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Complex and interactive effects of ocean acidification and warming on the life span of a marine trematode parasite

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ABSTRACT

Human activities have caused an increase in atmospheric CO_2 over the last 250 years, leading to unprecedented rates of change in seawater pH and temperature. These global scale processes are now commonly referred to as ocean acidification and warming, and have the potential to substantially alter the physiological performance of many marine organisms. It is vital that the effects of ocean acidification and warming on marine organisms are explored so that we can predict how marine communities may change in future. In particular, the effect of ocean acidification and warming on host-parasite dynamics is poorly understood, despite the ecological importance of these relationships. Here, we explore the response of one himasthlid trematode, *Himasthla* sp., an abundant and broadly distributed species of marine parasite, to combinations of elevated temperature and pCO_2 that represent physiological extremes, pre-industrial conditions, and end of century predictions. Specifically, we quantified the life span of the free-living cercarial stage under elevated temperature and pCO_2, focussing our research on functional life span (the time cercariae spend actively swimming) and absolute life span (the period before death). We found that the effects of temperature and pCO_2 were complex and interactive. Overall, increased temperature negatively affected functional and absolute life span, e.g. across all pCO_2 treatments the average time to 50% cessation of active swimming was approximately 8 h at 5 °C, 6 h at 15 °C, 4 h at 25 °C, and 2 h at 40 °C. The effect of pCO_2, which significantly affected absolute life span, was highly variable across temperature treatments. These results strongly suggest that ocean acidification and warming may alter the transmission success of trematode cercariae, and potentially reduce the input of cercariae to marine zooplankton. Either outcome could substantially alter the community structure of coastal marine systems.

1. Introduction

The rapid production of CO_2 since the Industrial Revolution, primarily through fossil fuel combustion, has dramatically increased atmospheric pCO_2, and caused a concomitant increase in CO_2 absorbed by the oceans. The cumulative effect of adding such vast quantities of CO_2 to the atmosphere and ocean is an increase in air and water temperatures (Intergovernmental Panel on Climate Change IPCC, 2014) and a decrease in seawater pH (Caldeira and Wickett, 2003). As there has been no significant reduction in CO_2 emissions since the advent of the Industrial Revolution, nor is there any globally coordinated effort to do so currently, these alterations to the marine environment are predicted to become more extreme by the end of the current century (Intergovernmental Panel on Climate Change IPCC, 2014). In addition, recent research has shown that the global ocean has actually absorbed more atmospheric heat energy than previously thought, which could lead to a further acceleration of warming (Cheng et al., 2019). These stressors, and their combined effects, have the potential to change many aspects of life in the ocean, and have been identified as clear research priorities for marine scientists (Halpern et al., 2008; Rudd, 2014).

Given the potentially wide-ranging physiological effects of ocean acidification and warming (OAW) on individual species (see meta-analysis in Harvey et al., 2013), biological interactions that underpin community structure will likely also be altered by these stressors. Interactions such as competition and predator-prey dynamics have been studied under acidified and warmed conditions (see review in Nagelkerken and Munday, 2016). In each case, the underlying assumption has been that the outcome of any inter-specific interaction may shift, e.g. a strengthening, weakening, or reversal of competitive dominance, if one species in an interacting pair proves to be more susceptible to changing abiotic conditions than the other (Kordas et al., 2011). In this context,

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identifying how interacting species are affected by environmental change is vital to understand how ecosystem and community structure may shift in the very near future. Despite this increased interest in the effects of OAW on biological interactions, there has been little work conducted on the effect of these stressors on parasitism, one of the most common life history strategies on the planet, which by definition involves the interaction of two species (Poulin and Morand, 2014).

