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Temperature and pCO_2 jointly affect the emergence and survival of cercariae from a snail host: implications for future parasitic infections in the Humboldt Current system

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ABSTRACT

Ocean warming and acidification are general consequences of rising atmospheric CO_2 concentrations. In addition to future predictions, highly productive systems such as the Humboldt Current System are characterized by important variations in both temperature and pCO_2 level, but how these physical–chemical ocean changes might influence the transmission and survival of parasites has not been assessed. This study experimentally evaluated the effects of temperature (14, 18 and 25 °C) and the combined effects of temperature (~15 and 20 °C) and pCO_2 level (~500 and 1400 microatmospheres (µatm) on the emergence and survival of two species of marine trematodes—Echinostomatidae gen. sp. and Philophthalmidae gen. sp.—both of which infect the intertidal snail *Echinolittorina peruviana*. Snails were collected from intertidal rocky pools in a year-round upwelling area of the northern Humboldt Current System (23°S). Two experiments assessed parasite emergence and survival by simulating emersion-immersion tidal cycles. To assess parasite survival, 2 h old cercariae (on average) were taken from a pool of infected snails incubated at 20–25 °C, and their mortality was recorded every 6 h until all the cercariae were dead. For both species, a trade-off between high emergence and low survival of cercariae was observed in the high temperature treatment. Species-specific responses to the combination of temperature and pCO_2 levels were also observed: the emergence of Echinostomatidae cercariae was highest at 20 °C regardless of the pCO_2 levels. By contrast, the emergence of Philophthalmidae cercariae was highest at elevated pCO_2 (15 and 20 °C), suggesting that CO_2 may react synergistically with temperature, increasing transmission success of this parasite in coastal ecosystems of the Humboldt Current System where water temperature and pH are expected to decrease. In conclusion, our results suggest that integrating temperature-pCO_2 interactions in parasite studies is essential for understanding the consequence of climate change in future marine ecosystem health.

1. Introduction

Anthropogenic CO_2 emissions have increased considerably in the past 250 years and have driven considerable changes in the global climate (e.g., patterns of ocean circulation and precipitation, rising sea levels, ocean warming), known collectively as climate change (Doney et al., 2012). Two important, direct consequences of this climate perturbation are increased ocean temperature (ocean warming) and ocean acidification (OA) (Doney et al., 2012). Estimations of future oceanic CO_2 concentrations under more pessimistic scenarios suggest a warming of 3.7 °C and a decline of 0.2–0.4 pH units by the end of this century (IPCC, 2014). However, in upwelling areas such as those in the Humboldt Current System (HCS), ocean temperatures are expected to decrease as upwelling favorable winds increase due to climate change (Bakun and Weeks, 2008), thereby promoting the upwel-

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ling of cold water that has low levels of dissolved oxygen but is supersaturated with CO_2, resulting in a lower seawater pH (Torres et al., 2011).

The coastal areas of the HCS are characterized by geographic and temporal variations in key environmental variables related to climate change such as temperature (~15–21 °C between 18°S and 23°S; ~11–13 °C between 33°S and 41°S during autumn and summer periods) (Camus, 2001; Sobarzo et al., 2007) and partial pressure of CO_2 (pCO_2, >400 microatmospheres (μatm) between 21°S and 37°S and decreased pCO_2 levels southward, ~37°S during the austral spring/summer period) (Torres et al., 2011, Vargas et al., 2017). Physical/chemical gradients within coastal habitats can affect the behavior (e.g., predator avoidance), physiology (e.g., metabolic rate), and adaptive potential of native marine populations (e.g. Melatunan et al., 2011; Manríquez et al., 2013; Aguilera et al., 2016). For example, Lardies et al. (2014) and Vargas et al. (2015) assessed reactions of key physiological traits (oxygen consumption, clearance and ingestion rates) of benthic mollusk species inhabiting different geographic localities of the HCS under elevated pCO_2. These studies found that the species sensitivity to pCO_2 variations depended on their geographic origin. Furthermore, those studies suggest that species residing in highly variable pCO_2 southern areas (ca. 39°S) are inherently more tolerant of OA-like conditions than those inhabiting the northern areas (above 30°S) where the pCO_2 variability is lower.

Intertidal rocky pools are characterized by temporal and spatial short-term fluctuations in their local environment, especially by daily variations in tide cycles and solar radiation (Hernández et al., 2002). Therefore, marine organisms living in the intertidal zone (e.g. gastropods) face substantial temporal variation in habitat suitability, in that they are exposed to extreme fluctuations in temperature, desiccation and oxygen (Morris and Taylor, 1983; Mouritsen and Poulin, 2002). For example, in the Chañaral Bay (26°15′S) in northern Chile, the rocky pools had mean values (±S.D.) of temperature and pH between 15.7 ± 2.2 and 19.3 ± 3.5 °C, and 7.79 ± 0.07 and 8.10 ± 0.06, respectively, during the autumn and summer (Andrade et al., 2006). In these partially closed systems, parasitism is recognized as a major factor contributing to the structure of intertidal communities, regulation of host populations, and ecosystem biodiversity (Mouritsen and Poulin, 2002; Poulin and Mouritsen, 2006). Yet, parasites are also susceptible to the strong ambient variations of rocky pools, which may directly or indirectly influence their prevalence, intensity and abundance (Pietrock and Marcogliese, 2003). Low ambient pH conditions can increase the metabolic demands of acid–base regulation for parasites, thus influencing other traits such as transmission (Harland et al., 2015) and survival (MacLeod and Poulin, 2015). In parallel, higher temperatures may increase stress in the organisms, making them more susceptible to diseases caused by opportunistic pathogens (Lafferty et al., 2004).

Trematodes are the most common parasite group found in coastal ecosystems (Mouritsen and Poulin, 2002), and most use mollusks as their first intermediate host; crustaceans, mollusks, fish and polychaete worms as a second intermediate host and vertebrates (birds, fishes, or mammals) as a definitive host (Cribb, 2005; Leung et al., 2009). The free-living stages (i.e. eggs, miracidia and cercarial larvae) of trematodes are directly affected by various abiotic parameters that influence their emergence (Koprivnikar and Poulin, 2009), transmission (Lei and Poulin, 2011), and survival (Studer and Poulin, 2013). For example, the production and emergence of cercarial larvae (infective stage) from snails is accelerated by an increase in the ambient temperature, and their survival is reduced by a deviation from their optimum environmental parameters (Poulin, 2006; Thieltges and Rick, 2006). In addition, lower aquatic pH levels may influence the energy budget that cercariae require to maintain homeostasis, reducing their longevity and thus decreasing the likelihood the parasite reaches its next host within its life-cycle (Macleod and Poulin, 2012). Furthermore, experimental studies have shown that physiological responses of parasites to OA and ocean warming can also vary depending on the parasite taxa involved (e.g. Poulin, 2006; Koprivnikar and Poulin, 2009; Koprivnikar et al., 2010; Macleod and Poulin, 2015).

Knowing more about the intra- and inter-specific parasite responses to natural environmental fluctuations can help us better understand their responses to future oceanic conditions associated with climate change. In this study, we experimentally tested the single effect of temperature (14, 18 and 25 °C), and the combined effect of temperature (~15 and 20 °C) and pCO_2 level (~500 and 1400 μatm) on the emergence and survival of two marine trematode species, which parasitise the intertidal snail *Echinolittorina peruviana*. These snails commonly inhabit the supratidal and upper intertidal shores of the southeastern Pacific, between Panamá and central Chile (Guzmán et al., 1998), where they are exposed to significant thermal fluctuations. Particularly in those supratidal and upper intertidal microhabitats at Antofagasta, Chile, the temperature can increase 3–5 °C above sea temperature during the daytime ebb tides together with pH reductions from mean values (±S.D.) of 7.730 (0.013) to 7.514 (0.077) (P.H. Manríquez, unpublished data). Since those changes are reverted when the pools are filled by bulk water, this suggests that species inhabiting those pools are exposed to acute high variability in terms of temperature and pCO_2 levels. However in the long-term, sessile or low-movement species inhabiting those microhabitats will be exposed to the global trend of warming and acidification. Additionally, high pCO_2 (low pH conditions) can generate physiological stress in host snails (Kroeker et al., 2010; Melatunan et al., 2011), compromising their immune response and triggering an increase in cercarial production (Guilloteau et al., 2016); and consequent cercarial emergence. Therefore, it is expected that the combined exposure to chronic high temperature and pCO_2 levels would have a synergistic impact on the emergence and survival of these parasites.

