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ORIGINAL PAPER

Effects of temperature, salinity, and water level on the emergence of marine cercariae

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Abstract With the prospect of large-scale environmental changes, there is an urgent need to obtain information regarding environmental influences acting on the emergence of cercariae in marine systems. We investigated the response of trematodes of the intertidal snail *Zeacumantus subcarinatus* to altered temperature, salinity, and water level. The emergence of one trematode species, *Maritrema novaezealandensis* (Microphallidae), showed a weak trend to decrease with increased temperature; whereas, the emergence of a second species, *Philophthalmus* sp. (Philophthalmidae), increased at warmer temperatures. Both species exhibited increased cercarial emergence at the lowest salinity used (30 PSU). More *M. novaezealandensis* cercariae emerged when snails were kept partially submerged. In contrast, emergence of *Philophthalmus* sp. increased when snails were completely submerged. These results may reflect different transmission strategies employed by the two trematode species. Based on this model, we propose that trematode parasitism in intertidal zones is likely to be impacted by various changes in the marine environment resulting from global warming.

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Introduction

Various environmental factors may impact the emergence of larval trematodes (cercariae) from their molluscan first intermediate hosts. For example, the effects of temperature on cercarial emergence are well documented. Many trematode species display increased emergence in response to increased temperature (Lo and Lee 1996; Lyholt and Buchmann 1996; Mouritsen 2002a); however, decreased emergence in response to increased temperature has also been observed for *Renicola roscovita* and *Maritrema novaezealandensis*, using the molluscs *Littorina littorea* and *Zeacumantus subcarinatus*, respectively (Thieltges and Rick 2006; Koprivnikar and Poulin 2009).

Temperature-mediated cercarial emergence has largely been studied in freshwater systems, with relatively little known regarding temperature effects on the emergence of marine trematodes. However, abiotic conditions in marine environments, particularly intertidal systems, fluctuate in other ways that could affect cercarial emergence. A field study by Fingerut et al. (2003) determined that tidal currents are a predictable signal that determines both submersion time of the first intermediate hosts (*Cerithidea californica*) as well as the emergence and aquatic transport of trematode larvae in a Californian intertidal estuary. Laboratory studies using the same host and trematode species (*Himasthla rhigedana*, *Renicola buchanani*, *Euhaplorchis californensis*, and *Microphallid* sp.) showed that cercariae emerged only if snails were totally submerged (Fingerut et al. 2003). In contrast, Mouritsen (2002a) found that water level had no effect on the emergence of *Maritrema subdolum* from its molluscan host (*Hydrobia ulvae*).

Salinity is one of the most important marine environmental factors as it varies considerably in intertidal zones

and estuaries while being relatively constant in open seas (Berger and Kharazova 1997). Most marine systems have a salinity level of approximately 35 PSU, equivalent to 35 parts per thousand (Knauss 1978). In contrast, the salinity levels of intertidal zones and estuaries can fluctuate in response to tidal levels and freshwater input. For example, intertidal mudflats often have pools of water that can experience greatly elevated salinity as water evaporates at low tide or, alternatively, decreased salinity in areas of high rainfall as freshwater input increases during low tide. While salinity clearly has the potential to influence cercarial emergence in marine environments, little is known about its importance. Mouritsen (2002a) found greater cercarial emergence at higher salinity levels but only at elevated temperatures. However, it appears that cercarial emergence generally increases with increasing salinity within a range of naturally occurring values (Rees 1948; Sindermann 1960; Sindermann and Farrin 1962).

As relatively little is known about the effects of temperature on the emergence of marine cercariae and even less about the importance of water level and salinity, it is important to determine the effects of these environmental factors, particularly given that all three are likely to be impacted by global climate change (Marcogliese 2001). In this study, we examine the effects of temperature, water level, and salinity on the emergence of two marine trematode species from a New Zealand intertidal mud snail in order to determine their relative importance both within and between trematode species.

Materials and methods

Trematode species used

We chose to examine the two trematode species found to be most common while screening for infected snails (see below). The first, *M. novaezealandensis* (Microphallidae), uses the intertidal mud snail *Z. subcarinatus* as the first intermediate host, various crabs and amphipods as second intermediate hosts, and red-billed gulls as a definitive host (Martorelli et al. 2004). The life cycle of the second trematode, *Philophthalmus* sp. (Philophthalmidae), has not yet been elucidated, but this trematode uses *Z. subcarinatus* as its first intermediate host, encysts as metacercariae on substrate (including the plastic sides of experimental wells), and infects the eyes of vertebrate final hosts (Martorelli et al. 2008).

