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Spatial variation in parasite-induced mortality in an amphipod: shore height versus exposure history

A. E. Bates · R. Poulin · M. D. Lamare

Abstract Characterizing the causes of spatial and temporal variation in parasite-induced mortality under natural conditions is crucial to better understanding the factors driving host population dynamics. Our goal was to quantify this variation in the amphipod *Paracalliope novizealandiae*, a second intermediate host of the trematode, *Maritrema novaezealandensis*. If infection and development of trematode metacercariae are benign, we expected mature metacercariae to accumulate within amphipods inhabiting high infestation areas. In field samples, intensity levels of mature metacercariae decreased linearly when amphipods harbored >5 immature metacercariae. This finding is consistent with the hypothesis that the parasite can be detrimental at high intensities of infection. Short-term field experiments showed that host survival also declines with the intensity of new infections and drops below 80% when early stage metacercariae reach 10 amphipod^{-1}. However, parasite effects varied over space and time. High-shore amphipods suffered an increased risk of infection in the summer and a lower likelihood of survival: there was a 10–30% decrease in survivorship for any given infection intensity at high- versus low-shore locations. We also tested for differences in the susceptibility of naive and exposed populations using transplant experiments, and found that naive amphipods acquired greater parasite loads (on average, 4.7 vs. 2.8 metacercariae amphipod^{-1}). Because survival decreases rapidly with infection intensity of both early- and late-stage metacercariae, naive populations would suffer considerably if the parasite were to increase its range. Our results indicate that trematode infections cause high mortality in amphipods during summer months under natural conditions, and emphasize that the effects of parasitism vary at local spatial scales and with exposure history.

Keywords *Paracalliope novizealandiae* · *Maritrema novaezealandensis* · Microphallidae · Parasite · Susceptibility · Naive host

Introduction

Parasites can regulate the population dynamics of their host species by causing mortality of individuals (Tompkins and Begon 1999) and by decreasing fecundity (e.g., Hudson et al. 1998). Although early theory predicted that virulent parasites should be selected against, because usually parasites that kill their host also die, the lifetime reproductive success of parasites depends upon interactions between survival, reproduction, and transmission success (Ewald 1983; May and Anderson 1983; Anderson and May 1982). There are numerous theoretical (e.g., Adler and Kretzschmar 1992; Anderson and May 1978; May and Anderson 1978) and experimental studies that document parasite-induced intensity-dependent mortality (reviewed in Tompkins et al. 2002).

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The effect of parasites on host populations varies, such that the impact of a parasite species on a host population can differ by locality or time. This results, in part, from environmental variability. For instance, the transmission and developmental rates of many parasites are positively related to temperature leading to higher prevalence (percent of the population with the infection), infection intensity and, at the extreme, host mortality (e.g., Harvell et al. 1999). There is also a growing understanding that biotic factors modulate the impact of parasites on their hosts, and that infection prevalence and parasite effects can vary with community patterns. For example, sympatric species can impact the success of transmission stages and subsequent infection rates (Thieltges et al. 2008). Mouritsen and Poulin (2003) provide an example of such a case where the intensity of trematode infections in a cockle host was reduced in the presence of anemones that prey upon the parasite's transmission stages. Such findings suggest strongly that parasitism and its effects must show considerable spatial and temporal heterogeneity. Indeed, a meta-analysis of published data on infection levels by trematode parasites in crustaceans and bivalves indicates that, although variation among host populations of the same species is somewhat constrained, there is still substantial spatial variation in the infection risk faced by individual hosts resulting from local abiotic or biotic factors (Thieltges et al. 2009).

