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Parasitized snails take the heat: a case of host manipulation?

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Abstract Infection-induced changes in a host's thermal physiology can represent (1) a generalized host response to infection, (2) a pathological side-effect of infection, or (3), provided the parasite's development is temperature-dependent, a subtle case of host manipulation. This study investigates parasite-induced changes in the thermal biology of a first intermediate host infected by two castrating trematodes (genera *Maritrema* and *Philophthalmus*) using laboratory experiments and field surveys. The heat tolerance and temperatures selected by the snail, *Zeacumantus subcarinatus*, displayed alterations upon infection that differed between the two trematodes. Upon heating, snails infected by *Maritrema* sustained activity for longer durations than uninfected snails, followed by a more rapid recovery, and selected higher temperatures in a thermal gradient. These snails were also relatively abundant in high shore localities in the summer only, corresponding with seasonal elevated microhabitat temperatures. By contrast, *Philophthalmus*-infected snails fell rapidly into a coma upon heating and did not display altered thermal preferences. The respective heat tolerance of each trematode corresponded with the thermal responses induced in the snail: *Maritrema* survived exposure to 40°C, while *Philophthalmus* was less heat tolerant. Although both trematodes infect the same tissues, *Philophthalmus* leads to a reduction in the host's thermal tolerance, a response consistent with a pathological side effect. By contrast, *Maritrema* induces heat tolerance in the snail and withstood exposure to high heat. As the developmental rate and infectivity of *Maritrema* increase with temperature up to 25°C, one adaptive explanation for our findings is that *Maritrema* manipulates the snail's thermal responses to exploit warm microhabitats.

Keywords Temperature · Tolerance limit · Thermal preference · Trematode · *Zeacumantus* · *Maritrema*

Introduction

It is widely recognized that behavioral changes in parasitized hosts can be tied to physiological changes with varying adaptive outcomes. For instance, increased thermal preferences or selection of warmer habitats by invertebrates upon infection with microbial parasites increases body temperature and can kill invading microbes and/or boost immune responses, a phenomenon termed behavioral fever (e.g., Bronstein and Conner 1984; Myhre et al. 1997; Bernheim and Kluger 1976). Even when mortality is unavoidable, fever can prolong host longevity, thus permitting reproduction. For instance, Elliot et al. (2002) demonstrated that locusts infected by a fungal pathogen in

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microhabitats where they were able to raise their body temperatures to fever levels prolonged their lifespan and consequently produced one clutch of viable offspring before death. Although less well documented, selection of cool temperatures upon infection can also retard parasite development (e.g., Moore and Freehling 2002). However, alterations in host behavior may simply be a side-effect of the infection and benefit neither the host nor parasite (Campbell et al. 2010; Kavaliers and Colwell 1992; Lefcort and Bayne 1991; Holmes and Zohar 1990; Minchella 1985; Ewald 1980).

In other cases, behavioral alteration upon infection can benefit the parasite and, in such cases, is often interpreted as a manipulation of the host's response (Poulin 2010). For instance, some microbial parasites cause shifts in their host's thermal preference that favor transmission (Fialho and Schall 1995; Watson et al. 1993). Conceivably, because the developmental rate of many parasites is positively related to temperature (Harvell et al. 2002, 1999), transmission success could be increased if ectothermic hosts occupy warmer habitats. Yet, interpreting cases of altered thermal choice are challenging, because parasites can also increase their host's thermal vulnerability (Fredensborg et al. 2005; McDaniel 1969; Vernberg and Vernberg 1963). Thus, elevated thermal preferences may be tied to a decrease in host survivorship due to greater physiological costs, or indirectly, for example, by increasing malaise and enhancing predation risk (Lefcort and Eiger 1993).

Identifying how temperature influences a parasite's development and subsequent outcome of the infection is critical to the appropriate interpretation of thermal biology data (Campbell et al. 2010). The challenge is to link changes in the thermal behavior of infected hosts to fitness benefits for the host or parasite, a daunting task in field conditions. As a first step, testing for differences in how similar parasites influence the heat tolerance and behavior of the same host may identify cases that are consistent with the following scenarios: (1) generalized host response to infection, (2) pathological side-effect of infection, or (3) host manipulation. Moreover, when the host is permanently castrated by its parasite, any alteration that leads to greater host survival and/or enhanced parasite development can clearly only benefit the parasite (Hechinger et al. 2009), thus eliminating the need to consider the host's fitness.