Here, we investigate the effects of OAW on trematode parasites, a ubiquitous component of all coastal marine ecosystems. Trematodes have complex life cycles commonly involving three hosts and two free-living life stages (Galaktionov and Dobrovolskij, 2003). The first intermediate host of the trematode life cycle is typically a gastropod, within which free-living cercariae are produced asexually by sporocysts or rediae and periodically released to infect the second intermediate host, e.g. gastropods, crustaceans, bivalves, or fish. Upon successfully infecting an appropriate secondary host, cercariae will form metacercariae and await a predation event to transfer infection to the final definitive host, often a shore bird, fish or mammal, the site of sexual reproduction by adult trematodes (Galaktionov and Dobrovolskij, 2003). Eggs produced by adult trematodes are released from the definitive host via its faeces, hatching to become free-living miracidia which infect a new first intermediate host, thus completing the life cycle. Due to the complexity of this multi-host life cycle, a single trematode species can affect a community at multiple trophic levels via one or more of its hosts (Marcogliese and Cone, 1997). Trematode parasites can therefore regulate host populations through host castration, sublethal and lethal effects (Lafferty and Kuris, 2009) or by mediating competition interactions between the host and other sympatric species (Combes, 1996). In this way, a change in parasite prevalence caused by OAW could lead to a corresponding change in host mortality, fecundity, predation risk, and/or competitive dynamics of host individuals and host populations.

To date, only a small number of experiments have been completed on the effect of climate change on trematodes, most of these being single stressor experiments on cercariae (Poulin, 2006; Thieltges and Rick, 2006; Studer et al., 2012a, 2012b). Cercariae are a crucial dispersal phase of the trematode life-cycle, responsible for locating, identifying, and infecting suitable target hosts (Morley, 2011). Cercariae emerge from snail hosts when conditions are optimal for locating and infecting the next host in their life cycle (Combes et al., 1994). This often means that cercariae emerge from snail hosts at low tide, triggered by rising temperatures of tide pool seawater. This results in cercariae emerging into relatively small volumes of water, increasing the probability of encountering their desired host. Cercariae emerge from infected snails in large numbers, some species producing up to several thousand per day (Poulin, 2006), and make a significant contribution to the zooplankton biomass, providing a substantial amount of energy to non-host predators (Thieltges et al., 2013). They are also lecithotrophic, i.e. they rely on finite energy reserves, which may contribute more energy to marine food chains than planktotrophic zooplankton (Morley, 2012).

Cercariae are appropriate subjects for climate change research because they may represent the weak link in the trematode life cycle (Pietrock and Marcogliese, 2003), as they are directly exposed to changing abiotic conditions in the environment. Several studies have examined cercarial responses to climate drivers, e.g. ocean acidification (Harland et al., 2015; MacLeod and Poulin, 2015), ocean warming (see reviews in Poulin, 2006; Morley and Lewis, 2013), and there are many studies that examine the effect of temperature on cercariae from a purely physiological perspective (see Section 4). Cercariae exposed to decreased pH exhibited decreased life spans (MacLeod and Poulin, 2015), while the magnitude of the negative effect was highly species-specific. Similarly, elevated temperature caused cercariae life spans to be significantly reduced (Thieltges and Rick, 2006; Studer et al., 2010). Although the effect of pH and temperature on cercarial survival have both been examined to some extent, there remain many aspects of cercarial biology that are unexplored in the context of multi-stressor simulations of climate change (MacLeod, 2017). Through effects on cercariae, changing oceanic pH and temperature may affect parasite population dynamics, parasite-host relationships, and parasite-parasite competition within an ecosystem.

Here, we describe a multi-stressor experiment that spanned a wide range of temperatures: 5 °C, 15 °C, 25 °C, 40 °C; and six pCO_2 levels: 280 ppm, 317 ppm, 455 ppm, 587 ppm, 853 ppm, and 1600 ppm. During exposure, we measured the functional and the absolute life span of cercariae: we defined the functional life span as the period immediately after emergence from the snail, during which the cercaria swims constantly and with precise and repeated body movements; and the absolute life span as the more conventional definition, i.e. the period prior to death (Pechenik and Fried, 1995). In these experiments, we used cercariae of the trematode parasite *Himasthla* sp. (Family Himasthlidae), which is found in intertidal ecosystems throughout the Pacific northwest (Tkach et al., 2016). *Himasthla* sp. uses the marine snail *Littorina scutulata* as its first intermediate host, and a range of marine bivalves as second intermediate hosts (Ching, 1991).

Our objectives were to answer a series of key ecological questions: (i) will OAW reduce the transmission window of cercariae by reducing their functional life span? (ii) will OAW reduce the time cercariae persist as food items in the intertidal marine environment by reducing their absolute life span? (iii) are cercariae better adapted to pre-industrial pCO_2 values relative to current conditions? and (iv) do cercariae possess the tolerance to abiotic conditions exhibited by adult trematodes which are exposed to the warm, acidic microenvironment of the definitive host?

