected conspecifics.

## 2. Methods

In July 2013, *Z. subcarinatus* snails were collected from Otago Harbour, New Zealand, and subsequently screened for trematode infection by exposing snails to physical conditions that trigger cercarial emergence: warmed seawater (25 °C) and constant light. Four size-matched groups of snails (uninfected or infected with the trematodes *Maritrema novaezealandensis*, *Philophthalmus* sp. or *Acanthoparyphium* sp.) were exposed to three pH treatments (pH<sub>T</sub> 8.1, 7.6 and 7.4, 12.5 °C, total hydrogen scale) that corresponded to the average seawater pH at the collection site of *Z. subcarinatus* (pH 8.085 [14]), and predicted conditions for the years 2100 (pH 7.6) and 2300 (pH 7.4) reported in the 2014 Intergovernmental Panel on Climate Change Report [15]. All snails were maintained for 90 days in three potentiometrically regulated OA simulation tanks acidified with 100% CO<sub>2</sub> gas (see [16] for a detailed description of this system). Dissolved inorganic carbon (DIC) and total alkalinity (*A<sub>T</sub>*) were regularly measured throughout the study to monitor seawater carbonate chemistry (electronic supplementary material, table S1). Within pH treatments, 30 tagged snails belonging to each infection category, i.e. uninfected or infected with *M. novaezealandensis*, *Acanthoparyphium* sp. or *Philophthalmus* sp., were randomly allocated to one of five cylindrical nylon mesh chambers (height: 8 cm, diameter: 8.5 cm, 20 chambers in total). To account for any unrecorded and unwanted variation in the performance of a particular culture tank and associated apparatus, i.e. tank

effect, the pH assigned to each culture tank was changed at 30 and 60 days, and the snails transferred between tanks. Consequently, all snails experienced constant pH conditions and spent equal amounts of time in each culture tank. During the 90 day trial, snails were fed *Ulva* spp. ad libitum, and light levels were standardized across all tanks.

Snail survival was monitored weekly throughout the 90 day exposure to acidified or unmodified seawater. If snails failed to respond to physical stimuli, i.e. a blunt probe touching the operculum, then their tag number was noted and they were returned to the culture tanks. If they failed to respond to stimuli the following week, then they were classified as dead, and the time (post-exposure) they first failed to respond was used to calculate cumulative mortality for the corresponding infection category/pH combination.

Snail mortality was analysed using three Cox proportional hazard mixed-effect models. These models provided a hazard response based on the time of death of individuals associated with a given pH treatment or infection category. Infection category, pH and time of death for each individual that died during the 90 day experiment were recorded, in addition to censoring data to indicate snails that were alive at the end of the study (i.e. right-censored data). The first model analysed the effect of pH on snail survival within each infection category, whereas the second model analysed snail survival using pooled data from each pH treatment, i.e. by excluding infection status. The third model compared the mortality of infected and uninfected snails within pH treatments. In all models, 'chamber ID' was included as a random effect to compensate for some snails being maintained in the same chamber. All analyses were completed with the *coxme* package [17] using R v. 3.1.0 [18].

## 3. Results

Mortality was observed in all infection categories over the 90 day trial (figure 1 and table 1), although only the survival of

**Table 1.** Total mortality (%) and mean survival time (days, d) of snails from each infection category and in pooled data after a 90 day exposure to acidified or unmodified seawater.

	pH <sub>T</sub> 8.1		pH <sub>T</sub> 7.6		pH <sub>T</sub> 7.4	
	mortality (%)	mean survival (d)	mortality (%)	mean survival (d)	mortality (%)	mean survival (d)
uninfected	3	88	28	83	22	76
<i>Acanthoparyphium</i> sp.	7	88	3	88	13	88
<i>Philophthalmus</i> sp.	13	82	13	83	17	82
<i>M. novaezealandensis</i>	33	73	23	74	31	72
pooled data	14	83	17	82	21	79

**Table 2.** Cox proportional hazard analysis of the effect of pH on the survival of snails from each infection category and pooled data. Bold text indicates significant values ( $p < 0.05$ ).