In this study, we investigate parasite-induced changes in the thermal responses of a first intermediate host infected by two species of castrating trematodes using complementary experiments and field surveys. Specifically, we test whether different parasites infecting the same host species induce changes in their host's thermal biology that match their respective thermal tolerances.

Materials and methods

Host–parasite species' biology

Zeacumantus subcarinatus (Sowerby 1855) is an abundant soft-sediment and rocky shore intertidal snail that is the first intermediate host to several trematode castrators including the microphallid, *Maritrema novaezealandensis* Martorelli 2004 (hereafter, *Maritrema*). *Maritrema* cercariae are produced by sporocysts and leave the snail to encyst in crustaceans (Martorelli et al. 2004). The philophthalmid, *Philophthalmus* sp. (probably *P. burrili* Howell and Bearup 1967; hereafter, *Philophthalmus*), also commonly infects the snail (Martorelli et al. 2008). In this case, rediae produce cercariae which emerge to encyst on hard surfaces, such as mollusc shells or crustacean exoskeletons. Both trematodes complete their life cycle following ingestion of metacercariae by shorebirds where each trematode species reaches sexual maturity and produces eggs which, when shed into the water column, release miracidia to continue the infection cycle. *Maritrema* and *Philophthalmus* can be found in single or double infections in the snail (i.e., both species can co-infect the same individual) where they replace the host's gonad and digestive gland tissues.

Experiments

Adult *Zeacumantus subcarinatus* (~15 mm in shell length) were collected in Lower Portobello Bay, Otago Harbour, New Zealand (45°52′S, 170°42′E) in May (heat tolerance experiment) and June 2009 (temperature selection experiment). Following transport to the laboratory, a preliminary infection status was assigned (to ensure a relatively even distribution of specimens across each infection group; the prevalence of snails infected by the two trematode species can vary by an order of magnitude). Snails were exposed individually in Petri dishes to 30°C and high illumination (eight 40-W daylight spectrum fluorescent tubes) in a temperature control chamber for 3 h to induce shedding of cercariae. Animals that did not shed cercariae were assigned to the uninfected group. In those snails that did shed, cercariae were identified using light microscopy and their host snails were housed according to their infection status (*Maritrema*, *Philophthalmus*, or uninfected). Animals were held in flow-through aquaria at 10–14°C for 4 weeks prior to the start of experiments (1) to increase the likelihood that snails infected by a trematode would host mature cercarial stages, and (2) to acclimate experimental snails to the same temperature regime. Sea lettuce (*Ulva* spp.) was provided ad libitum. Similarly sized animals from the different infection treatments were selected haphazardly (by eye); however, shell length was also measured to the nearest 0.5 mm with calipers to include as a factor in our

statistical analyses. We did not observe snail mortality during experiments.

To investigate heat tolerance, we used multiple-lines of evidence and tested for infection-related differences in (1) *heat-hardening response*, i.e., a rapid, transitory increase in heat tolerance following brief exposures to near-lethal temperatures that persists on the order of days (Bowler 2005; Maness and Hutchison 1980), (2) time to onset of heat coma (sensu McMahon 1976 as applied to gastropods: active crawling was arrested, the foot curled, and the specimen was unresponsive to touch) upon exposure to high temperature, and (3) time to recover mobility following this heat exposure. To control temperature, snails were contained individually in seawater-filled polystyrene well plates (BD Falcon 351143; 3 × 4 wells per plate; 6.0-ml wells) that were floated in a water bath at the appropriate temperature and monitored with a digital thermometer (VWR, ±0.2°C).

To induce a heat-hardening (defined above), 20 specimens from each infection treatment were exposed to 10 (control), 35 and 40°C for 1 h, 2 days preceding experimental exposure to high heat. Temperature treatments were selected based on the following rationale: 10°C = ambient aquaria temperatures, 35°C = maximum temperature experienced by *Z. subcarinatus* in field conditions (based on point measurements of upper shore tide pools in the summer months in Lower Portobello Bay), and 40°C = extreme but sub-lethal temperature based on pilot experiments. Heat tolerance was quantified 2 days post-heat-hardening by exposing snails to fluids heated from 35 to 39°C in 1°C increments where each heating increment lasted 15 min (note that dissolved oxygen saturation in seawater approaches a minimum threshold at these temperatures; Benson and Krause 1984). A cumulative heating design was selected to ensure that all snails, regardless of infection status, could be scored as exhibiting a response (determined in pilot experiments): the time taken for each specimen to show signs of a heat coma. Immediately following exposure to 39°C, all animals were cooled to 15°C and monitored for activity. The time at which each animal initiated active crawling was recorded as the recovery time.