2. Materials and methods

2.1. Study area

The study was conducted in San Jorge bay (23°30′S), a semi-enclosed system oriented to the south of the Península de Mejillones, Antofagasta, northern Chile (Valdés et al., 2011). Although this bay is under the direct influence of year-round upwelling and the environmental fluctuations this process generates, surface waters are warmer than adjacent coastal areas (Piñones et al., 2007). Biological activity, upwelling and environmental factors (e.g. seasonality) can produce variations in carbonate parameters, leading to a wide range (300 to 1000 μatm) in pCO_2 levels in coastal upwelling areas off the northern Chilean coast (Torres et al., 1999, 2002; Lefèvre et al., 2002; Vargas et al., 2017). In the rocky intertidal habitats of San Jorge Bay, variations in seawater temperature depend mainly on the intertidal level and the season (Pulgar et al., 2005). The average daily seawater temperature in the upper intertidal area is higher in summer (15 to 30 °C) than in winter (14 to 20 °C) (Pulgar et al., 2005, 2007).

2.2. Effects of temperature on the emergence and survival of cercariae

2.2.1. Snail collection, acclimatisation and experimental design

Between 14 April and 9 June in 2017, 935 individuals of *E. peruviana* (7.0–14.6 mm in shell length) were collected from the upper rocky intertidal zone of Coloso in San Jorge Bay (23°45′S; 70°28′W). This sampling was carried out during the lowest daily tide. The snails were collected by hand, placed in plastic containers, and transported immediately to the laboratory (Universidad de Antofagasta, Chile), where the snails were distributed in plastic wells and examined for parasites. Three experimental series ('ES') for three temperature treatment levels (i.e., 14, 18 and 25 °C) were performed. Details of the snail sample sizes used in each ES are given in Table 1.

To determine the proportion of infected individuals (prevalence), each snail was put into a separate well (4 cm diameter) and kept dry for 30 min, after which 30 ml of seawater were added to each well. The emergence of cercariae was verified after 6 h of immersion by direct observation under a stereomicroscope (Leica MZ6, Leica Microsystem, Wetzlar, Germany). Infected snails were maintained for an extra period of 5 days at room temperature (approximately 18–20 °C) in aerated seawater and fed with sea lettuce (*Ulva* spp.). The identification of trematode species was made based on cercarial morphology (Martorelli, 2003).

After 5 days of acclimatisation, the infected snails from each temperature treatment (see Table 1) were randomly allocated to two cylindrical plastic aquaria (n = 6 aquaria in total, with 10 snails per aquarium). Each plastic aquarium (10 cm diameter) was filled with 200 ml of filtered seawater (at 1.0 μm), snails were fed with sea lettuce (*Ulva* spp.), and maintained under the three experimental temperature levels (14, 18 or 25 °C) for a 48 h period. During this period, any cercariae which emerged from the snails were not counted.

2.2.2. Emergence of cercariae

For each temperature treatment, the snails were individually placed in plastic wells (7.4 cm diameter, 100 ml) and incubated in a cooling chamber, with 20 replicates used per treatment. These snails were maintained for 4 days at 12:12 h dry:immersion cycles (in total, 48 h of emersion and immersion each). In the emersion cycle, the plastic wells were incubated at the treatment air temperature and the immersion cycles were performed by adding seawater at the same temperature. The emerged cercariae were counted at 24 h intervals during the immersion cycles (i.e., at 24, 48, 72 and 96 h) by using the same stereomicroscope described in Section 2.2.1.

2.2.3. Survival of cercariae

Experiments were run separately for two trematode species (the most prevalent species). At each experimental temperature, 45 cercariae (2 h old on average) were taken from a pool of infected snails, were distributed among three plastic wells (4 cm diameter, with 15 cercariae each) filled with 30 ml of aerated seawater and incubated at 25 °C (used in the 2.2.2. emergence cercariae experiment) in a constant temperature chamber. To avoid handling stress affecting the cercariae, the water in the wells was renewed every 24 h with new water at the same temperature, thus avoiding a change in salinity and temperature in the wells. Cercarial mortalities were checked with a stereomicroscope at intervals of 12 h until all cercariae were found dead (~60 h). Stimulation with a fine needle was used to check for and confirm a cercarial death, following Anderson and Whitfield (1975). For Philophthalmidae gen. sp. three categories were generated: alive cercariae, dead cercariae that were differentiated by their lack of motility, and encysted cercariae (metacercariae) which were considered to be alive in the survival analysis.

2.3. Combined effects of temperature and pCO_2 on cercarial emergence and survival

2.3.1. Snail collection, acclimatisation and experimental design

A total of 890 snails were collected in August 2017 from the study site. The infected snails (n = 130) were identified as before (described in Section 2.2.1) and transported under wet conditions to the Universidad Católica del Norte, Coquimbo, Chile (29°58′S). Prior to the experiment, individuals were kept for 5 days in an aquarium, immersed in a temperature-controlled water table (~20 °C) with running seawater and fed with sea lettuce (*Ulva* spp.). After this period the infected snails were randomly allocated to 12 plastic boxes that each contained one 12-well multiwell plate (accommodating one snail per well), and semi-immersed in each type of temperature-controlled water table (~15 veraus 20 °C) and maintained under the two experimental pCO_2 levels (500 versus 1400 μatm) for a 48 h period. We chose 15 °C as the annual mean temperature during the austral winter in the collection site (P.H. Manríquez unpublished data). However, the 5 °C warming represents the mean temperature increase for coastal waters of northern Chile during intense El Niño events (Torres et al., 2003) or acute warming of intertidal pools at Antofagasta during summer months (P.H. Manríquez unpublished data). In terms of pCO_2 levels, the higher level (1400 μatm) represents the worst-case scenario for the beginning of the next century (Meinshausen et al., 2011; IPCC, 2014), at a site where average values never reach above 1000 μatm (Vargas et al., 2017). However, the control level (500 μatm) is near the average coastal values recorded during most of the year at the collection site (Vargas et al., 2017; our unpublished observations).

During the experimental period, the snails were stimulated to shed cercariae before the experiment was conducted, essentially emptying the snails of cercariae and standardizing the amount of cercariae shed per snail. Any cercariae which emerged during this period were not counted. The lid of each 12-well multiwell plate was modified by drilling one hole (2 cm in diameter) above each well (12 wells per plate) and a piece of 250-μm mesh placed between the lid and the plate was used to retain the released cercariae (Fig. 1). For each combination of temperature and pCO_2

Table 1
Mean (±S.D.) and median values of cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. from the snail *Echinolittorina peruviana*, with its mean shell length (SL ± S.D.), for each temperature level (14 °C, 18 °C and 25 °C) and experimental series (ES1–3).

ES (total N)	Temperature (°C)	Mean ± S.D.	Median (50%)	SL ± S.D. (mm)
1 (265)	14 (n = 23)	1.5 ± 3.1	0	9.98 ± 1.45
	18 (n = 23)	6.6 ± 13.3	0	9.43 ± 1.29
	25 (n = 20)	111.5 ± 139.3	67	9.66 ± 1.28
2 (320)	14 (n = 22)	0.3 ± 1.1	0	9.43 ± 1.13
	18 (n = 23)	12.9 ± 17.1	4	10.14 ± 1.11
	25 (n = 22)	111.1 ± 168.0	55	9.75 ± 0.80
3 (350)	14 (n = 22)	3.0 ± 14.3	0	10.26 ± 1.38
	18 (n = 23)	7.0 ± 11.3	1	9.84 ± 1.36
	25 (n = 22)	130.8 ± 127.8	63	10.86 ± 1.17

N, total number snails collected; n, number of snails used for each treatment.