Sampling and screening

Approximately 800 *Z. subcarinatus* individuals were collected from Lower Portobello Bay (45°47′S, 170°42′E) on the South Island of New Zealand from November 2006 to February 2007. Snails were haphazardly collected by hand at low tide from the low water mark to approximately three fourths of the way toward the high tide margin. After transportation to the University of Otago (Dunedin), snails were kept in the laboratory in 2 L plastic containers filled with artificial seawater at 35 PSU containing 200–300 individuals each. Snails were kept at ambient temperature (15–23°C) with a natural photoperiod and were supplied with sea lettuce (*Ulva reticulata*) collected from Otago Harbour.

To screen for infected individuals, groups of five snails were placed in individual wells of cell-well plates containing 3 ml of artificial seawater (35 PSU) each and kept in an incubator overnight at 28°C before being examined the next morning for the emergence of cercariae. Snails infected by *M. novaezealandensis* were 9.2–15.5 mm in shell length, while snails infected by *Philophthalmus* sp. were 10.9–19.9 mm in shell length, with all snails considered to be adults. Individuals found to be infected were then kept in separate containers in the same conditions as described above.

Experimental procedure

The experiments were conducted in an environmental chamber set at 15°C with a 14:10 light: dark cycle. In addition, two incubators were used within the chamber. Both had glass doors to allow exposure to the light conditions. One was set at 20°C, and the other was set at 25°C.

Two experiments were conducted: the first examined the respective and combined effects of salinity and temperature, while the second focused on the respective and combined effects of water level and temperature. Cell-well plates consisting of 12 wells (6 ml each) were used. For the salinity experiment, each well was filled with 3 ml of artificial seawater at 30, 35, or 40 PSU using solutions made with Red Sea Salt® and distilled water. The lowest concentration is in keeping with lower values recorded for intertidal mudflats (e.g., Cheng et al. 1993), while the highest concentration can occur as a result of evaporation in tide pools during low tide. For each trematode species, 36 infected snails were randomly assigned to the three salinities, resulting in 12 infected snails for each salinity level. For the water level experiment, each well was filled with either 3 ml (low level) or 6 ml (high level) of artificial seawater at 35 PSU. The low water level left part of the snail exposed to air; whereas, snails in the high water level treatment were fully submerged. Snails in the low water treatment were thus able to partially emerge from the water, but snails in the high water treatment were prevented from doing so by lids. For each trematode species, 24 infected snails were randomly assigned to the two water levels, resulting in 12 infected snails for each water level.

Experiments were run separately for the two trematode species; however, the salinity and water level experiments

for each species were conducted simultaneously. The experiment using *Philophthalmus* sp. began at the beginning of February 2007, with the plates kept at 15°C for 24 h. After this time-period, snails were individually transferred into new cell-well plates in which the water was pre-warmed to 20°C. Cercariae and encysted metacercariae left in the wells of the initial plates were then counted using a dissection microscope. After 24 h at 20°C, snails were transferred to new plates containing water pre-warmed to 25°C, with the same counting procedure. The snails were subsequently transferred back down to 20°C for 24 h, and finally down to 15°C in order to avoid abrupt and potentially stressful temperature changes that could influence host mortality, but cercariae were not counted. At that time, snail length was measured using callipers and snails were returned to their original containers.

The exact same procedure was used for *M. novaezealandensis*-infected snails at the beginning of March with one exception: given the small size of *M. novaezealandensis* and the large numbers of cercariae typically emerging from this species, 1-ml aliquots were used as subsamples after agitation of the wells. All cercariae in the wells were counted during the first day of counting for each experiment and trial to ensure that the subsampling was representative. The experiment was repeated for both trematode species at the end of March using other infected individuals from Lower Portobello Bay found during the initial screening process.

Statistical analyses

The following procedures were performed separately for the two trematode species, as well as for each experimental trial. Infected snails from which cercariae emerged during screening but not during the experiments were excluded from the analysis. Snail size can potentially have an effect on the number of cercariae emerging (Poulin 2006). As such, a value of one was added to the number of cercariae emerged from each snail at each temperature, and the number of cercariae shed was divided by snail length (in millimetre). This was followed by a square-root transformation of the data in order to meet the assumption of normality. These data were then entered into a general linear model analysis, using repeated-measures ANOVA. Salinity or water level was used as the between-subjects factor to determine whether cercarial emergence differed among/between levels, while temperature was used as the within-subjects factor to determine whether temperature had an influence on the number of cercariae emerging from each snail. Post hoc tests (Tukey honestly significant difference) were performed where appropriate. All analyses were done using Statistical Package for the Social Sciences 16.0.