Following the experiment, snails were dissected to confirm their infection status and at this point co-infections of *Maritrema* and *Philophthalmus* were identified; thus, sample size was not even across the infection groups. During dissection, activity of *Maritrema* cercariae (the sporocysts are not mobile) and *Philophthalmus* rediae and cercariae were examined from each infected snail 24 h following the experiment (to assess differences in the thermal tolerance of the two trematodes). At least 25 rediae and cercariae of both species were scored as active or moribund based on lack of evident movement and lack of response to touch with a metal probe.

Selected temperatures were quantified along a horizontal temperature gradient (5–35°C), maintained by cooling one end of an aluminium aquarium with a re-circulating water bath and heating the opposite end with an electric heating element (Bates et al. 2010). Animals had unrestricted access along a slot (33 cm long × 1.0 cm wide × 1.5 cm high) in the gradient aquarium. Black plastic was draped over the chamber during trials to exclude light as a selection cue. Individual snails were placed mid-chamber at 15°C. The temperature corresponding to the position of each snail after 90 min (most animals were stationary after this time period, as determined in pilot experiments) was measured with a digital thermometer probe (VWR, ±0.2°C). For each treatment group (snails infected with *Maritrema* or *Philophthalmus*, and uninfected snails), 25 snails were tested by alternating individuals from each treatment group over the course of 14 days in the experimental chamber. Infection status was confirmed post-experiment for each snail by dissection. Because only two snails with a double infection were included, these specimens were excluded from the dataset.

Field surveys

Sampling was conducted in summer (January) and spring (September) of 2009 during low tide series at five haphazardly placed transects (GPS coordinates are in Supplementary Table 1) spaced along a 3.5-km stretch of soft-sediment shore on the Otago Peninsula, New Zealand. Fixed positions along each transect represented low (0.1–0.3 m above MLW) and high (0.7–0.9 m) shore heights. At each location, a ~25 × 10 m stretch of shore was searched until a minimum of 24 and maximum of 30 snails >15 mm in shell length were collected (as limited by the search time available within a low tide interval). In one case (transect #5: summer), the density of snails was remarkably high in both the high and low shore, and 96 specimens were haphazardly sampled from each shore height to include a locality with a large sample size in the dataset. Snails were transported to the laboratory and dissected under a light microscope to confirm their infection status.

Statistical analyses

All analyses were conducted in R (2.9.1) (alpha = 0.05). Assumptions of normality (Kolmogorov–Smirnov Test) and homogeneity of variances (Bartlett's Test) were tested prior to analyses as required. Analysis of covariance (ANCOVA) tested for differences in the response time of the snails with heat-hardening temperature and infection status as factors and shell length as a covariate; time to onset of heat coma upon exposure to high temperature and time to recover mobility following heat expo-

sure were treated in separate analyses. In each analysis, the full model was simplified before performing treatment contrasts against the control, by removing non-significant factors and interaction terms; the log-likelihood of the full model was then compared to the restricted model to ensure that simplification did not significantly reduce the model fit. A binomial distribution test identified significant differences in the proportion of rediae and cercariae that remained active (vs. moribund) when found in single and double infections for each of the heat-hardening treatments. ANCOVA tested for significant differences in selected temperature with infection status as a factor and shell length as a covariate. A paired *t* test was used to test for significant differences in the prevalence of infected snails from low versus high shore positions ($n = 5$) in the two seasons.

Results

Heat tolerance

Exposure to 35 and 40°C fluids for 1 h, 2 days preceding experimental treatments, heightened the thermal tolerance of *Zeacumantus subcarinatus*, irrespective of infection status (Fig. 1; Table 1). The magnitude of the heat-hardening response did not differ between healthy and infected snails or with size (the interaction terms between the heat-hardening and infection treatments, and shell length were not significant). However, *Maritrema*-infected snails were highly resilient to short-term heat-exposure. In comparison to uninfected snails, snails with single infections of *Maritrema* displayed significantly longer durations where activity was observed when exposed to high heat (35–39°C), as well as a more rapid recovery upon cooling, in both the control (held at 10°C prior to the experiment) and heat-hardened treatments (prior exposure to 35 or 40°C: Fig. 1; Table 1). In contrast, snails with single infections of *Philophthalmus* fell into a heat coma more rapidly, but displayed recovery times upon subsequent cooling that did not differ significantly from uninfected snails. Snails with double trematode infections had similar activity patterns to uninfected snails upon heating and cooling (Fig. 1, Table 1).