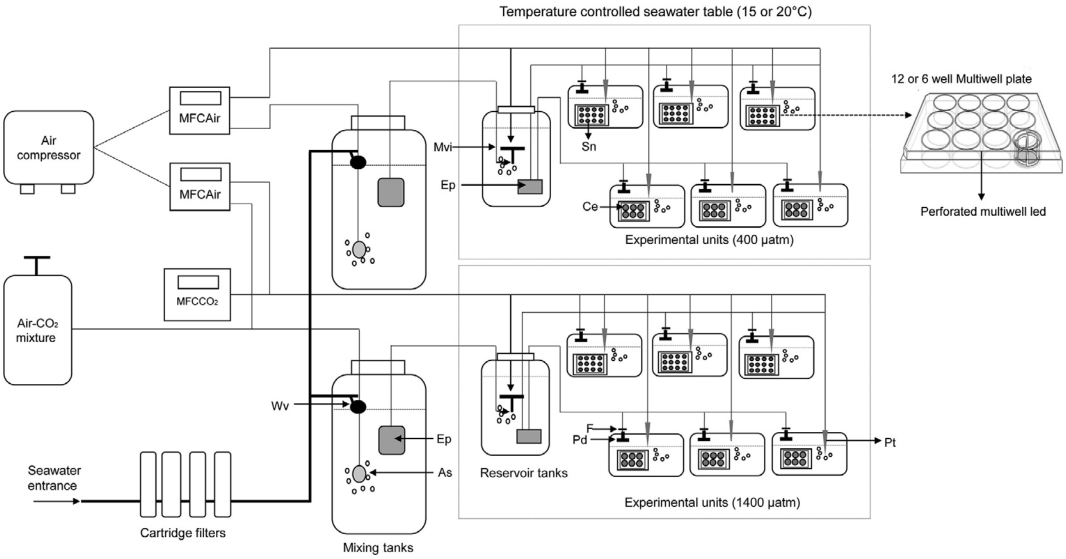

Fig. 1. Seawater-CO_2 equilibration system used to evaluate the combined effects of pCO_2 and temperature levels on the survival and emergence of cercariae of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. A flow-through CO_2 mixing system, as described by Torres et al. (2013), was used to manipulate pCO_2 levels in the experimental aquaria. Briefly, mass flow controllers (Aalborg®, Denmark, model GFC) were used to precisely mix CO_2-free air with pure CO_2 gas, which was then bubbled into four large (230 l), independent plastic reservoir tanks filled with 1.0 μm filtered seawater (FSW). Using an electric pump (Ep), treated seawater was delivered from the reservoir tank to equilibration tanks immersed in the temperature controlled seawater tables (~15 or 20 °C) by an electric pump. Within each equilibration tank, treated seawater was mixed with additional mixed gas using a Mazzei® Venturi injector (model 484) and then delivered to each experimental unit using another electric pump. A large air stone (As) placed at the bottom of each reservoir tank was used to release the CO_2-air mixture. Moreover, plastic tubing and pipette tips (Pt) were used to deliver and release the CO_2-air mixture into the aquaria containing the multiwell plate with experimental snails (Sn) or cercariae (Ce). At the entrance of each aquarium a disposable syringe-driven 0.22 μm filter (F) and a plastic dripper (Pd) were placed to regulate the entrance of treated seawater from the equilibrations tanks. The lid of each multiwell plate was modified by drilling one hole (2 cm in diameter) above each well and a piece of a 250-μm mesh placed between the lid and the plate was used to retain the released cercaria. All the experimental units were assigned and semi-immersed in two separate seawater tables (dashed line box), set at 15 and 20 °C, filled with seawater, and connected to two thermostatically controlled heat exchanger units sets at the corresponding temperatures. In the upper part of each mixing tank, a water level control float valve (Wv) was placed as a mechanical feedback mechanism to regulate the filling of the tanks with fresh seawater.

levels, three replicates were used, for 12 in total (i.e., four treatment combinations).

Each plastic box (11 cm × 8.1 cm × 16.1 cm, 800 ml) was filled with filtered seawater (at 1.0 μm) conditioned to the required pCO_2 levels (500 and 1400 μatm). Each plastic box was modified by making two holes (each 2 cm in diameter); one hole for the air and CO_2 mixture intake, and another hole for the water input located on the upper side of the box. A third hole was located at the right side, at 6.1 cm above the base of the box, and used as an overflow to maintain the seawater level in the boxes. The air and CO_2 mixture, and water supply, were delivered to each box by using pipette tips (100 μl) connected via flexible silicone tubing. The water supply was controlled by a plastic drip-feed system, set to deliver water at a rate of 1 L h^{-1} (Fig. 1).

2.3.2. Seawater CO_2 mixing system

In our study, a flow-through CO_2 mixing system (Torres et al., 2013) was used to generate seawater at the two pCO_2 treatment levels: ~500 and 1400 μatm, respectively, for low and elevated pCO_2. Those nominal levels are in line with coastal ocean conditions observed in the upwelling areas of northern Chile (20°S–33°S) (Vargas et al., 2017).

For each experimental temperature (15 and 20 °C), two independent plastic reservoirs tanks (230 L) were filled with filtered seawater (at 1.0 μm). To obtain the CO_2 levels, seawater was equilibrated with atmospheric air, while the experimental CO_2 level was generated by blending air and pure CO_2 using a mass flow controller (MFC, model GFC, Aalborg, Denmark). From the plastic reservoir tanks, treated seawater was delivered to 20 L equilibration vessels immersed in the temperature-controlled water tables (~15 and 20 °C) by an electric pump. Then the seawater in the equilibration vessels was delivered to the aquaria containing the experimental snails by using a Mazzei Venturi injector (model 484, Mazzei Injector Company, Bakersfield, United States) (Fig. 1). These aquaria were used to maintain the seawater temperature at two level, ~15 and 20 °C, to thereby simulate the annual seawater temperature variations—average daily temperature in winter and summer, respectively—at high intertidal rocky pools in northern Chile (Pulgar et al., 2005, 2007).

Seawater parameters, namely pH, total alkalinity (TA), temperature, and salinity, were measured in each aquarium at the beginning and the end of each experiment (Table 2). Seawater samples from each aquarium were collected by using 60 ml plastic syringes and then transferred to a 25 ml closed cell at 25 °C. Seawater pH was measured at 25 °C using a pH meter (Metrohm model 713, Metrohm AG, Herisau, Switzerland) and a glass-combined double-junction Ag/AgCl electrode (Metrohm model 6.0219.100, Metrohm AG, Herisau, Switzerland) calibrated with 8.089 Tris buffer (buffer based on 2-amino-2-hydroxymethyl-1,3-propanediol) at 25 °C (DOE, 1994). Values of pH are reported on the total hydrogen ion scale (DOE, 1994). Temperature and salinity were measured using a CTD instrument (Ocean Seven, 304 Plus, Brugherio, Italy). Seawater samples for TA were stored in 250 ml plastic bottles and poisoned with a saturated $HgCl_2$ solution. TA was measured using an automated open cell titration system, described in detail by Haraldsson et al. (1997) and the accuracy was verified using certified reference material (CRM) supplied by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA). The pH, TA, salinity and temperature variables were used to calculate the rest of the carbonate system parameters (pCO_2 and dissolved inorganic carbon (DIC)) using CO2SYS software (http://cdiac.ornl.gov/oceans/co2rprt.html), set with the Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987).

Table 2
Mean values (±S.E.) of the seawater (SW) utilized to characterize the carbonate chemistry under contrasting temperature and $p\mathrm{CO}_2$ levels. Low and high $p\mathrm{CO}_2$ levels are based on those from Vargas et al. (2017) for the northern Chilean coast for the summer and winters months.