Results

Salinity

Multivariate tests indicated a significant effect of temperature on cercarial output for *Philophthalmus* sp. for both the first (Wilks' $\lambda = 0.296$, $F_{2,32} = 37.963$, $P < 0.0001$) and second salinity trials (Wilks' $\lambda = 0.419$, $F_{2,32} = 22.2$, $P < 0.0001$), as the number of cercariae emerging increased with increasing temperature (Fig. 1 and Table 1). Multivariate tests showed no significant interaction of temperature

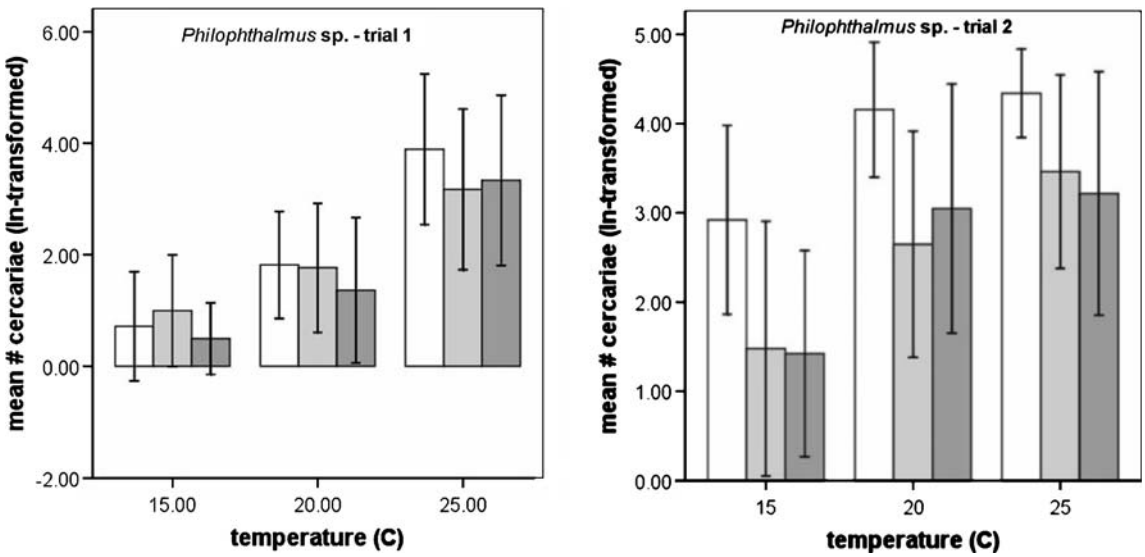

Fig. 1 Mean cercarial output of *Philophthalmus* sp. in response to temperature and salinity for both trials (30 PSU: *white bars*, 35 PSU: *light grey bars*, and 40 PSU: *dark grey bars*). Size corrected and transformed; number of cercariae shed are given ± SE

Table 1 Emergence of cercariae (raw data) from snails in response to increasing water temperature from separate experiments examining effects of salinity and water level on emergence

			Salinity		Water level	
			Trial 1	Trial 2	Trial 1	Trial 2
Maritrema novaezealandensis	15°C	Mean	53.8	55.2	52.6	27.1
		SD	±96.8	±64.1	±48.0	±25.4
		Range	0–516	0–249	0–186	0–99
		Number	36	31	24	24
	20°C	Mean	79.8	26.7	50.5	9.8
		SD	±143.3	±45.8	±49.4	±17.3
		Range	0–732	0–219	0–189	0–63
		Number	36	31	24	24
	25°C	Mean	104.8	12.7	51.8	11.5
		SD	±127.1	±18.4	±71.8	±19.7
		Range	0–495	0–63	0–354	0–81
		Number	36	31	24	24
Philophthalmus sp.	15°C	Mean	2.3	16.1	2.9	16.8
		SD	±3.7	±29.6	±6.0	±28.1
		Range	0–13	0–160	0–29	0–133
		Number	36	36	24	24
	20°C	Mean	8.4	46.2	3.0	27.8
		SD	±10.9	±41.3	±4.9	±33.2
		Range	0–48	0–155	0–23	0–113
		Number	36	36	24	24
	25°C	Mean	56.4	61.8	49.8	56.5
		SD	±46.0	±51.3	±62.8	±53.7
		Range	0–193	2–184	0–260	2–186
		Number	36	36	24	24

Note that data are combined for all salinity and water levels, respectively, for each temperature

and salinity for either the first trial (Wilks' $\lambda = 0.924$, $F_{4,64} = 0.649$, $P = 0.649$), or the second trial (Wilks' $\lambda = 0.922$, $F_{4,64} = 0.665$, $P = 0.619$).