Maritrema was relatively heat tolerant and its cercarial stages were always active in spite of its host snail being exposed to temperatures of 40°C during the pre-experiment heat-hardening treatment, followed by experimental exposure to increasing temperatures from 35 to 39°C over 75 min. By contrast, activity of *Philophthalmus* rediae and cercariae depended upon whether *Maritrema* was also present.

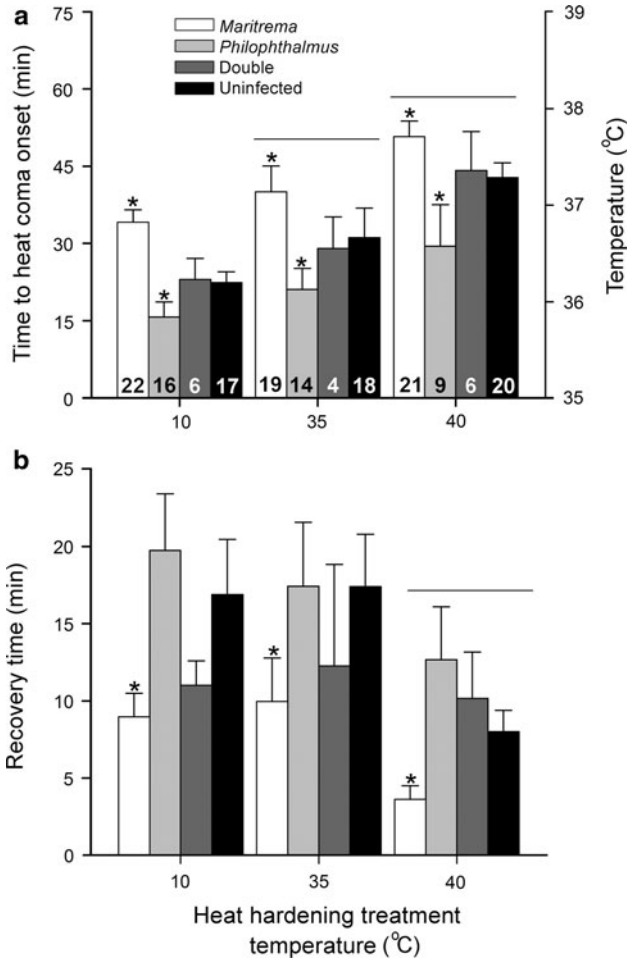

Fig. 1 Snails (*Zeacumantus subcarinatus*) infected with single infections of *Maritrema* and *Philophthalmus*, double infections of the two trematodes, and uninfected specimens exposed to high heat following 3 heat-hardening treatments: control (held at 10°C) or 60 min of heat exposure (35 or 40°C) 2 days prior to experiment. **a** Time to onset of heat coma (mean + 1 SE); corresponding temperature is shown on the right *y*-axis. Temperature was increased from 35 to 39°C in 1°C increments every 15 min for a total of 75 min. **b** Recovery time (mean + 1 SE) of these same snails upon cooling. *Asterisks* indicate infection treatments with significantly different activity responses from uninfected animals. Heat-hardening treatments distinguished by a *line* are significantly different than the control (10°C). Results of statistical analyses (ANCOVA) are summarized in Table 1 and were conducted independently for the two response variables. Sample size (number of individual snails observed) for both panels are shown at the base of each *bar* in (**a**)

In double infections, *Philophthalmus* from snails exposed to all heat treatments remained active. However, when *Philophthalmus* occurred as the sole parasite, the proportion of snails with active rediae and cercariae dropped dramatically with increasing heat exposure (binomial distribution test: *P* value <0.0001; Fig. 2). The rediae and cercariae from *Philophthalmus* infecting those snails exposed to 40°C were moribund.