2. Materials and methods

2.1. Snail collection and screening for parasitic infection

We collected approximately 10,000 *L. scutulata* snails from the rocky intertidal zone at Bluestone Point, Vancouver Island, Canada (48°49′12.3″N 125°09′51.5″W), between May and July, 2017. *Littorina scutulata* have been documented as hosts to various trematode parasites in British Columbia, Canada (Ching, 1991). We gathered snails haphazardly from the intertidal zone at low tide, preferentially selecting larger individuals as they showed a greater rate of infection (personal observation). After collection, snails were transported to the University of British Columbia, housed within a recirculating seawater table, and provided with either *Fucus distichus* or *Ulva* spp. ad libitum. The snails were then allowed to acclimate in the seawater table for 1 week before screening for infection.

To detect trematode infection, snails were placed in 24-well plastic culture plates filled with seawater and moved to incubators (Panasonic MIR 154, USA) set at 25 °C under strong light for approximately 12 h, conditions which stimulate cercarial release (MacLeod and Poulin, 2015). After incubation, each well was inspected under a stereo-microscope for the presence of cercariae; a snail was considered infected if cercariae were found within the well and uninfected if no cercariae were present. To facilitate tracking individual snails, numbered, colour-coded tags (The Bee Works, Ontario, Canada) were attached to the shells of infected snails before those were returned to the aquaria. Of the five species of trematode parasites found at Bluestone Point, we chose *Himasthla* sp. to use in our experiments, as it was the most abundant (see Fig. 1 for image of a cercaria). We identified this species

Fig. 1. Image of a *Himasthla* sp. cercaria. Scale bar = 50 μm.

by comparing morphological parameters with published descriptions of trematodes that infect *L. scutulata* (Ching, 1991). Morphological parameters were recorded using ImageJ software to analyse photographs taken using a compound microscope (Leica DMIL LED, Canada) and digital camera (Leica MC120HD, Canada – see Supplementary Table S1).

2.2. Experimental apparatus (OAW simulation)

The experimental apparatus consisted of four temperature-controlled incubators (Panasonic MIR 154) fitted with a custom designed CO_2 delivery system. In order to establish precise pCO_2 treatment conditions, ambient air was stripped of moisture and CO_2 using a desiccant (WA Hammond, Drierite, USA) and Soda Lime (Ormond Veterinary Supply Ltd., Ontario, Canada), respectively, and mixed with CO_2 enriched air (15% CO_2, PraxAir Canada Inc., Canada) using Smart-Trak® mass flow controllers (Sierra Instruments, Inc., USA). The resultant CO_2/air mixture was pumped into a small Perspex chamber (30 cm × 22 cm × 25 cm) inside each of the four incubators to minimize the volume of gas required to achieve pCO_2 treatment levels. A Qubit S151 CO_2 gas analyser (Qubit Systems, Kingston, Ontario, Canada) was used to monitor pCO_2 concentrations within the chambers during the experiment. In addition to monitoring pCO_2 with the gas analyser, we measured the pH of seawater-filled blank wells, placed in the chambers with the test wells, throughout each trial. pH was measured using a hand-held pH meter (Oakton pH 450 (±0.01 pH), Oakton Instruments, IL USA) calibrated with two saltwater buffers, as described in Macleod et al. (2015), to ensure that our pCO_2 manipulation had successfully simulated the effects of altered atmospheric CO_2 on seawater chemistry (see Table 1 and Supplementary Fig. S1).