Tests of within-subjects effects for *Philophthalmus* sp. indicated a significant effect of temperature for the first trial ($F_{2,66} = 58.686$, $P < 0.0001$), but no significant interaction of temperature and salinity ($F_{4,66} = 1.128$, $P = 0.351$). Similarly, tests of within-subjects effects for the second trial indicate a significant effect of temperature ($F_{2,66} = 28.105$, $P < 0.0001$), but no significant interaction of temperature and salinity ($F_{4,64} = 0.581$, $P = 0.678$).

Tests of between-subjects effects for *Philophthalmus* sp. indicated no significant effect of salinity on cercarial output for the first trial ($F_{2,33} = 0.888$, $P = 0.421$; Table 2). However, salinity had significant effects in the second trial ($F_{2,33} = 8.668$, $P = 0.001$), with Tukey post hoc tests indicating that more cercariae emerged at 30 than 35 PSU ($P = 0.002$) and more emerged at 30 than 40 PSU ($P = 0.004$).

Multivariate tests for *M. novaezealandensis* indicated a significant effect of temperature on cercarial output for the first trial (Wilks' $\lambda = 0.87$, $F_{2,32} = 3.835$, $P = 0.032$), as the number of cercariae emerging increased with increasing temperature (Fig. 2). Temperature also had a significant effect in the second salinity trial (Wilks' $\lambda = 0.484$, $F_{2,27} = 14.384$, $P < 0.0001$); however, the number of cercariae emerging decreased with increasing temperature. Multivariate tests showed no significant interaction of temperature and salinity for the first trial (Wilks' $\lambda = 0.916$, $F_{4,64} = 0.714$, $P = 0.585$); however, the interaction was significant for the second trial (Wilks' $\lambda = 0.684$, $F_{4,54} = 2.818$, $P = 0.034$), as cercarial output decreased with increased temperature but more so at 30 PSU.

Tests of within-subjects effects for *M. novaezealandensis* indicated a significant effect of temperature for the first trial ($F_{2,66} = 3.508$, $P = 0.036$), but no significant interaction of temperature and salinity ($F_{4,66} = 0.671$, $P = 0.614$). In contrast, tests of within-subjects effects for the second trial indicated a significant effect of temperature ($F_{2,56} = 12.313$, $P < 0.0001$), as well as a significant interaction of temperature and salinity ($F_{4,56} = 3.466$, $P = 0.013$).

Tests of between-subjects effects for *M. novaezealandensis* indicated a significant effect of salinity on cercarial output for the first trial ($F_{2,33} = 3.548$, $P = 0.04$), with Tukey post hoc tests indicating that more cercariae emerged at 30 than 35 PSU ($P = 0.033$). However, salinity

Table 2 Emergence of cercariae (raw data) from snails in response to salinity (PSU= practical salinity units) and water level in cell wells

		Salinity		Water level	
		Trial 1	Trial 2	Trial 1	Trial 2
Maritrema novaezealandensis		30 PSU	30 PSU	50%	50%
	Mean	133.6	40.0	44.6	22.4
	SD	±172.9	±56.2	±44.1	±20.7
	Range	0–732	0–228	0–189	0–81
	Number	36	33	36	33
		35 PSU	35 PSU	100%	100%
	Mean	43.6	26.9	58.7	10.8
	SD	±71.9	±45.6	±66.8	±22.3
	Range	0–276	0–249	0–354	0–99
	Number	36	33	36	39
		40 PSU	40 PSU		
	Mean	61.2	26.8		
	SD	±87.4	±45.6		
	Range	0–447	0–219		
	Number	36	33		
Philophthalmus sp.		30 PSU	30 PSU	50%	50%
	Mean	27.9	64.5	20.7	17.5
	SD	±43.2	±47.0	±51.4	±22.5
	Range	0–193	3–160	0–260	0–86
	Number	36	36	36	33
		35 PSU	35 PSU	100%	100%
	Mean	19.1	28.8	16.4	47.4
	SD	±31.4	±39.7	±16.4	±50.7
	Range	0–136	0–174	0–119	0–186
	Number	36	36	36	39
		40 PSU	40 PSU		
	Mean	20.0	30.7		
	SD	±34.1	±41.5		
	Range	0–141	0–184		
	Number	36	36		

Note that data are combined for all temperatures for each salinity and water level, respectively

itself had no significant effect in the second trial ($F_{2,28}$ = 0.293, P = 0.748) despite the significant interaction with temperature.