	$\chi^2$	d.f.	$p$
uninfected	9.04	2	<b>0.01</b>
<i>Acanthoparyphium</i> sp.	2.03	2	0.36
<i>Philophthalmus</i> sp.	0.2	2	0.9
<i>M. novaezealandensis</i>	0.48	2	0.78
pooled data	2.29	2	0.31

uninfected snails was significantly reduced by pH (figure 1a and table 2). Uninfected snails exhibited a significantly increased probability of death in the pH<sub>T</sub> 7.6 ( $z = 2.178$ ,  $p = 0.0294$ ) and pH<sub>T</sub> 7.4 ( $z = 1.988$ ,  $p = 0.0468$ ) treatments relative to control conditions (pH<sub>T</sub> 8.1). In contrast, infected snails exhibited little difference in mortality between pH treatments (figure 1b–d), with the greatest total mortality and lowest mean survival time in *M. novaezealandensis*-infected snails, and the lowest mortality and greatest mean survival time in *Philophthalmus* sp.- and *Acanthoparyphium* sp.-infected snails (table 1). The mortality of uninfected snails increased relative to infected snails in acidified treatments (electronic supplementary material, figure S1), although this increase was only significant between uninfected and *Acanthoparyphium* sp.-infected snails at pH<sub>T</sub> 7.6 ( $z = 2.064$ ,  $p = 0.039$ ). Pooled data showed a non-significant linear increase in mortality as pH decreased and a corresponding reduction in mean survival time (tables 1 and 2).

## 4. Discussion

Exposure to acidified seawater caused a significant decrease in the survival of uninfected snails, and had little effect on the survival of infected individuals. The most likely explanation for this differential mortality is that infected snails may have more energy available to compensate for the increased metabolic costs of acid–base regulation and calcification. Trematode infection invariably causes castration in host snails and may reduce the overall energy budget of infected individuals [19–22], i.e. reproductive activity requires more

energy in uninfected individuals than the parasite consumes in infected individuals. Previous research has suggested that potential reductions in the energy budget of infected snails can cause gigantism [9,11], although there is no clear consensus among parasitologists.

As trematode prevalence can be extremely high in some snail populations (e.g. 80% [13]), OA-mediated increases in the mortality of uninfected snails (relative to infected individuals) may significantly reduce the reproductive output of heavily parasitized snail species, particularly when we consider competition for food resources between reproductive (uninfected) and non-reproductive (infected) conspecifics. Of course, there are many other aspects of host–parasite interactions affected by OA that could cause ecologically relevant changes to host species, e.g. reduced parasite survival [23], altered parasite transmission success [24] and compromised host calcification [9]. Clearly, more research is needed to fully characterize the combined effects of OA and infection in marine ecosystems.

We acknowledge that the culture of all snails exposed to a given pH treatment in a single tank was not ideal (see critique in [25]). However, the transfer of snails between tanks at 30 and 60 days would have distributed any potential ‘tank effect’ uniformly across all groups of snails, and previous analyses of data generated using this simulation system supported the treatment of each snail as an independent replicate [9]. Despite the limitations of this study, our results demonstrate that parasitic infection has the potential to significantly alter the response of marine organisms exposed to simulated OA. Our analysis of pooled snail data also clearly showed that without the inclusion of infection status, we would not have detected the significant effect of pH on the mortality of uninfected *Z. subcarinatus*, i.e. the true response of this species. The confounding effect of parasitic infection described here may lead to marine species being mistakenly categorized as tolerant of acidified conditions, when, in fact, this response is an artefact of parasitic infection. Our results strongly support the growing consensus that parasitic infection must be incorporated into experimental design to accurately assess the ecological impact of OA [3,26].

**Ethics.** The experimental organisms used in this study are not subject to ethics approval but were treated as humanely as possible.

**Data accessibility.** The datasets supporting this article are available in the Dryad repository at <http://dx.doi.org/10.5061/dryad.h8j57d>.

**Authors' contributions.** C.M. and R.P. designed the study; C.M. conducted the experiments, analysed the data and wrote the manuscript with input from R.P. Both C.M. and R.P. agree to be held accountable for the content therein and approve the final version of the manuscript.