2.3. Experimental design

Six pCO_2 and four temperature treatments were used in this fully crossed experiment: 280 ppm, 317 ppm, 455 ppm, 587 ppm, 853 ppm, and 1600 ppm, and 5 °C, 15 °C, 25 °C, and 40 °C. Control pCO_2 (317 ppm) was based on in situ conditions measured at Bluestone Point during daylight (Sunday et al., 2011; K. Anderson, 2018. Algal-herbivore interactions in a high carbon world: direct and indirect effects through individuals, populations, and communities. PhD Thesis, University of British Columbia, Canada). Three of the elevated pCO_2 treatments were chosen by calculating the predicted increase in atmospheric CO_2 (IPCC, 2014) relative to the current global average (400 ppm) and adding that value to in situ CO_2 measurements: 455 ppm (RCP4.5), 587 ppm (RCP6.0), 853 ppm (RCP8.5). An extreme treatment (1600 ppm) was included to examine the physiological limits of cercariae, and a pre-industrial pCO_2 (280 ppm) treatment to better understand biological changes that may have occurred since the beginning of the Industrial Revolution.

Temperature treatments (5 °C, 15 °C and 25 °C) were selected to cover the range of in situ values experienced, recorded at the collection site (K. Anderson, PhD Thesis, cited earlier). We also chose a high temperature treatment of 40 °C, which was chosen over a logically spaced high treatment of 35 °C because the body temperature of most shore birds is approximately 40 °C (McNab, 1966), and theoretically, cercariae of himasthlid parasites that use a bird as their definitive host should have the genetic ability to survive temperatures the adult parasite would experience (Vernberg, 1961). Pilot trials indicated that 50% of *Himasthla* sp. cercariae ceased swimming after 15 h and died after approximately 25 h under ambient conditions (400 ppm and 20 °C), so we chose to monitor cercariae for 28 h at 4 h intervals.

Table 1
Abiotic characteristics of modified seawater in all temperature/pCO_2 combinations.

Temperature (°C)	Target pCO_2 (ppm)	Measured pCO_2 (ppm)	pH (S.D.)	DIC (μmol/kg)	Salinity (S.D.)
5	280		7.94(0.083)	1680.77	34.6(0.54)
	317		7.86(0.043)	1686.13	33.2(1.30)
	455		7.84(0.061)	1675.74	35.2(1.09)
	587		7.75(0.068)	1679.78	30.4(1.51)
	853		7.67(0.020)	1700.88	27.6(1.91)
	1600		7.46(0.029)	1684.82	34.8(2.16)
15	280		7.95(0.030)	1678.51	36.2(1.03)
	317		7.88(0.022)	1682.73	33.4(1.34)
	455		7.85(0.033)	1668.52	34.0(0.70)
	587		7.76(0.059)	1678.08	29.0(1.00)
	853		7.68(0.091)	1706.71	29.5(1.70)
	1600		7.45(0.039)	1721.81	33.8(1.09)
25	280	272.07(15.23)	7.96(0.025)	1694.51	30.8(1.42)
	317	314.78(15.93)	7.92(0.010)	1714.67	31.4(1.67)
	455	452.64(18.69)	7.90(0.036)	1720.04	33.8(1.16)
	587	570.64(18.10)	7.82(0.04)	1694.64	29.6(1.14)
	853	860.85(16.99)	7.70(0.002)	1740.45	30.1(1.38)
	1600	1642.42(31.05)	7.54(0.074)	1719.24	34.4(1.81)
40	280		8.08(0.050)	1723.8	35.4(1.57)
	317		8.00(0.010)	1751.34	35.2(1.42)
	455		7.95(0.013)	1741.36	32.6(1.94)
	587		7.86(0.013)	1743.01	32.6(1.40)
	853		7.77(0.029)	1780.81	35.1(1.03)
	1600		7.61(0.09)	1735.98	33.8(1.14)

DIC, Dissolved Inorganic Carbon.

2.4. Exposure trials

Twelve survival trials were conducted from August 24th 2017 to January 3rd 2018 with at least 7 days between each trial so that the parasites could generate more cercariae within snail hosts. Trials were conducted by setting four incubators to the four treatment temperatures, and supplying each incubator with one of the treatment pCO_2 levels, such that cercariae were exposed to each pCO_2 treatment (6) at each temperature treatment (4) for a total of 24 groups. Some trials were repeated on multiple dates (280 ppm, 317 ppm, 455 ppm, and 587 ppm), allowing us to compare results from the same parasite individual and rule out any effects of time (see Section 2.5 and Supplementary Table S2).