Table 1 ANCOVA full model results summary for temperature selection experiments (*a*) and treatment contrasts against uninfected snails exposed to 10°C prior to the experiment for the restricted model (*b*)

(a)

Factor	*df*	Sum sq	Mean sq	*F* value	*P*
Response:time to heat coma onset					
Inf	3	9,117	3,039	11.23	<0.0001
Heat	2	8,092	4,046	14.96	<0.0001
Size	1	644	644	2.38	0.13
Inf × heat	6	942	157	0.58	0.75
Inf × size	3	205	68	0.25	0.86
Heat × size	2	486	243	0.89	0.41
Inf × heat × size	6	1,262	210	0.78	0.59
Residuals	147	39,777	271		
Response:recovery time					
Inf	3	2,431	810	6.58	0.00033
Heat	2	1,666	833	6.76	0.0015
Size	1	23	23	0.19	0.66
Inf × heat	6	258	43	0.34	0.91
Inf × size	3	174	58	0.47	0.70
Heat × size	2	224	112	0.91	0.41
Inf × heat × size	6	478	79	0.65	0.69
Residuals	147	18,101	123		

(b)

	Coef	SE	*t* value	*P*
Response:time to heat coma onset				
Intercept	24.29	2.83	8.58	<0.0001
M	9.45	3.00	3.15	0.0019
P	−8.32	3.41	−2.44	0.016
MP	4.50	4.72	0.95	0.34
35°C	6.87	3.03	2.27	0.024
40°C	16.77	3.02	5.53	<0.0001
Response:recovery time				
Intercept	16.08	1.89	8.52	<0.0001
M	−6.28	2.00	−3.14	0.0020
P	2.58	2.27	1.13	0.26
MP	−2.55	3.14	−0.81	0.42
35°C	0.34	2.01	0.17	0.86
40°C	−6.52	2.02	8.58	0.0015

The two factors are: (1) infection status (*Inf*): single infections of *Maritrema* (*M*) and *Philophthalmus* (*P*), double infections of *Maritrema* and *Philophthalmus* (*MP*), and uninfected snails, and (2) heat-hardening treatment (*Heat*), pre-experimental exposure to 35 and 40, and the control, (10°C). Shell length (*Size*) was included as a covariate in the full model. *Intercept* represents the mean for the control treatment (corrected for unbalanced data); contrast coefficients are the difference in the mean of each infection treatment intercept versus the control

Sum sq Sum of squares, *Mean sq* mean squares, *Coef* treatment contrast coefficient, *SE* standard error

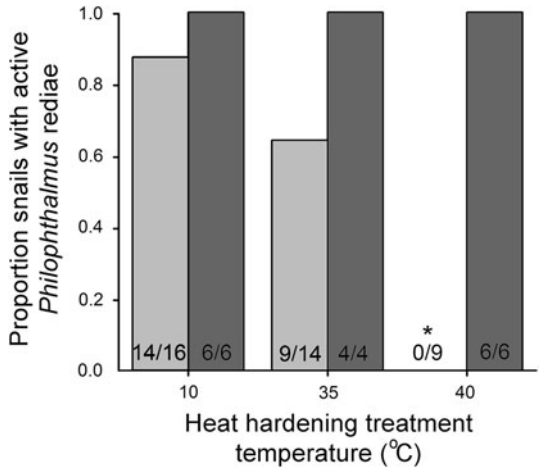

Fig. 2 Proportion of snails with active *Philophtalmus* rediae in single infections (*light gray*) or double infections with *Maritrema* (*dark gray*) observed in host dissection after 75 min of heat exposure for different heat-hardening treatments: control (10°C) or prior heat exposure (35 or 40°C for 60 min). The individual binomial distribution probability for the difference in the observed frequency of active *Philophthalmus* between double and single infections was calculated separately for each heat-hardening treatment. *P value <0.0001 based on the binomial distribution test

Temperature selection

Maritrema-infected snails selected significantly higher temperatures [mean selected temperature (°C) ± 1 SD: 20.5 ± 5.4] than snails infected with *Philophthalmus* (15.1 ± 5.5) or healthy snails (17.5 ± 5.8) (Fig. 3; Table 2). Although shell length of snails did not differ between treatments [mean (mm) ± 1 SD for snails infected with *Maritrema*: 14.3 ± 0.9 or *Philophthalmus*: 14.7 ± 0.9, and uninfected snails: 15.0 ± 1.2], larger snails selected significantly cooler temperatures (Table 2).