Water level

Multivariate tests indicated a significant effect of temperature on cercarial output for *Philophthalmus* sp. for both the first (Wilks' λ = 0.418, $F_{2,21}$ = 14.614, P<0.0001) and second water level trials (Wilks' λ = 0.572, $F_{2,21}$ = 16.431, P = 0.001), as the number of cercariae emerging increased with increasing temperature (Fig. 3). Multivariate tests showed no significant interaction of temperature and water level for either the first trial (Wilks' λ = 0.995, $F_{2,21}$ = 0.048, P = 0.954), or the second trial (Wilks' λ = 0.911, $F_{2,21}$ = 2.155, P = 0.156).

Tests of within-subjects effects for *Philophthalmus* sp. indicated a significant effect of temperature for the first trial ($F_{2,44}$ = 24.627, P < 0.0001), but no significant interaction of temperature and water level ($F_{2,44}$ = 0.085, P = 0.918). Similarly, tests of within-subjects effects for the second trial indicate a significant effect of temperature ($F_{2,44}$ = 16.431, P < 0.0001), but no significant interaction of temperature and water level ($F_{2,44}$ = 2.155, P = 0.128).

Tests of between-subjects effects for *Philophthalmus* sp. indicated no significant effect of water level on cercariae output for the first trial ($F_{1,22}$ = 1.262, P = 0.273), although there was a slight trend for more cercariae to emerge from snails kept at the high water level. However, water level was significant for the second trial ($F_{1,22}$ = 7.141, P = 0.014), as more cercariae emerged from snails kept at the high water level.

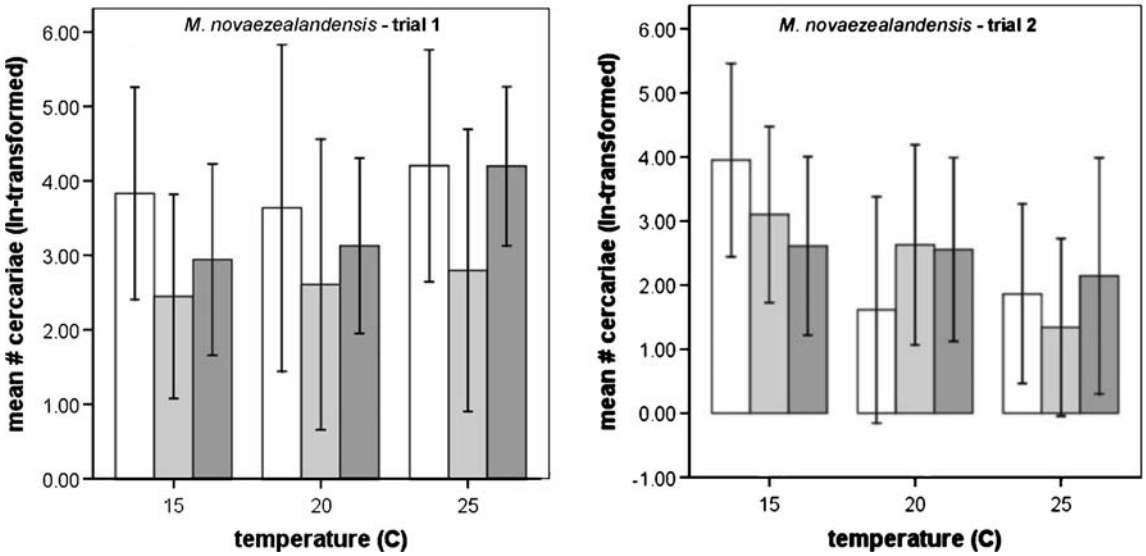

Fig. 2 Mean cercarial output of *Maritrema novaezealandensis* in response to temperature and salinity for both trials (30 PSU: *white bars*, 35 PSU: *light grey bars*, and 40 PSU: *dark grey bars*). Size corrected and transformed; number of cercariae shed are given ± SE