All snails infected with *Himasthla* sp. were stimulated to shed cercariae as described in the screening procedure (Section 2.1), although snails were kept in the incubator for a shorter time and monitored more frequently to standardise the age of cercariae used in our trials (<1h). Individual cercariae were pipetted into seawater-filled wells of a 96-well culture plate (volume 360 μL), and in total 40 cercariae were used from each infected snail with 10 per treatment combination. Not all infected snails shed cercariae before each trial, which lead to a different number of parasite individuals being tested in any given trial (see Supplementary Table S3); typically, snails are initially infected by one miracidium which then produces rediae/sporocysts of a single genotype. Consequently, all of the cercariae produced by these sporocysts or rediae also have the same genotype, such that the number of infected snails corresponds with the number of parasite individuals. After transferring cercariae to the 96-well culture plates, they were moved to the Perspex chambers within the incubators, marking the start of the 28 h trial. Every 4 h, cercariae were examined under a stereomicroscope and given a score from 1 to 3: 1 – alive and fully functional (i.e. still swimming as they were when they were released from the snail); 2 – alive but no longer able to swim properly (i.e. crawling along the bottom of the well); and 3 – non-motile, presumed dead. Most cercariae exhibit one of two common methods of motion: continuous swimming or swim-pause-swim behaviour (see Morley, 2011). During preliminary observations of cercariae, we observed that this species moves continually when alive; consequently, we found that observing each cercariae for 5 s was sufficient to observe movements indicating its alive/dead status. We considered a score of 2 to indicate that a cercaria was functionally dead, i.e. they were unlikely to be able to find a suitable host. If a cercaria scored 3, it was considered dead in both the functional and absolute sense. Also during each inspection, small volumes (<5 μL) of low salinity seawater were added to each well to avoid elevated salinity caused by evaporation. To monitor changes in seawater chemistry, 24-well culture plates (volume 3.4 mL) filled with seawater were placed in each incubator at the same time as the cercariae. At the end of each trial, 15 mL of seawater were taken from these test plates, transferred to Falcon tubes (FisherScientific), fixed with mercuric chloride (RICCA Chemical Company, TX, USA), and stored at room temperature. After all trials had been completed, these samples were analysed for dissolved inorganic carbon (DIC) content (Apollo Sci-Tech [Model AS-C3], USA). Seawater from each 24 well plate was also used to record pH_T and salinity (Table 1).

2.5. Data analysis

The cercarial life span (functional and absolute) was analysed using a Cox Proportional Hazard mixed effect model, using the function *coxme* in the *coxme* package (Therneau, 2012, Mixed Effects Cox Models, http://r-forge.r-project.org) in R (Version 3.4.0). This model provides a hazard response based on the time of death of individuals associated with a given treatment combination. Here, we used this technique to analyse times of functional and absolute death. As cercariae from each snail were assumed to be genetic clones (Galaktionov and Dobrovolskij, 2003), and therefore not independent, an average functional or absolute life span was calculated from the 10 cercariae associated with each parasite individual in each treatment combination. Further, because individual parasites were used in multiple trials, "Parasite ID" was included as a random effect, to account for repeated measurements of the same individual. In our analysis, temperature and pCO_2 were used as fixed effects, plus the interaction of these factors, and time of functional or absolute death was used as the response variable. As described above, some trials were repeated to increase replication and allow for a test of the effect of trial date. Consequently, we ran our model with and without these duplicate snail data; no difference was found between analyses. Additionally, the absolute life span of one trematode parasite tested in duplicate at 280 ppm, 317 ppm, and 587 ppm pCO_2 was analysed in order to identify any unidentified differences between duplicate trials. Here, survival models were used to test for the effect of "Trial" within each temperature/pCO_2 combination, e.g. for data generated at 5 °C we used a Cox proportional hazards model to test for a significant effect of "Trial" on absolute life span.

3. Results

Overall, the negative effect of elevated temperature on the life span of cercariae, functional and absolute, was more pronounced than the effect of different levels of pCO_2. Our 40 °C treatment resulted in the lowest life spans at all pCO_2 levels (Fig. 2A and B). Although the 5 °C treatment showed the longest functional life spans, in all other combinations, the effects of pCO_2 and temperature did not produce a straightforward effect on functional or absolute life span (Fig. 2A and B). For both functional and absolute data, temperature had a significant effect, while only absolute life span was significantly affected by pCO_2 and the interaction between the two fixed effects (Table 2).