	Time	pH at 25 °C (pH units)	Temperature (°C)	Alkalinity (μmol kg^{-1})	$p\mathrm{CO}_2$ in situ (μatm)	[CO_3^{2-}] in situ (μmol kg^{-1} SW)	Salinity (PSU)
[a]Natural SW	T0	7.78 ± 0.04	14.21 ± 0.12	2263.94 ± 13.87	528.67 ± 55.68	121.68 ± 11.11	34.50 ± 0.04
[a]Natural SW	TF	7.80 ± 0.05	14.43 ± 0.57	2244.33 ± 38.13	497.94 ± 78.01	126.86 ± 11.75	34.59 ± 0.42
Expt. Emergence							
Low $p\mathrm{CO}_2$ (∼15 °C)	T0	7.79 ± 0.02	13.92 ± 0.03	2254.48 ± 17.11	504.41 ± 36.41	123.69 ± 5.04	34.58 ± 0.01
Low $p\mathrm{CO}_2$ (∼15 °C)	TF	7.72 ± 0.04	14.13 ± 0.03	2261.48 ± 9.92	611.68 ± 54.44	107.89 ± 8.65	34.42 ± 0.05
High $p\mathrm{CO}_2$ (∼15 °C)	T0	7.44 ± 0.02	13.93 ± 0.04	2278.36 ± 5.34	1293.58 ± 60.02	58.26 ± 2.22	34.88 ± 0.23
High $p\mathrm{CO}_2$ (∼15 °C)	TF	7.47 ± 0.01	14.05 ± 0.05	2288.30 ± 26.50	1208.79 ± 49.79	62.33 ± 0.97	34.64 ± 0.14
Low $p\mathrm{CO}_2$ (∼20 °C)	T0	7.87 ± 0.06	19.67 ± 0.11	2237.19 ± 11.67	504.74 ± 93.01	150.37 ± 18.75	35.17 ± 0.32
Low $p\mathrm{CO}_2$ (∼20 °C)	TF	7.81 ± 0.08	19.74 ± 0.22	2266.87 ± 17.09	608.22 ± 134.50	134.53 ± 21.91	34.62 ± 0.19
High $p\mathrm{CO}_2$ (∼20 °C)	T0	7.50 ± 0.02	19.51 ± 0.04	2308.33 ± 33.63	1357.23 ± 43.11	71.33 ± 4.00	34.94 ± 0.32
High $p\mathrm{CO}_2$ (∼20 °C)	TF	7.56 ± 0.04	19.73 ± 0.32	2272.81 ± 14.83	1162.72 ± 100.33	79.21 ± 6.81	34.41 ± 0.10
Expt. Survival							
Low $p\mathrm{CO}_2$ (∼15 °C)	T0	7.78 ± 0.02	14.33 ± 0.07	2257.30 ± 7.06	526.92 ± 31.56	121.60 ± 5.49	34.58 ± 0.04
Low $p\mathrm{CO}_2$ (∼15 °C)	TF	7.78 ± 0.04	13.98 ± 0.19	2279.65 ± 7.45	524.17 ± 63.26	123.58 ± 10.15	34.77 ± 0.07
High $p\mathrm{CO}_2$ (∼15 °C)	T0	7.47 ± 0.01	14.37 ± 0.01	2278.13 ± 10.39	1193.82 ± 30.30	63.06 ± 0.86	34.45 ± 0.06
High $p\mathrm{CO}_2$ (∼15 °C)	TF	7.45 ± 0.01	14.11 ± 0.06	2269.56 ± 13.03	1241.03 ± 32.44	60.21 ± 1.53	34.73 ± 0.35
Low $p\mathrm{CO}_2$ (∼20 °C)	T0	7.86 ± 0.05	19.65 ± 0.05	2315.65 ± 91.94	535.42 ± 66.53	153.37 ± 22.71	35.48 ± 0.44
Low $p\mathrm{CO}_2$ (∼20 °C)	TF	7.86 ± 0.02	20.10 ± 0.10	2279.96 ± 7.99	540.78 ± 33.69	147.35 ± 7.17	34.57 ± 0.11
High $p\mathrm{CO}_2$ (∼20 °C)	T0	7.54 ± 0.01	19.66 ± 0.06	2304.10 ± 21.24	1239.75 ± 25.98	76.98 ± 2.10	34.80 ± 0.14
High $p\mathrm{CO}_2$ (∼20 °C)	TF	7.54 ± 0.02	20.17 ± 0.13	2276.88 ± 2.36	1247.51 ± 63.23	77.13 ± 3.28	35.39 ± 0.42

[a] Parameters of the natural SW used to renew the reservoir tanks during the experiments were measured twice per week in near-shore water samples collected during daylight hours; therefore, the average temperature did not include day/night variations.Exp., experiment; T0, initial of the experiment; TF, final of the experiment; PSU, Practical Salinity Unit.

2.3.3. Emergence of cercariae

The infected snails (n = 130) were randomly assigned to the same four experimental treatment combinations, resulting in 10–12 infected snails used for replication (three replicates per treatment). Each trial consisted of 6 h long simulated emersion-immersion tidal cycles on the Chilean coast (in total, 60 h dry and 60 h immersion). In the emersion cycle, the multi-well plates were incubated at room temperature (approximately 18 °C) and the immersion cycles were performed within a mesocosm setup, in which the snails were exposed to each different experimental treatment for 5 days. The emerged cercariae were counted at 12 h intervals during the immersion cycle—i.e., 12, 24, 36, 48, 60, 72, 84, 90 and 120 h—under a stereomicroscope (Nikon SMZ-10, Nikon, Tokyo, Japan).

2.3.4. Survival of cercariae

For each experimental treatment, ∼90 cercariae of Echinostomatidae gen. sp. (average age: 2 h) were taken from a pool of infected snails incubated at 20 °C and 400 μatm (used in the cercariae emergence experiment described in Section 2.3.3) and distributed among 12 plastic boxes (11 cm × 8.1 cm × 16.1 cm, 800 ml) containing one 6 well multiwell plate (cercariae were located only in three wells, so 5–12 cercariae per well). Each plastic box was semi-submerged in one of two temperature-controlled water tables (∼15 °C versus 20 °C) and maintained at one of two experimental $p\mathrm{CO}_2$ levels (∼500 versus 1400 μatm) (Fig. 1). Each multiwell plate had been modified as per Section 2.4.1. For each treatment three replicates were used (n = 12 replicates in total). As described earlier, the survival curves for cercariae were obtained by checking their mortality at regular intervals of 12 h until all had died (∼60 h; following Anderson and Whitfield, 1975).

At the end of each experiment, each snail was sexed and its shell length (SL) measured with a vernier caliper (within 0.5-mm accuracy).

2.4. Statistical analysis

Analyses were conducted separately for each parasite species. The effects of temperature on the emergence of cercariae (count data) were analyzed in Generalized Linear Models (GLMs) with repeated measures, using the Poisson distribution for the response variable and the log link function (Venables and Ripley, 2002). The combined effects of temperature, time and $p\mathrm{CO}_2$ levels on cercarial emergence were analyzed by using nested GLMs (with $p\mathrm{CO}_2$ nested in temperature), with the negative binomial distribution used for the response variable and log link function. Goodness of fit was evaluated through deviance (D^2) that accounts for the percentage of variance explained by the model. Post-hoc analyses were performed with Tukey's Honestly Significant Difference (HSD) test (Day and Quinn, 1989), and the above analyses were implemented in R-Studio v1.1.383 software.

The shell length of the snails was compared among the three experimental series by one-way ANOVA; the SL variable was ln-transformed to meet assumptions of homogeneity of variances and normality. The Spearman correlation (r_s) was used to evaluate the association between the SL of snails and the number of emerged cercariae per host (Zar, 1999) as it has previously been reported that there are positive associations between SL and cercarial output (Poulin, 2006). In addition to obtained survival curves, tables of contingency (Chi-square tests) were performed to statistically evaluate the differences in larval survival at selected temperatures and times (Experiment 1) and at different $p\mathrm{CO}_2$ levels and times (Experiment 2). These statistical analyses were performed using Statistica v10.0 software. $P < 0.05$ were considered significant for all statistical analyses.

3. Results

Three digenean species, representing the families Echinostomatidae, Philophthalmidae and Schistosomatidae, were recorded as infesting the examined snails collected in northern Chile at Antofagasta. The Echinostomatidae gen. sp. cercariae were distinguished by having their anterior end modified and armed with spines (Kostadinova, 2005); Philophthalmidae gen. sp. cercariae were distinguished by having an adhesion gland in the posterior of their tail (Kanev et al., 2005); and Schistosomatidae gen. sp. were distinguished by having a forked (bifurcated) tail (Khalil, 2001). The experiments in this study used only the most prevalent species, Echinostomatidae gen. sp. and Philophthalmidae gen. sp.

3.1. Effects of temperature on cercarial emergence and survival

3.1.1. Characteristics of the sampled snails

From the collected snails ($n = 935$), 200 individuals (21.4%) were infected with one or more species of trematodes. Echinostomatidae gen. sp. was the most abundant and prevalent species in the snails (17.7%), followed by Philophthalmidae gen. sp. (3.6%), and Schistosomatidae gen. sp. (0.1%). The co-occurrence of two cercarial species in a snail was only observed in five snails (0.5%). At 14 °C, the emergence of cercarial species was almost zero; only 1–3 snails produced cercariae (Table 1). Therefore, the statistical analyses considered only data for temperatures 18 and 25 °C.

3.1.2. Emergence of cercariae

The number of emerged cercariae for both parasite species was significantly higher in the high temperature treatment (25 °C), but it varied considerably among individual snails (Table 3). The exposure time significantly affected the emergence of Philophthalmidae gen. sp. but not Echinostomatidae gen. sp. (except for ES2) (Fig. 2, Table 3).

Snail SL ranged from 7.0–13.2 mm in ES1, 7.1–12.6 mm SL in ES2, and 7.5–13.5 mm SL in ES3 (Table 1), and it differed significantly among the experimental series (one-way ANOVA; $F_{2, 196} = 4.38$, $P = 0.01$). However, cercarial emergence was not significantly correlated with SL of the snails in any of the experimental series (ES1 $r_s = -0.09$, ES2 $r_s = 0.14$ and ES3 $r_s = 0.18$, all $P > 0.05$).