In situ distribution of parasitized snails

The prevalence of *Maritrema* infections was significantly higher in snails collected from the upper intertidal during the summer: high shore (0.7–0.9 m) versus low shore (0.1–0.3 m) was, respectively, 42 versus 15% (Fig. 4). Environmental temperature measured over a week in early summer differed markedly between the high and low shore as follows: temperature at shore heights >1.0 m ranged from ~11 to 30°C, while temperature at 0.1 m was relative constant (~11 to 13°C; Bates et al. 2010). In contrast, prevalence did not differ significantly at these same sites in the early spring, when environmental temperatures across the shore were also similar: e.g., mean (±range) temperature at 1.0 and 0.5 m was, respectively, 9.2 ± 6.5 and 8.8 ± 8.5 (Bates et al. 2010).

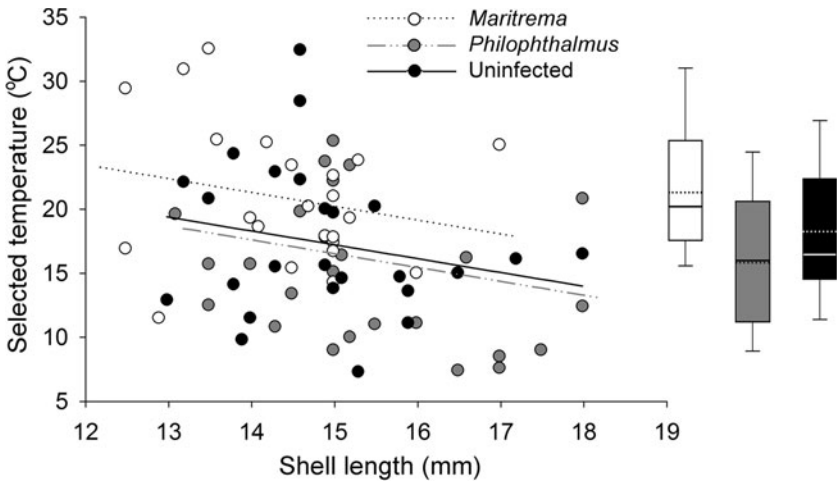

Fig. 3 Temperatures selected by snails infected with single infections of *Maritrema* and *Philophthalmus*, and uninfected ($n = 30$ specimens per infection group) in a horizontal temperature gradient versus shell length. Median (*solid lines*) and mean (*dotted lines*) for each infection group are shown in the *box plots*. Table 2 provides ANCOVA results and regression parameters

Table 2 ANCOVA full model results for temperature selection experiments (*a*) and treatment contrasts against uninfected snails for the restricted model which included shell length (size) as a covariate and infection status (*b*) (*Inf*: *Maritrema*, *Philophthalmus* and uninfected) as a factor

(a)

Factor	*df*	Sum sq	Mean sq	*F* value	*P*
Size	1	353	353	11.81	0.0010
Inf	2	183	91	3.06	0.051
Size × inf	2	6	3	0.094	0.91
Residuals	71	2,063	3		

(b)

	Coef	SE	*t* value	*P*
Intercept	33.41	8.14	4.10	0.00011
Size	−1.08	0.55	−1.97	0.052
Maritrema	3.07	1.53	2.01	0.042
Philophthalmus	−1.68	1.58	−1.06	0.29

Intercept represents the regression intercept for the control treatment; contrast coefficients are the difference between each infection treatment intercept and the control. The regression slope for all treatments is −1.08; interaction terms were not included in the restricted model summary (*b*)

Sum sq Sum of squares, *Mean sq* mean squares, *Coef* treatment contrast coefficient, *SE* standard error

Philophthalmus-infected snails and those with double infections displayed similar infection prevalence with shore height in both seasons.