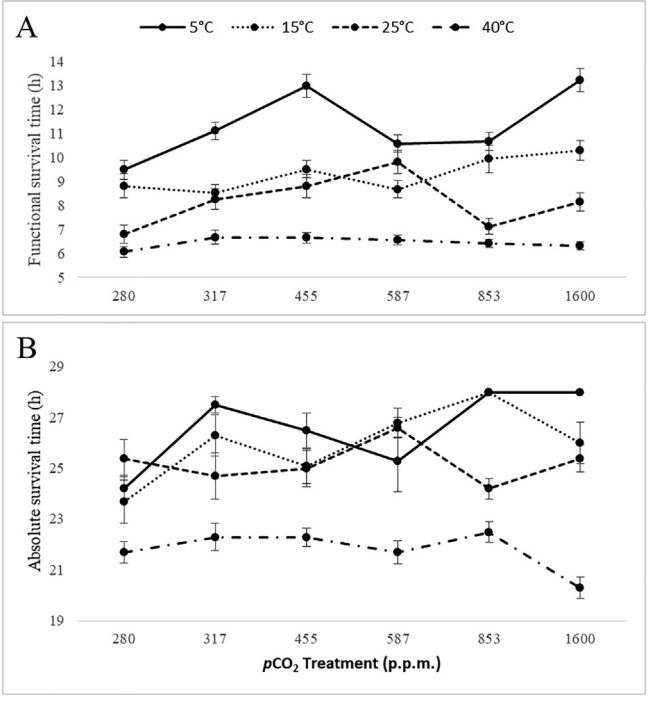

Fig. 2. Mean life span (±S.E.) time of cercariae maintained at four temperatures and six pCO_2 levels. (A) Functional life span and (B) absolute life span.

Table 2
Cox proportional hazard mixed effect analysis output of pCO_2 and temperature effects on the survival of *Himasthla* sp. cercariae. Bold text indicates significant values ($P < 0.05$).

		|z|	*P*
Functional life span	Temp	6.32	**<0.001**
	pCO_2	1.57	0.120
	Temp:pCO_2	1.43	0.150
Absolute life span	Temp	8.21	**<0.001**
	pCO_2	3.08	**0.002**
	Temp:pCO_2	3.37	**<0.001**

Functional life spans varied between 6 and 13 h, with the maximum found in the 5 °C treatment and the minimum in the 40 °C treatment. At 5 °C, there was an initial increase in life span as pCO_2 rose from pre-industrial levels to 455 ppm (RCP4.5), followed by a reduction at 587 ppm (RCP6.0) and 853 ppm (RCP8.5) before increasing again at 1600 ppm. At 15 °C, the functional life span was approximately 9 h, although there appeared to be an overall increase in longevity as pCO_2 increased. At 25 °C, the functional life span exhibited a gradual increase as pCO_2 increased until 587 ppm (RCP6.0), after which point it decreased dramatically. Finally, at 40 °C, the functional life span was uniformly low (approximately 6 h) at all pCO_2 levels.

The absolute life span of cercariae varied between 20 and 28 + h across treatments, although many cercariae were alive at the end of the 28 h trial, particularly at 5 °C (see Supplementary Figs. S2–S4). Similarly to functional life span, the absolute life span at 40 °C was the lowest at all pCO_2 levels. However, there was considerable overlap in absolute life span between 5 °C, 15 °C, and 25 °C across all pCO_2 levels (Fig. 2). The significant temperature × pCO_2 interaction likely arose because the rank order of temperature treatments varied with CO_2 concentration (compare 280 and 317 ppm), and because cercariae exposed to 40 °C did especially poorly at the highest CO_2 concentration.

Analysis of a cercariae taken from a single snail used in duplicate trials (three pCO_2 levels (317 ppm, 455 ppm, and 587 ppm) at up to four temperatures for a total of nine treatment combinations showed no significant differences in absolute life span between trials for any treatment combination, except at 40 °C at 587 ppm, where the *P* value was 0.05 (See Supplementary Tables S2 and S3).