3.1.3. Survival of cercariae

Cercarial survival of both trematodes species was significantly affected by temperature treatment (Table 4). At a high temperature (25 °C), mortality of Echinostomatidae gen. sp. increased from 36 h of incubation through to 60 h, when 100% of cercariae died (Fig. 3), while mortality of Philophthalmidae gen. sp. varied from 20% at 24 h to 50% at 48 h (Fig. 4). However, survival was not significantly affected by the low temperature treatment (14 °C) (Fig. 4); 90% remained alive even after 72 h of incubation. In the case of Philophthalmidae gen. sp., after 24 h of incubation 100% of cercariae had formed a metacercarial cyst on an open substrate at low temperature (14 °C); however, encystment success was evidently lower in the high temperature treatment (25 °C) (Fig. 4).

3.2. Combined effects of temperature and $p\mathrm{CO}_2$ on the emergence and survival of cercariae

3.2.1. Characteristics of the sampled snails

From the collected snails ($n = 890$), 130 (14.6%) were parasitized with one or more species of trematodes; Echinostomatidae gen. sp. was the most prevalent (13.3%), followed by Philophthalmidae gen. sp. (1.01%) and Schistosomatidae gen. sp. (0.3%). Co-occurrences of two species were observed in 12 snails (1.3%).

3.2.2. Emergence of cercariae

The temperature and $p\mathrm{CO}_2$ levels, together with exposure times, significantly affected cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. (Table 5). However, the deviance (D^2) was low (2.14%, Table 5). Cercarial emergence of Echinostomatidae gen. sp. was significantly higher at 20 °C than at 15 °C (Fig. 5), generating emission pulses every 12 h. At a low temperature and an elevated $p\mathrm{CO}_2$ (i.e., 15 °C + 1400 µatm), the emergence of Philophthalmidae gen. sp. cercariae was higher than at a low $p\mathrm{CO}_2$ level at 15 °C (Fig. 5) (Tukey's HSD test; $P < 0.001$).

The SL of snails ranged from 7.84–14.65 mm (Table 6) and was significantly different among the treatments ($F_{3, 126} = 4.74$, $P < 0.01$). However, the emergence of the cercariae was not significantly correlated with snail SL (all $P > 0.05$).

3.2.3. Survival of cercariae

Significant effects of temperature, exposure time and $p\mathrm{CO}_2$ level on cercarial survival were detected for Echinostomatidae gen. sp (Table 4). The survival time of its cercariae was shorter under increased temperature and $p\mathrm{CO}_2$ levels (20 °C + 1400 $p\mathrm{CO}_2$, Fig. 6), with mortality that varied from 60% at 48 h to 100% at 66 h (Fig. 6). Survival of the larval stage of the trematodes was not significantly affected by low temperature and $p\mathrm{CO}_2$ (15 °C + 500 $p\mathrm{CO}_2$) (Fig. 6). Since low numbers of emerged cercariae of Philophthalmidae were recorded, this species was not considered for this experiment.

4. Discussion

The free-living larval stages of trematodes are affected by natural factors such as temperature, salinity, pH, oxygen, water mineral content (hardness), light and ultraviolet radiation (Pietrock and Marcogliese, 2003). However, there are only two studies evaluating the effects of pH on the survival and emergence of larval trematode species (MacLeod and Poulin, 2015; Guilloteau et al., 2016) and

Table 3

Results of Generalized Linear Models (GLMs, repeated measures) for the effect of temperature (Te) and exposure time on cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. from the snail *Echinolittorina peruviana*. Statistically significant relationships ($P \leq 0.05$) are indicated in bold.

		Echinostomatidae				Philophthalmidae			
	Factor	Z value	P	AIC	D^2	Z value	P	AIC	D^2
S1	Te	18.337	**<0.001**	3460.6	42.14	14.422	**<0.001**	290.23	82.23
	Time	0.737	0.461			5.147	**<0.001**		
	Suj	4.509	**<0.001**			−1.177	0.239		
	Suj × Time	−0.752	0.452			−6.476	**<0.001**		
S2	Te	29.178	**<0.001**	5394.2	34.31	−5.617	**<0.001**	904.25	66.66
	Time	−2.989	**<0.01**			−6.751	**<0.001**		
	Suj	−5.022	**<0.001**			5.210	**<0.001**		
	Suj × Time	−1420	0.156			6.729	**<0.001**		
S3	Te	16.421	**<0.001**	3357.3	48.50				
	Time	0.611	0.541						
	Suj	7.933	**<0.001**						
	Suj × Time	−1.354	0.176						

S1–3: experimental series, AIC: Akaike criterion, D^2 (%): deviance, and Suj: Subject (= snail).

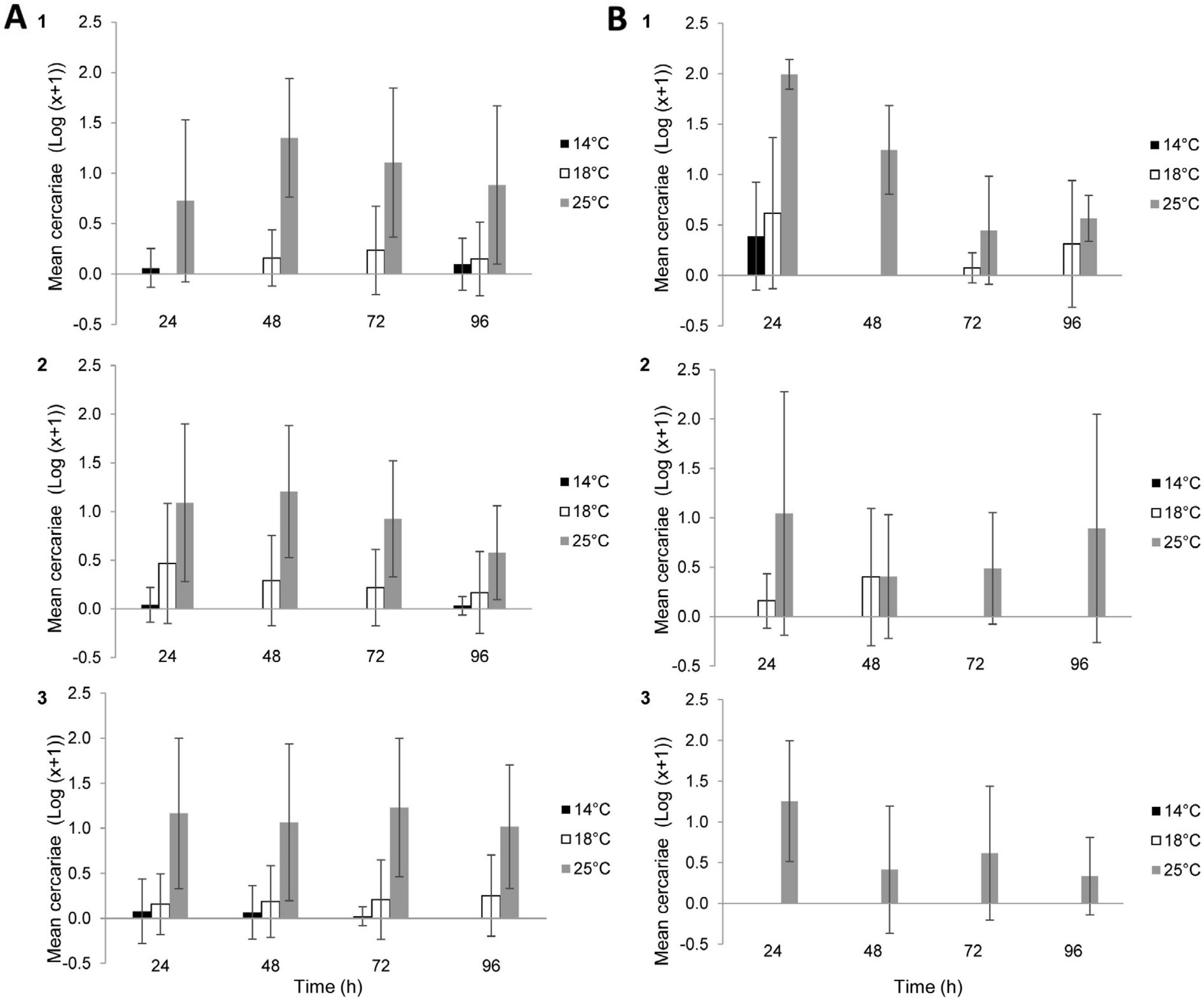

Fig. 2. Mean cercarial (±S.D.) emergence of Echinostomatidae gen. sp. (A) and Philophthalmidae gen. sp. (B), from the snail *Echinolittorina peruviana* in response to temperature for each experimental series (1, 2, and 3). Temperature treatments were 14 °C (dark bars), 18 °C (white bars), and 25 °C (gray bars).