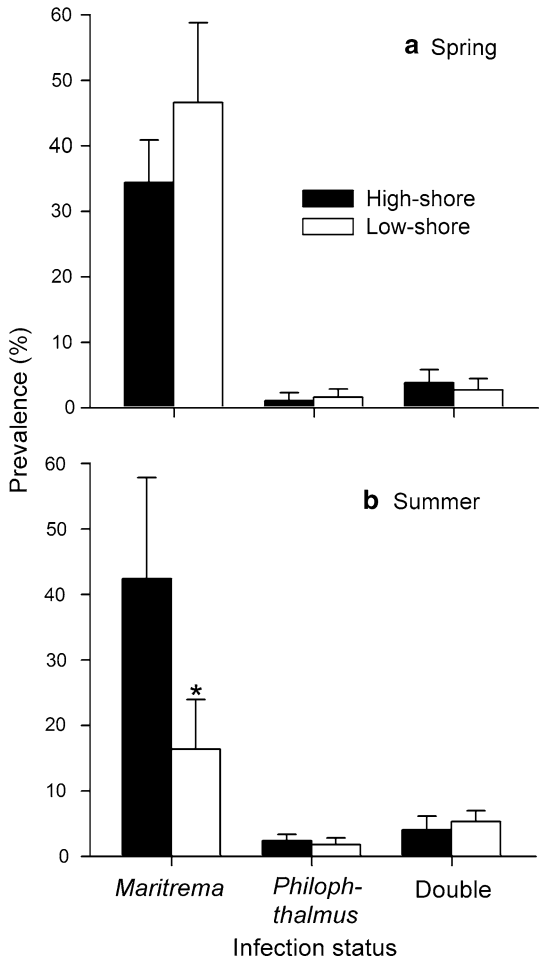

Fig. 4 Prevalence of single infections of *Matritrema* and *Philophthalmus*, and double infections of the two trematode species, in snails in the high and low shore at 6 locations in Otago Harbour sampled in the austral **a** spring and **b** summer of 2009. Prevalence of *Maritrema* infected snails was significantly lower in the low shore (*asterisks*) during the summer based on paired *t* test results: $t = 2.86$, $P = 0.023$

Discussion

Our results provide the first case, to our knowledge, of a metazoan parasite increasing the thermal tolerance of its host. We propose that this comparatively high tolerance to short-term elevated temperatures may allow the snail, *Zeacumantus subcarinatus*, to select relatively warm microhabitats and persist in high shore locations during summer months. In addition, we exposed cold-acclimated

animals to a heat stress to mimic real-world variability, as would be encountered by these intertidal species on extreme warm days following the winter season. Our results indicate that snails infected by *Maritrema* may better tolerate heat stress during spring months, a time of year when warm temperatures can cause widespread mortality in diseased animals acclimated to cool climate conditions (e.g., Bates et al. 2009).

All snails exposed to 35 and 40°C, regardless of infection status, exhibited increased thermal tolerance. Thus, infection does not appear to interfere with the inherent capability of the snail to respond to heat stress, such as the heat shock response—a molecular process associated with the synthesis of heat shock proteins (hsps) and molecular chaperones elicited following acute sub-lethal heat injury (Bowler 2005). However, snails infected with *Maritrema* and *Philophthalmus* had significantly different thermal tolerance and preferences. Thus, the heat responses of snails are affected by infection in a parasite species-specific way. Surprisingly, because infected molluscs tend to be relatively vulnerable to thermal stress (e.g., Lauckner 1980, 1983; Tallmark and Norrgren 1976; Lee and Cheng 1971; McDaniel 1969; Vernberg and Vernberg 1963), snails parasitized by *Maritrema* were relatively responsive when exposed to extreme heat in comparison to uninfected individuals. In addition, these snails sought warmer temperatures in laboratory experiments over a range of sizes and preferred upper intertidal microhabitats during the summer months only. By contrast, snails infected with *Philophthalmus* showed reduced levels of activity and prolonged recovery upon heating. However, the temperature selection of *Philophthalmus*-infected snails did not differ significantly from that of uninfected snails in both laboratory experiments and in the field. Snails infected by both trematode species displayed responses to heat that did not differ from uninfected individuals, suggesting a compromise alteration of host thermal responses. Moreover, the heat tolerance of the two trematode species mirrored the patterns displayed by their hosts: *Maritrema* appears to be more heat tolerant than *Philophthalmus*. *Maritrema* cercariae survived fluids heated to 40°C (for 1 h), whereas the rediae and cercariae of *Philophthalmus* were moribund following similar exposures, except when they co-occurred with *Maritrema* (as a double infection).

Thus, because the thermal responses of *Zeacumantus subcarinatus* differ upon infection by the two trematode species, it is unlikely that the alterations we observed represent a generalized host response to infection or castration. Instead, elevated temperature tolerance and preference in the snail may be adaptive for *Maritrema*, but not for *Philophthalmus*. *Maritrema* achieves higher rates of multiplication and displays an increasing ability to infect its next host with temperature up to 25°C, based on experimental maintenance of *Maritrema*-infected snails at elevated temperatures over periods of several weeks (Studer et al. 2010). Any takeover of the host's thermal responses that leads to the occupation of warmer localities would presumably favor *Maritrema*'s cercarial output and infectivity. Additionally, *Maritrema* and the snails it infects have relatively high thermal tolerance, which may confer a survival advantage in preferred upper intertidal microhabitats where exposure to extreme heat is a risk. For instance, aerially exposed dark-colored snails can absorb solar heat to the extent that their body temperatures reach 50°C, in spite of air temperatures being much cooler (Marshall et al. 2010).