4. Discussion

Previous research that investigated the effects of climate change on cercariae has predominantly focussed on increased water temperature. In fact, from a purely physiological perspective, there is an extensive literature on the cercarial response to thermal stress (see review by Morley, 2011). Generally, cercarial survival and activity is negatively affected by elevated temperatures, putatively explained by increased rates of energy consumption. Far fewer studies have investigated the effects of acidified seawater on cercarial survival, although of the two publications that have done so (Koprivnikar et al., 2010; MacLeod and Poulin, 2015), the effect of pH was also negative. To date, only a single paper has investigated the combined effects of OAW on cercarial survival (Leiva et al., 2019), which reported that acidification and warming had synergistic, negative effects.

In this experiment, we further develop the investigation of OAW on cercariae by partitioning cercarial life span into functional and absolute categories: the functional life span being the period during which we assume the cercariae are most likely to infect the next host in the life-cycle, based on swimming activity; and the absolute life span to determine the time that cercariae persist in their habitat as a component of the zooplankton community. Working with a himasthlid species greatly facilitated this approach, as most members of this family exhibit constant swimming behaviour, rather than periodic bouts of swimming activity, until energy stores are depleted (Morley, 2012). *Himasthla* sp. cercariae exhibited altered functional and absolute life spans when exposed to different combinations of temperature and pCO_2. We found that increased temperature was strongly correlated with reduced cercarial life span, both functional and absolute. This reduction is likely caused by the lecithotrophic life history of cercariae, i.e. they do not feed after emerging from their snail host (Galaktionov and Dobrovolskij, 2003). Consequently, cercariae have relatively short life spans, typically lasting between 60 and 160 h under ideal conditions (e.g. Lo and Lee, 1996; McCarthy, 1999), although cercarial longevity is sensitive to temperature variability (Morley, 2011). As expected, we observed a linear decrease in functional and absolute life spans in response to increasing temperatures, agreeing with the findings of previous research. As with the studies discussed above, we assume that the elevated temperatures accelerated physiological processes and increased the rate of glycogen consumption by cercariae. Of note, the cercariae maintained in 40 °C seawater lived for much longer than expected; some even remained alive in the absolute sense at the end of the trial (28 h).

Although the effect of temperature was largely consistent with theoretically-based predictions, the effect of pCO_2, particularly as it interacted with temperature, was more complex. Again, taking the lecithotrophic lifestyle of cercariae into consideration, it is logical to expect that an increased pCO_2 level, and therefore decreased pH, would increase energy utilisation. Many studies have found that the change in hydrogen ion concentration that underpins reduced pH can cause physiological challenges that require additional energy consumption to rectify/moderate (e.g. oxygen consumption – Queirós et al., 2015; heart rate – Lim and Harley, 2018). Analogous research conducted on the lecithotrophic life-stages of non-parasitic organisms has repeatedly shown that exposure to reduced pH and associated acid-base challenges has resulted in increased energetic expenditure (Dupont et al., 2010a). It is interesting to note, however, that the lecithotrophic strategy is actually thought to be more tolerant of stressors associated with OAW than the planktotrophic strategy used by the majority of zooplanktonic life-stages of non-parasitic organisms (Dupont et al., 2010b).

Despite our expectations, we did not find a consistent effect of pCO_2 across temperatures on functional or absolute cercarial life spans. For example, the cercariae exposed to extreme pCO_2 levels did not consistently exhibit shorter life spans, functional or absolute, than those at present-day or pre-industrial conditions. In fact, in some trials the cercariae exposed to extreme pCO_2 levels were found to have the longest life spans, again both functional and absolute. These trends suggest that while the combined effects of OAW do affect cercariae, the nature of the interactions is specific to the exact combination, e.g. high temperature/low pH versus low temperature/high pH. The underlying mechanism may be physical, i.e. the difference in CO_2 solubility in low versus high temperatures, which would lead to lower pH at lower temperatures, or biological, i.e. there are combinations of abiotic factors that cercariae can withstand, while other combinations are less tolerable.