Table 4
Results of tables of contingency (Chi-square tests) for the differences in larval survival of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. at selected temperatures and at several times (24, 48, 72 and 96 h; Experiment # 1) and at different pCO_2 levels and at several times (12, 24, 36, 48, 60, 72, 84, 90 and 120 h; Experiment # 2). Statistically significant relationships ($P \leq 0.05$) are indicated in bold.

		Echinostomatidae			Philophthalmidae		
	Experimental Series	Chi^2	GL	*P*	Chi^2	GL	*P*
Experiment # 1	S1	114.478	8	**<<0.001**	13.840	8	0.086
	S2	105.119	8	**<<0.001**	200.317	8	**<<0.001**
	S3	60.551	8	**<<0.001**	41.496	8	**<<0.001**
Experiment # 2		67.705	5[a]	**<<0.001**			

[a] Analysis was performed only at temperature 20 °C because larval survival was 100% at 15 °C.

there are not previously published studies that evaluate the combined effect of pH and temperature on cercarial survival and emergence. Our model species during low tides is sporadically exposed to high temperatures similar to that used in our experiment (20 °C). However, in our study the same high temperature (20 °C) had a positive effect on cercarial emergence for both Philophthalmidae gen. sp. and Echinostomatidae gen. sp., which may be associated with that metabolic processes would occur at a faster rate at elevated temperatures in the parasite and host snails (Poulin, 2006; Koprivnikar and Poulin, 2009; Koprivnikar et al., 2010; Castro-Rojas et al., 2015). Meanwhile, species-specific responses to the combination of temperature and pCO_2 were also observed. The emergence of Echinostomatidae cercariae was higher at 20 °C regardless of the pCO_2 levels. By contrast, the emergence of Philophthalmidae cercariae was higher at elevated pCO_2 levels (15 and 20 °C). Hence, these results might be useful for predicting parasite infection levels (and transmission rates) in marine organisms living in coastal ecosystems such as that of the HCS.

A trade-off between high cercarial emergence and diminished survival at high temperature was revealed in our study. Survival of Philophthalmidae gen. sp. and Echinostomatidae gen. sp. cercariae were both diminished at a higher temperature (25 °C) than

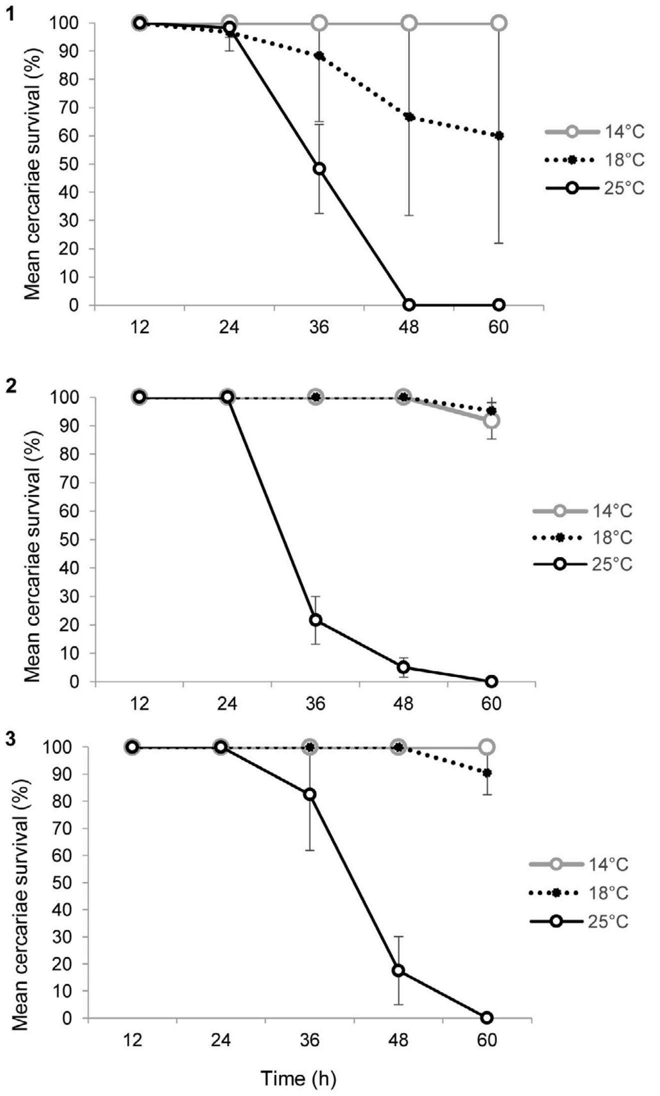

Fig. 3. Mean percentage (±S.D.) of surviving Echinostomatidae gen. sp. cercariae after 60 h of incubation in response to temperature for each experimental series (1, 2, and 3). Temperature treatments were 14 °C (gray line), 18 °C (dotted line), and 25 °C (black line).

at the lower temperatures (14 and 18 °C), where mortality was very low or even negligible over the study period. This appears to be a direct consequence of greater cercarial activity at higher temperatures, which would accelerate depletion of their energetic reserves in the form of glycogen losses (McCarthy, 1999; Poulin, 2006). However, Philophthalmidae gen. sp. cercariae were more temperature-resistant than were Echinostomatidae gen. sp. cercariae; more than 60% of the former stayed alive after 66 h of exposure to the high temperature. Specific responses of parasites exposed to high temperatures have also been reported for *Philophthalmus* sp. and *Maritrema* sp. infecting the snail *Zeacumantus subcarinatus*, where *Maritrema* cercariae were more heat tolerant than *Philophthalmus* cercariae (Bates et al., 2011).

The philophthalmid trematodes do not use a second intermediate host, forming a protective cyst on an open substrate, losing their tail in this process and becoming metacercariae (Prinz et al., 2011). The protective cyst may be a strategy for this species to survive adverse but temporary stressful environmental conditions, thereby increasing its probability of successful transmission (Morley, 2015). This study determined that at high temperatures, increased cercarial activity left less energy available for the process of cyst formation, which may explain the reduced encystment of Philophthalmidae gen. sp. cercariae we observed at 25 °C.

Guilloteau et al. (2016) demonstrated that cercarial production of *Philophthalmus* sp. and *Parorchis* sp. (family Philopthalmidae) in the snails *Z. subcarinatus* and *Austrolittorina cinca*, respectively (maintained at 25 °C), was positively affected by low pH conditions (= high pCO_2 level); that is, cercarial production increased. In our study, the emergence of the Philophthalmidae gen. sp. cercariae was also greater at elevated pCO_2 level (= low pH) but at 15 °C, while the emergence of Echinostomatidae gen. sp. was not affected by the pCO_2 levels (500 or 1400 μatm) at 20 °C. Low temperatures combined with high pCO_2 make seawater more acidic compared with the 20 °C treatment (see Table 2), given that the temperature affects the solubility of CO_2 in seawater (Teng et al., 1996; Fabry et al., 2009). Thus, the divergent pattern of cercarial emergence between these larval species could suggest differential tolerances at high pCO_2. It has been shown that host species of mollusks and crustaceans are vulnerable to stressors associated with OA that can affect a variety of physiological processes such as growth, calcification rates, development and survival (Kroeker et al., 2010). Guilloteau et al. (2016) determined low pH seawater may drive the host to use its energy to maintain essential physiological processes, leaving less energy for the parasite colony to subsist on. Therefore, in response to the combination of increased pCO_2 and low temperature, the trematode parasites may boost the release (which does not necessarily imply higher output) of cercariae from the snail host.

It has been determined that trematode larvae emerge from intermediate host snails according to a temporal rhythm which is synchronized with the presence of the next host (e.g. Toledo et al., 1998; Poulin, 2006; Castro-Rojas et al., 2015). The cercarial emergence of the Echinostomatidae gen. sp. increased significantly at high levels of temperature and pCO_2, generating emission pulses approximately every 12 h. Due to their relatively short lifespans (median survival of 24 to 48 h), many trematodes optimize cercarial emergence to enhance the probability of successful transmission (Toledo et al., 1998). Therefore, the pulses we found could facilitate host-parasite encounters, diminishing the energetic costs incurred by parasites upon emergence. Additionally, the temperature and pH fluctuations are often quite extreme in intertidal pools, mainly because water exchange is generally lower and restricted at the high tidal zone (Findlay et al., 2010). For gastropods infected with digenean larvae, cercarial emergence has been linked at high tidal levels (Fingerut et al., 2003; Castro-Rojas et al., 2015). Therefore, these extreme environmental conditions may favor cercarial emergence during tidal inundation, which should augment their dispersion and likelihood of contacting a host. Nevertheless, many mechanisms both endogenous (e.g., gene expressions) and exogenous (e.g., light/dark cycle, chemical signals) in the parasite and/or their hosts may also be involved (Toledo et al., 1998; Théron, 2015). To disentangle their relative roles, more studies of this phenomenon are obviously needed.