Multivariate tests for *M. novaezealandensis* indicated no significant effect of temperature on cercarial output for the first trial (Wilks' $\lambda = 0.997$, $F_{2,21} = 0.034$, $P = 0.966$), although there was a slight trend for a decrease in emergence with increased temperature at the low water level (Fig. 4). Temperature had a significant effect in the second water level trial (Wilks' $\lambda = 0.649$, $F_{2,21} = 5.667$, $P = 0.011$), as the number of cercariae emerging decreased with increasing temperature. Multivariate tests showed no significant interaction of temperature and water level for the first trial (Wilks' $\lambda = 0.996$, $F_{2,21} = 0.039$, $P = 0.962$), or for the second trial (Wilks' $\lambda = 0.894$, $F_{2,21} = 1.246$, $P = 0.308$).

Tests of within-subjects effects for *M. novaezealandensis* indicated no significant effect of temperature for the first trial ($F_{2,44} = 0.049$, $P = 0.953$), as well as no significant interaction of temperature and water level ($F_{2,44} = 0.05$, $P = 0.951$). In contrast, tests of within-subjects effects for the second trial indicate a significant effect of temperature ($F_{2,44} = 7.523$, $P = 0.002$), but not a significant interaction of temperature and water level ($F_{2,44} = 0.533$, $P = 0.591$).

Tests of between-subjects effects for *M. novaezealandensis* indicated no significant effect of water level on cercarial output for the first trial ($F_{1,22} = 0.744$, $P = 0.398$), although there was a strong trend for more cercariae to emerge at the lower water level. However, water level had a significant

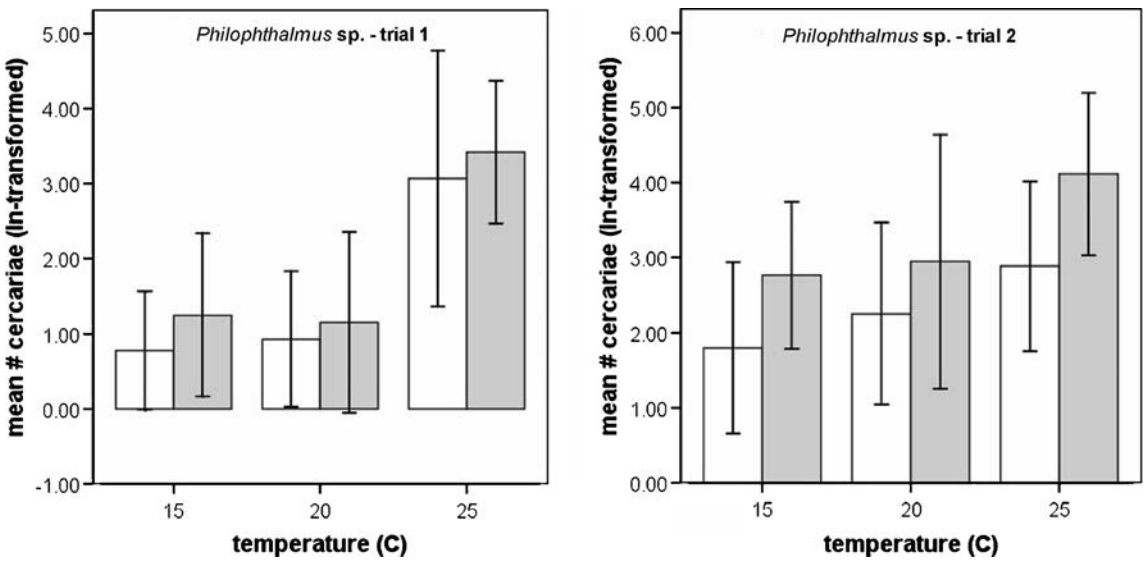

Fig. 3 Mean cercarial output of *Philophthalmus* sp. in response to temperature and water level for both trials (low water (50%): *white bars*, high water (100%): *grey bars*). Size corrected and transformed; number of cercariae shed are given ± SE