In contrast, although *Philophthalmus*-infected snails shed accumulated cercariae in greater numbers if placed at 25°C for days (Koprivnikar and Poulin 2009), there is no evidence that the parasite benefits from increased multiplication when housed at elevated temperatures for longer durations. As we show here, *Philophthalmus* also has a relatively lower thermal tolerance. Therefore, in the case of *Philophthalmus*, manipulation of its host's thermal preferences would not be adaptive. Instead, snails infected with *Philophthalmus* display characteristics which are typical of general infection-related responses to elevated heat, including decreased activity, that in other infected invertebrates relate to physiological alterations upon infection that may or may not be advantageous to either the host or parasite (Holmes and Zohar 1990; Ewald 1980). For instance, Lefcort and Bayne (1991) reported that the drop in the mean temperatures selected by one snail species (*Biomphalaria glabrata*) infected by a trematode (*Schistosoma mansoni*) may relate to higher levels of endogenous cytokines in association with parasite activation of the host's internal defence system, without obvious immediate adaptive significance.

Although *Zeacumantus subcarinatus* parasitized by *Maritrema* have higher heat tolerance and selected higher temperatures than uninfected snails, the long-term survivorship of infected snails decreased at higher temperatures. Fredensborg et al. (2005) housed *Z. subcarinatus* at 18 and 25°C for 40 weeks. While the survival curves of both uninfected and infected snails initially displayed a shallow decline at the two temperatures, exposure to elevated temperatures for multiple weeks is relatively costly for infected snails and is manifested earlier at 25 than at 18°C (~10 vs. 15 weeks). This result suggests that there may be a long-term temperature-dependent cost to infection, such as metabolically-driven higher nutritional demands in infected individuals at higher temperatures, leading to earlier death by starvation (Fredensborg et al. 2005). Elevated temperatures within the range normally encountered by snails can also relate to increased cercarial emergence and subsequent physical tissue damage to the host snail (Pan 1965). Yet snails infected with trematodes can survive for decades

under natural conditions (Curtis 2003). Long-term laboratory exposures to temperatures several degrees higher than what animals would encounter in nature because of daily and seasonal changes in temperature regime may not readily translate into the responses of animals to natural short-term variability. Importantly, differences in survival as a function of infection at short versus long time scales are very likely driven by different mechanisms. Sousa and Gleason (1989) found that differential mortality between snails infected and uninfected with trematodes was due to exposure to low levels of dissolved oxygen, and was accentuated at higher fluid temperatures for several, but not all, species of trematodes included in their study. Thus, oxygen limitation may be a critical factor driving short-term mortality in parasitized snails exposed to high heat (Pörtner 2001) and may also explain the temperature-induced pathological effect of infection by *Philopthalmus*, and/or the low heat tolerance displayed by *Philopthalmus* rediae.

Here, the physiological mechanism enabling snails infected by *Maritrema* to remain active for longer durations upon short-term exposure to high heat is unknown and deserves investigation. For instance, it is possible that *Maritrema* secretes and/or excretes a substance (e.g., hormone-like compounds and regulatory peptides; Thompson and Kavaliers 1994) that alters the heat tolerance of both its host and co-infecting *Philophthalmus*. This could have a direct effect, i.e., the compound may be secreted into the external milieu to interact with the snail and *Philophthalmus*. Alternatively, *Maritrema* may manipulate the snail's physiological response resulting in an altered host environment that, as a side effect, allows *Philophthalmus* to tolerate high heat conditions. Elevated heat tolerance upon infection corresponding to increased temperature selection in the laboratory and field is unprecedented in the literature and presents a compelling model system for future work.

In conclusion, the major implications from our study are: (1) the effects of related parasite species on the thermal responses of the same host cannot be generalized, and could lead to the spatial segregation or patchy distribution of conspecific hosts harboring different parasite species as they seek microhabitats with contrasting thermal properties; (2) temperature effects on host survival may differ with temporal scale; (3) it is possible that co-infection may have positive consequences for parasites under certain environmental parameters; and (4) host manipulation may include altered thermal tolerance and behavioral thermal selection.

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