Chemistry and temperature of the water column have a major impact on the planktonic larval development, growth and success of recruitment to adult populations (Kurihara, 2008; O'Donnell et al., 2008). For example, Talmage and Gobler (2011) showed that increases in temperature and CO_2 concentration significantly affect the larval survival of two species of bivalves, *Mercenaria mercenaria* and *Argopecten irradians*. Similarly, the free-living stages of parasites can resist broad deviations in pH and temperature, but if those deviate from a species-specific optimum it can affect parasite survival and/or reduce their infectivity (Pietrock and Marcogliese, 2003; MacLeod and Poulin, 2015). In Echinostomatidae species, several studies have demonstrated that elevated temperatures can affect larval survival (Shostak, 1993; McCarthy, 1999; Korprivnikar et al., 2010). In our study, the cercarial survival of Echi-

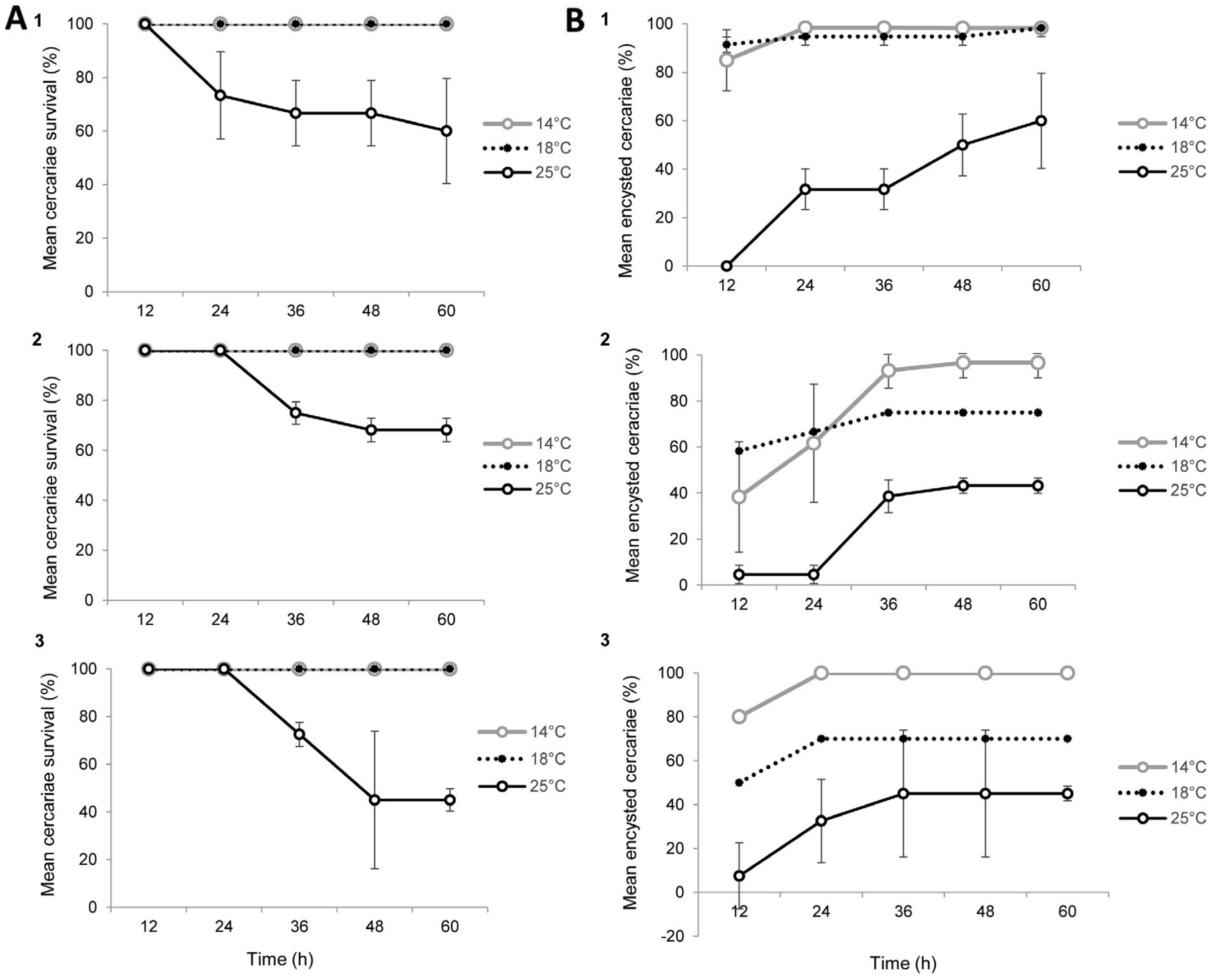

Fig. 4. Mean percentage (±S.D.) of cercarial survival (A) and mean percentage (±S.D.) of encysted cercariae (metacercariae) (B) of Philophthalmidae gen. sp. in response to temperature during each experimental series (1, 2, and 3). Temperature treatments were 14 °C (gray line), 18 °C (dotted line), and 25 °C (black line).

Table 5
Results of nested Generalized Linear Models on the effect of temperature (Te), *p*CO2, and time exposure to these conditions on cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. from the snail *Echinolittorina peruviana*. Statistically significant relationships ($P \leq 0.05$) are indicated in bold.

	Echinostomatidae					Philophthalmidae			
Factor	*Z* value	*P*	AIC	D^2	Factor	*Z* value	*P*	AIC	D^2
Te	3.671	**0.001**	6224.3	2.14	Time	−2.364	**0.1**	328.02	4.71
Time	3.857	**0.001**			pCO$_2$(Te(Time))	2.518	**0.01**		
pCO$_2$(Te)	2.079	**0.01**							

AIC, Akaike information criterion; D^2, (%) deviance.

nostomatidae gen. sp. decreased with elevated temperature and with elevated pCO$_2$, thus showing a negative synergistic effect of these two factors. Nonetheless, reductions in survival and other physiological aspects of parasites can vary distinctly between taxa (MacLeod and Poulin, 2015).

The free-living stages of trematodes are exposed to a highly variable environment in the intertidal zone, one that entails desiccation risks and fluctuations in the temperature, pH and oxygen conditions (Pietrock and Marcogliese, 2003; MacLeod and Poulin, 2012). The tolerance of these parasites to such environmental stresses may indicate sufficient phenotypic plasticity in certain species that enables them to survive in changing oceanic conditions (MacLeod and Poulin, 2015). These adaptive abilities are influenced by environmental conditions that marine organisms face throughout their life cycles, thus retaining the ability to adapt to a stressor when it becomes permanently present. Prosobranch gastropods, of the family Littorinidae, are highly tolerant of intertidal zone conditions and show different survival strategies such as shaping their groups to maintain their body temperature (Muñoz et al., 2005) and/or maintaining a decreased aerobic metabolism (Melatunan et al., 2011). Under natural conditions, the *E. peruviana* snails occurs in median and high intertidal zones, where it would experience long periods of emersion and would be affected by temperature changes and bouts of desiccation (Muñoz et al., 2005). It has also been suggested that free-living parasite life-stages can resist broad deviations in abiotic factors (Pietrock and Marcogliese, 2003), and some cercariae do tolerate a range of environmental conditions similar to that tolerated by their host (e.g., Shostak, 1993). This may well apply to the trematode species infecting the snail *E. peruviana*, but mainly to the