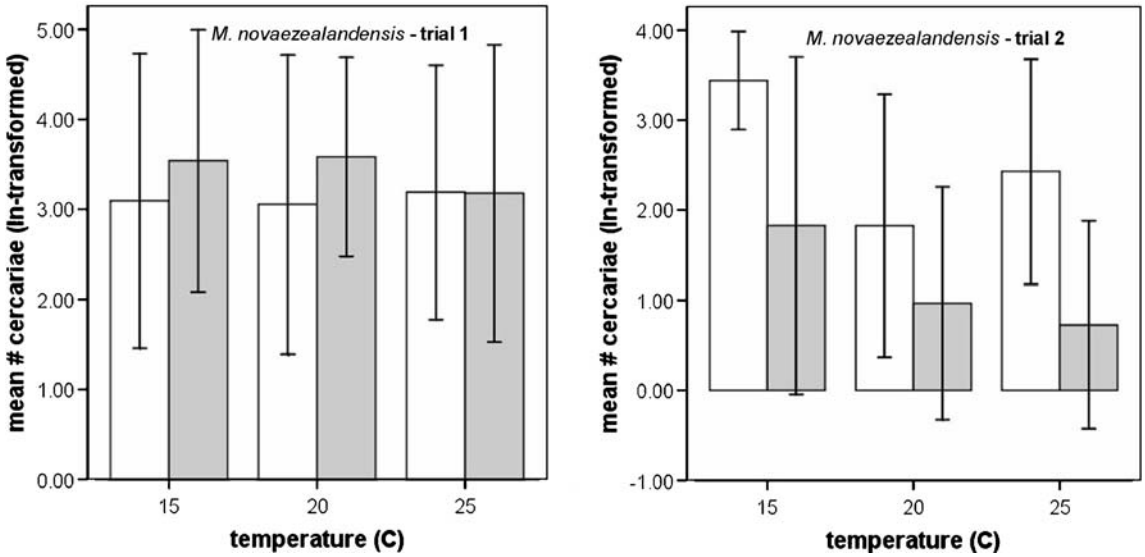

Fig. 4 Mean cercarial output of *Maritrema novaezealandensis* in response to temperature and water level for both trials (low water (50%): *white bars*, high water (100%): *grey bars*). Size corrected and transformed; number of cercariae shed are given ± SE

effect in the second trial ($F_{1,22} = 12.467$, $P = 0.002$), as more cercariae emerged from snails kept at the low water level.

Discussion

As expected, temperature had an effect on cercarial output for both *Philophthalmus* sp. and *M. novaezealandensis*; however, the effect appeared to differ between the two trematode species. Cercariae of *Philophthalmus* sp. consistently showed increased emergence in response to elevated temperature, reflecting the general trend for temperature effects reported in the literature (Lo and Lee 1996; Lyholt and Buchmann 1996; Mouritsen 2002a; Poulin 2006). In contrast, *M. novaezealandensis* cercariae most often displayed a decrease in emergence at elevated temperatures, as previously reported for this species (Koprivnikar and Poulin 2009), but in contrast to the findings of Fredensborg et al. (2005). Given that this earlier study used a different temperature range and the large variability in shedding seen here for this species, it is difficult to definitively make conclusions regarding temperature effects for *M. novaezealandensis*.

Water level appears to have an important, albeit opposite, effect on cercarial emergence of the two trematode species studied here. Significantly, more *Philophthalmus* sp. cercariae emerged from snails in the high water treatment in one trial, with a similar trend observed in the other trial. In contrast, significantly more *M. novaezealandensis* cercariae emerged from snails in the low water treatment in one trial, with a similar trend observed in the other trial. These results may reflect the optimal transmission strategies for each trematode species, as the timing of cercarial emergence is usually recognised as an adaptation by the parasite in order to reach the next host in the life cycle (Combes et al. 1994).

Philophthalmus sp. cercariae readily encyst as metacercariae in the aquatic environment. As this species is likely to have a life cycle similar to that of *Philophthalmus burrili* (Martorelli et al. 2008), the encysted metacercariae are ingested along with their substrate by avian final hosts and then excyst within the bird gut. Given the passive nature of this particular life cycle, it may be advantageous for cercariae to primarily emerge while the snail host is submerged so that cercariae are able to disperse and encyst on surfaces as widely as possible to maximise the chances of ingestion and transmission.