**Competing interests.** We have no competing interests.

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## References

- 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. (doi:10.1111/gcb.12179)
- Bockmon EE, Frieder CA, Navarro MO, White-Kershek LA, Dickson AG. 2013 Technical note: controlled experimental aquarium system for multi-stressor investigation of carbonate chemistry, oxygen saturation, and temperature. *Biogeosciences* **10**, 5967–5975. (doi:10.5194/bg-10-5967-2013)
- Kroeker KJ, Sanford E, Jellison BM, Gaylord B. 2014 Predicting the effects of ocean acidification on predator–prey interactions: a conceptual framework based on coastal molluscs. *Biol. Bull.* **226**, 211–222.
- Kroeker KJ, Micheli F, Gambi MC. 2012 Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nat. Clim. Change* **3**, 156–159. (doi:10.1038/nclimate1680)
- Burge CA *et al.* 2014 Climate change influences on marine infectious diseases: implications for management and society. *Annu. Rev. Mar. Sci.* **6**, 249–277. (doi:10.1146/annurev-marine-010213-135029)
- Hatcher MJ, Dick JTA, Dunn AM. 2014 Parasites that change predator or prey behaviour can have keystone effects on community composition. *Biol. Lett.* **10**, 20130879. (doi:10.1098/rsbl.2013.0879)
- Pan T-CF, Applebaum SL, Manahan DT. 2015 Experimental ocean acidification alters the allocation of metabolic energy. *Proc. Natl Acad. Sci. USA* **112**, 4696–4701. (doi:10.1073/pnas.1416967112)
- Kelly MW, Hofmann GE. 2013 Adaptation and the physiology of ocean acidification. *Funct. Ecol.* **27**, 980–990. (doi:10.1111/j.1365-2435.2012.02061.x)
- MacLeod CD, Poulin R. 2015 Interactive effects of parasitic infection and ocean acidification on the calcification of a marine gastropod. *Mar. Ecol. Prog. Ser.* **537**, 137–150. (doi:10.3354/meps11459)
- Bates AE, Leiterer F, Wiedeback ML, Poulin R. 2011 Parasitized snails take the heat: a case of host manipulation? *Oecologia* **167**, 613–621. (doi:10.1007/s00442-011-2014-0)
- Hay KB, Fredensborg BL, Poulin R. 2005 Trematode-induced alterations in shell shape of the mud snail *Zeacumantus subcarinatus* (Prosobranchia: Batillariidae). *J. Mar. Biol. Assoc. UK* **85**, 989–992. (doi:10.1017/S0025315405012002)
- Kamiya T, Poulin R. 2012 Parasite-induced behavioural changes to the trade-off between foraging and predator evasion in a marine snail. *J. Exp. Mar. Biol. Ecol.* **438**, 61–67. (doi:10.1016/j.jembe.2012.09.002)
- 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. (doi:10.3354/meps290109)
- MacLeod CD. 2015 The effects of ocean acidification on host–parasite associations. PhD thesis, University of Otago, New Zealand.
- Field CB *et al.* 2014 Summary for policymakers. In *Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, UK.
- MacLeod CD, Doyle HL, Currie KI. 2015 Technical note: maximising accuracy and minimising cost of a potentiometrically regulated ocean acidification simulation system. *Biogeosciences* **12**, 713–721. (doi:10.5194/bg-12-713-2015)
- Therneau T. 2012 *Mixed effects Cox models*. <http://r-forge.r-project.org>.
- R Development Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org>.
- McCarthy HO, Fitzpatrick SM, Irwin SWB. 2004 Parasite alteration of host shape: a quantitative approach to gigantism helps elucidate evolutionary advantages. *Parasitology* **128**, 7–14. (doi:10.1017/S0031182003004190)
- Mouritsen KN, Jensen KT. 1994 The enigma of gigantism: effect of larval trematodes on growth, fecundity, egestion and locomotion in *Hydrobia ulvae* (Pennant) (Gastropoda: Prosobranchia). *J. Exp. Mar. Biol. Ecol.* **181**, 53–66. (doi:10.1016/0022-0981(94)90103-1)
- Gorbushin AM. 1997 Field evidence of trematode-induced gigantism in *Hydrobia* spp. (Gastropoda: Prosobranchia). *J. Mar. Biol. Assoc. UK* **77**, 785–800. (doi:10.1017/S0025315400036195)
- Probst S, Kube J. 1999 Histopathological effects of larval trematode infections in mudsnails and their impact on host growth: what causes gigantism in *Hydrobia ventrosa* (Gastropoda: Prosobranchia)? *J. Exp. Mar. Biol. Ecol.* **238**, 49–68. (doi:10.1016/S0022-0981(99)00002-7)
- MacLeod CD, Poulin R. 2015 Differential tolerances to ocean acidification by parasites that share the same host. *Int. J. Parasitol.* **45**, 485–493. (doi:10.1016/j.ijpara.2015.02.007)
- Harland H, MacLeod C, Poulin R. 2015 Non-linear effects of ocean acidification on the transmission of a marine intertidal parasite. *Mar. Ecol. Prog. Ser.* **536**, 55–64. (doi:10.3354/meps11416)
- Cornwall CE, Hurd CL. 2015 Experimental design in ocean acidification research: problems and solutions. *ICES J. Mar. Sci. J. Cons.* **73**, 572–581. (doi:10.1093/icesjms/fsv118)
- Gaylord B *et al.* 2015 Ocean acidification through the lens of ecological theory. *Ecology* **96**, 3–15. (doi:10.1890/14-0802.1)