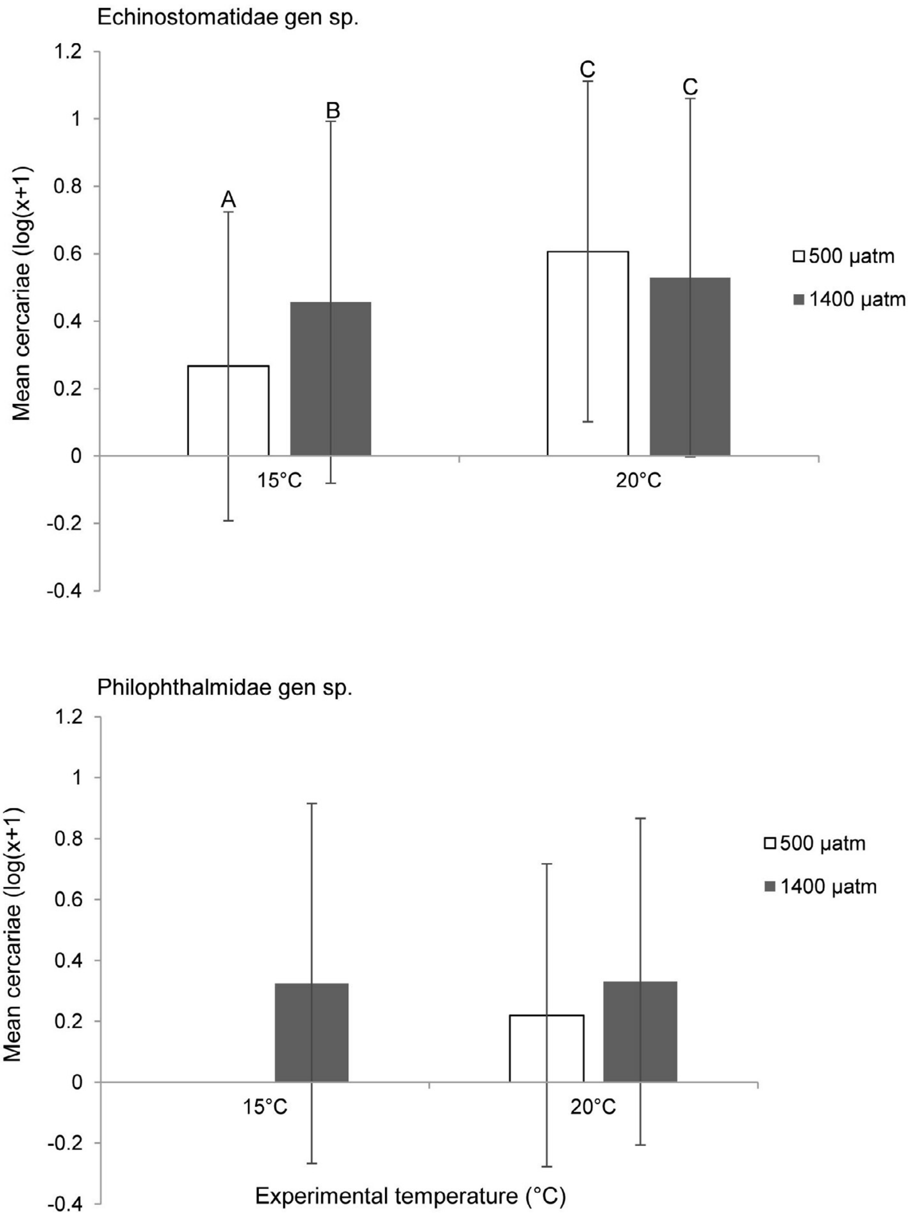

Fig. 5. Mean (±S.D.) cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. in response to temperature (15 °C and 20 °C) and partial pressure of CO_2 (pCO_2) levels (500 and 1400 µatm) at 1400 µatm (gray bars and gray line), 500 µatm (white bars and dotted line). Letters A, B and C indicate results of Tukey's Honestly Significant Difference tests. µatm, microatmospheres.

Table 6
Mean (±S.D.) and median cercarial emergence of Echinostomatidae gen. sp. and Philophthalmidae gen. sp. from the snail *Echinolittorina peruviana*, with its mean shell length (SL ± S.D.), for each combined treatment (A: 15 °C and 500 pCO_2, B: 15 °C and 1400 pCO_2, C: 20 °C and 500 pCO_2, and D: 20 °C and 1400 pCO_2) and replicate (R1, R2, and R3).

Treatment	Replicate	Mean ± SD	Median (50%)	SL ± SD (mm)
A	R1 (*n* = 12)	41.8 ± 47.9	18	11.31 ± 1.09
	R2 (*n* = 10)	33.2 ± 41.0	6	11.03 ± 1.11
	R3 (*n* = 10)	24.9 ± 30.5	10	11.68 ± 0.91
B	R1 (*n* = 10)	96.3 ± 138.3	36	11.60 ± 1.66
	R2 (*n* = 12)	88.4 ± 72.3	84	11.84 ± 0.99
	R3 (*n* = 10)	39.2 ± 27.5	26	11.83 ± 0.87
C	R1 (*n* = 12)	77.3 ± 44.4	83	10.02 ± 1.38
	R2 (*n* = 10)	81.1 ± 41.1	88	11.43 ± 0.93
	R3 (*n* = 12)	56.8 ± 52.0	39	10.80 ± 1.29
D	R1 (*n* = 11)	70.9 ± 42.6	13	11.19 ± 0.87
	R2 (*n* = 10)	69.3 ± 53.7	65	10.95 ± 0.83
	R3 (*n* = 11)	68.8 ± 77.2	49	11.17 ± 1.44

n, number of snails used in each treatment.

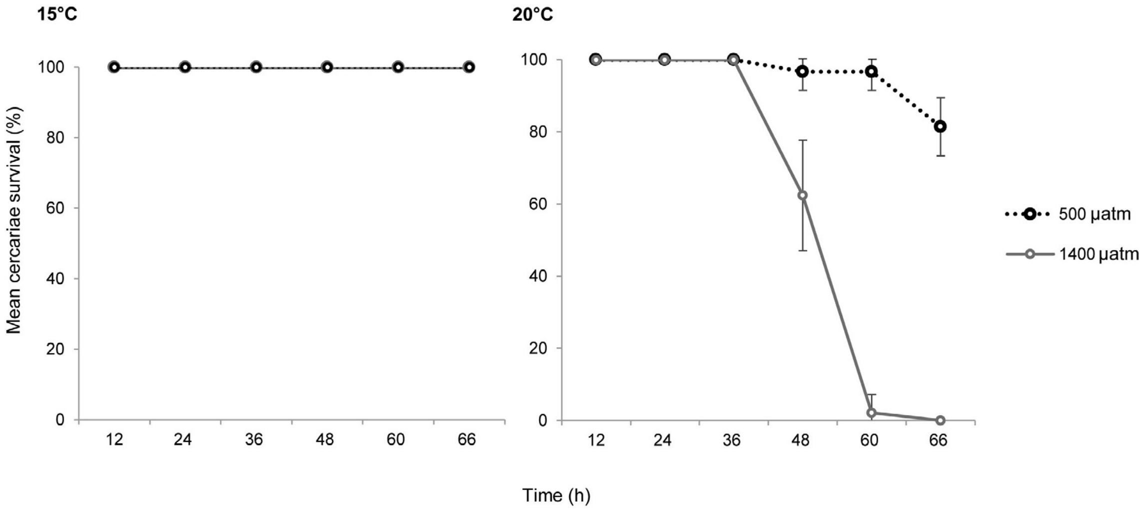

Fig. 6. Mean percentage (±S.D.) of surviving Echinostomatidae gen. sp. cercariae in response to temperature and partial pressure of CO_2 ($p\text{CO}_2$) levels at 1400 µatm (gray line), 500 µatm (dotted line). µatm: microatmospheres.

Philophthalmidae gen. sp. larvae that we found were highly tolerant to a wide range of temperatures and that showed increased cercarial emergence at elevated $p\text{CO}_2$. Therefore, in a near future scenario of climate change in HCS (i.e. increased $p\text{CO}_2$ and decreased temperature), we might expect greater cercarial emergence and transmission success to the next host by this trematode species in the intertidal environment.

In rocky intertidal pools, the biotic and abiotic factors—photosynthesis, respiration and daily variation in tides—tend to exert a greater impact in smaller volumes of water (Duarte et al., 2014). For example, Middelboe and Hansen (2007) reported that in marine environments with scarce water exchange the pH levels varied between mean values of 7.8 and 8.6. Additionally, Vargas et al. (2017) demonstrated the existence of contrasting spatial patterns in the $p\text{CO}_2$ levels among geographic latitudes along the Chilean coast, while Torres et al. (1999, 2002) have shown that episodic events of extremely high $p\text{CO}_2$ levels occur in the upwelling areas off central-northern Chile (30–40°S), reaching values of $\sim$1800 µatm. Nevertheless, natural spatio-temporal variability higher than the predicted values for the end of this century have been reported in some areas along the extensive HCS (Vargas et al., 2017). It is crucial to know the range of natural variations in temperature and $p\text{CO}_2$ in different coastal systems to better predict how both parasitic and host species will respond in the face of potential climate change scenarios. Since parasites play an important ecological role at the individual, population and ecosystem level (Mouritsen and Poulin, 2002; Poulin and Mouritsen, 2006), to determine the potential effect of near-term changes in temperature and $p\text{CO}_2$ levels on host-parasite interactions will facilitate a better understanding of future marine ecosystem health.

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