The contrast between the straightforward effect of temperature and the variable effect of pCO_2 are intriguing in the context of our initial expectation that both stressors would have a negative effect on cercarial life span. Here, we discuss two potential scenarios that could explain the tolerance *Himasthla* sp. cercariae exhibit to low pH conditions, and their ability to survive in 40 °C seawater for longer than expected. To complete its life cycle, an individual

trematode must survive high temperature and low pH microenvironments within host organisms: cercariae and metacercariae encounter relatively acidic conditions (7.4–7.5 pH) when exposed to extracellular fluid within the second intermediate bivalve host (Michaelidis et al., 2005; Zhao et al., 2017); and upon excystment of the metacercaria, the adult trematode is briefly exposed to the highly acidic stomach fluids of the avian host (1–4 pH, Lee et al., 2017), and the internal temperature of the final bird host can be as high as 40 °C (McNab, 1966). Consequently, one would predict that *Himasthla* sp. could have evolved the ability to survive both extreme heat and acidic conditions encountered within its various hosts. Our second explanation relates to the abiotic conditions directly experienced by cercariae in a tide pool habitat, as they emerge from the gastropod host. Respiration can drive a tide pool pH to extremes of 7.5 pH, especially at night when the buffering effect of photosynthesis is absent (e.g. McElroy et al., 2012). Similarly, during a hot summer day, the temperature in a tide pool can be as high as 28 °C (Pincebourde et al., 2009). Consequently, *Himasthla* sp. will be regularly exposed to these extreme abiotic conditions, and may have developed tolerance in order to survive long enough to locate and infect the next host in their life cycle.

Also contradictory to our expectations were the results concerning the effect of pre-industrial pCO_2 (280 ppm) on cercarial longevity. For the most part, the cercariae exposed to 280 ppm pCO_2 had increased mortality compared with control pCO_2. One might expect that cercariae should be better adapted to pre-industrial pCO_2 levels, given the short evolutionarily time period since the Industrial Revolution, but this was not clear from our data. This may indicate that adaptation to current conditions has already occurred, despite the short time-scale, or that acclimatisation or intergenerational effects have shifted the optima to conditions associated with ~400 ppm pCO_2.

We also observed that most cercariae lost coordinated swimming ability before the 12 h checkpoint. If swimming activity is an indicator of their ability to infect the next host in their life cycle, this may suggest that there is strong selection pressure for cercariae to be most active immediately after they are released while the tide is still out (between 4 and 8 h) and a weaker selection pressure for cercariae to be active after that time point. Theoretically, cercariae have the highest probability of finding and infecting an appropriate bivalve host during low tide since tide pools are small, relatively calm bodies of water compared with the turbulent conditions experienced at high tide, which would drive selection to make them most active during this time (Combes et al., 1994). Additionally, cercariae can be consumed by a wide variety of non-host predators, e.g. fish, crabs, anemones, barnacles and molluscs (Studer et al., 2013), and so the longer a cercariae is free swimming, the more likely it is to be consumed. This would again cause strong evolutionary pressure on cercariae to be most active in finding a host within the first few hours of being released, despite the fact that this would also simultaneously make them vulnerable to predation. Our findings that the post-functional period is also abbreviated by increased temperature and, in some cases, pCO_2, suggests that the cercarial component of the zooplankton community could be reduced as a consequence of OAW. Given the large numbers of cercariae produced by a single infected snail, any reduction in the absolute life span of cercariae could reduce the food available to planktivores, especially in locations with high parasite prevalence, causing reductions in the carrying capacity of these populations on a local scale.

The results of this study indicate that the effects of ocean acidification and warming on cercariae may not be as straightforward as expected, especially when compared with previous studies that reported a strong deleterious effect of reduced seawater pH on cercarial survival (MacLeod and Poulin, 2015). Since increased temperature showed a clear negative effect on cercarial life spans, we may expect the prevalence of these trematode species to decrease if rising temperatures were the only abiotic factor changing due to human activities. However, rising atmospheric CO_2 levels also affect cercarial life span in conjunction with warming, which lessens our predictive ability. Experimental manipulation of pCO_2 levels did not produce simple, linear patterns in terms of biological responses, and the nature of CO_2 as a driver is further complicated by interactions with temperature and species-specific responses, thus further experiments of this kind are recommended. Ultimately, however, it is highly likely that the combined effects of OAW will modify the performance of cercariae in the marine environment, which could significantly alter the role of parasites as either regulators of host populations or as a food resource for non-host species.

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Appendix A. Supplementary data

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