The life cycle of *M. novaezealandensis* is quite different, as cercariae actively infect their second intermediate hosts, various species of crabs and amphipods (Martorelli et al. 2004). Such a life cycle should favour a transmission strategy that maximises contact with the second intermediate hosts. Increased cercarial emergence at low water levels may do so, as cercariae emerge into a small volume of water, thereby optimising the concentration of cercariae and hence transmission rate (Mouritsen and Jensen 1997). As such, it would appear to be beneficial for *M. novaezealandensis* cercariae to emerge in intertidal pools during daytime at low tides (Mouritsen 2002b). Additionally, as water movements are insignificant in tidal pools, cercariae avoid dislodgment and interference with the swimming activity required in order to achieve contact with the next host (Mouritsen 2002a). It is interesting to note that the conditions which appear to promote cercarial emergence for this species, lower temperatures and decreased submergence, would not normally be expected to co-occur in low tide conditions. However, the mean annual temperature for

Lower Portobello Bay was 11.1°C ± 3.1 SD, with a mean temperature of 14.1°C ± 0.8 SD during the summer months (December to March) for 1971–2000 (www.niwascience.co.nz/edu/resources/climate).

Our results also clearly demonstrate that salinity is an important environmental factor impacting the emergence of marine trematode cercariae. Both species examined here exhibited similar responses, as generally more cercariae emerged from snails kept at the lowest salinity (30 PSU). It should be noted that the relatively small sample sizes and variability in cercarial emergence likely account for the fact that there were significant effects in some trials and only trends and interactions in other trials. The effect of salinity on the emergence of *M. novaezealandensis* cercariae was dependent on temperature in one trial, as significantly more cercariae emerged from snails in the low salinity treatment at the lowest temperature used. Mouritsen (2002a) also found such an interaction for *M. subdolum*; however, more cercariae emerged at higher salinity levels at elevated temperatures.

Salinity-mediated effects on cercarial emergence may be the result of trematode responses to changes in snail host physiology at altered salinity. For example, the most common reaction of marine mollusks to salinity change is a decrease of functional activity, including oxygen consumption and movement rate, which is kept at a comparatively low level until acclimation occurs after a few days (Berger and Kharazova 1997). Mouritsen (2002b) found that emergence of *M. subdolum* cercariae shows a positive relationship with host activity. Host activity was not monitored during this experiment but such a mechanism would suggest an increase rather than decrease of host activity at the lowest salinity level used. Other physiological changes in cells and tissues in response to salinity change, such as altered protein synthesis, or ion balance (Berger and Kharazova 1997), may also impact cercarial emergence.

Why physiological and behavioural responses may have occurred at the lowest salinity level in this experiment is unclear, but perhaps elevated salinities are less stressful to a certain point. It might be expected that intertidal mollusks would more routinely experience elevated rather than decreased salinities, particularly during sunny low tides when evaporation would be rapid in tidal pools, increasing salinity. Low salinities may only be experienced by the small proportion of individual mollusks located close to freshwater input sources. However, it might be expected that populations in areas of high rainfall (e.g., the Pacific Northwest of the USA and Canada) may routinely experience low salinities and show different responses to salinity fluctuations. Putting salinity in the context of optimal transmission strategy poses more of a challenge, as increased cercarial emergence by both species may be a response to host stress rather than a response to an environmental cue by the parasites. It would appear unlikely that intertidal zones are regularly subjected to lower than usual salinities such that this would provide a useful and relevant stimulus for cercarial emergence unless low tides routinely result in large freshwater input.

Our results show that while temperature has an important effect on the emergence of marine trematodes, other environmental factors showing temporal fluctuations, such as salinity and water level, have an influence as well. This is particularly important in the context of changes in the marine environment expected to result from global climate change that may impact parasitism (Marcogliese 2001). Clearly, effects of climate change on trematode parasitism in intertidal zones are not predictable solely in terms of temperature response. For example, decreased ocean salinity would appear to increase cercarial emergence of both *Philophthalmus* sp. and *M. novaezealandensis*, although perhaps only in the short-term if this is due to host stress during acclimation. It is also important to note that various trematode species will likely be differently impacted. Our study suggests that cercarial emergence of *Philophthalmus* sp. may be favoured under conditions of increased temperature and sea level compared to *M. novaezealandensis*, which exhibited decreased emergence under these same conditions.

Further study of environmentally mediated cercarial emergence is needed in order to determine the general importance of various abiotic factors in intertidal zones. This is critical not only because of the changing marine environment, but also because parasites are ubiquitous components of intertidal communities, able to regulate host population density, influence the diversity of the benthic community, and affect the structure of intertidal food webs (Poulin and Mouritsen 2006), as well as being an important path of energy flow in benthic systems (Thieltges et al. 2008). The alteration of abiotic factors impacting cercarial emergence could thus affect local parasite transmission and abundance. This in turn may have significant implications for intertidal ecosystems (Poulin and Mouritsen 2006), with possible collapses of certain host populations (Mouritsen et al. 2005).

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