The effects of parasites on host populations also relate to spatial and temporal variation in local parasite abundance. For instance, differences among host populations in past co-evolutionary history with a parasite can lead to differences in susceptibility. This is because selection for resistance can occur in host populations that are exposed to a parasite that reduces fitness, while conversely, because resistance can be costly (Moret and Schmid-Hempel 2000; Sheldon and Verhulst 1996), in the absence of parasites, hosts are predicted to invest less in resistance mechanisms (Lohse et al. 2006; Webster et al. 2004). Hasu et al. (2009) recently compared the susceptibility of isopod populations with different levels of exposure to an acanthocephalan parasite using controlled laboratory experiments. These authors found that, in general, parasitized populations were less susceptible to infection than unexposed populations. Yet how historical exposure to parasites relates to infection susceptibility, and in turn, survivorship, is poorly understood, especially in field conditions. This is largely because estimates of host susceptibility and survivorship are difficult to obtain in nature.

Paracalliope novizealandiae Dana, 1853 (Amphipoda: Crustacea) is an abundant amphipod in coastal sedimented habitats in New Zealand (Cummings et al. 1995) and is a major prey for a number of shorebirds. The amphipod is one of several crustacean species that acts as a second intermediate host to the microphallid trematode, *Maritrema novaezealandensis* Martorelli, 2004. Amphipods are infected by free-living cercariae that are shed directly into the water via the first intermediate host, *Zeacumantus subcarinatus* Sowerby, 1855 (Prosobranchia: Batillariidae) (Martorelli et al. 2004). The cercariae penetrate the cuticle wall directly and intensity-dependent mortality has been observed in *P. novizealandiae* following artificial infections in the laboratory (Fredensborg et al. 2004). Immediately after entering the host, migration and growth of metacercariae likely damage host organs and connective tissue, divert host resources toward tissue repair, and may decrease host survivorship (Meißner and Bick 1999). Mature metacercariae encyst after a growing period, a process that takes 4–5 weeks (Martorelli et al. 2004). Cysts themselves are relatively benign (e.g., Meißner and Schaarschmidt 2000; Meißner and Bick 1999; Jensen et al. 1998; Thomas et al. 1998) and are consequently predicted to accumulate in the host over time provided the host survives the earlier infection stages. The trematode's lifecycle is completed when shorebirds ingest crustaceans with mature metacercariae (Martorelli et al. 2004). This parasite–intermediate host system is particularly amenable to field studies that test for host survivorship under different scenarios because amphipods can be easily collected and manipulated, and prevalence of *M. novaezealandensis* infection varies widely among amphipod populations (e.g., Fredensborg et al. 2004). Furthermore, Bryan-Walker et al. (2007) found that amphipods from a parasite-free population (naive) acquired more metacercariae than those regularly exposed to the trematode. Their experimental data from laboratory trials indicate that exposed amphipods are better able to avoid infection via behavioral mechanisms such as grooming, and once infection has occurred, amphipods from the exposed population encapsulate and melanize (immune response) significantly greater numbers of metacercariae than naive individuals.

The main objective of the present study was to test for parasite-induced mortality in host populations under field conditions using survey and experimental approaches. Specifically, we tested the following hypotheses: (1) mature metacercariae will accumulate in amphipods over time, as would be predicted to occur if parasite-induced mortality is low, (2) the intensity of new infections is negatively related to survivorship and varies over space, and (3) naive populations suffer higher infection intensity and lower survivorship than populations naturally exposed to the parasite. Our field survey design further allowed us to characterize spatial and temporal variability in natural infection intensity.

Materials and methods

Infection intensity

Infection intensity is defined both in field surveys and experiments as the number of metacercariae per amphipod; mean infection intensity is thus the average number of metacercariae per host across a group of amphipods, with uninfected individuals included in this calculation.

Field surveys

Sampling was conducted monthly from October 2008 to April 2009 during low tide series at seven haphazardly placed transects spaced along a 3.5-km stretch of soft-sediment shore, labelled 1–7 (electronic supplementary material, ESM) on the Otago Peninsula, South Island, New Zealand (45°52′S, 170°42′E). Fixed positions along each transect represented low (0.1–0.3 m above MLW), medium (0.4–0.6 m) and high (0.7–0.9 m) shore heights; thus, because shore slope varied among sites, transect length ranged from ∼150 to 400 m. At each fixed location, amphipods were sampled using a 1-mm sieve and transported to the laboratory in a 10-L bucket with sea water. Fifteen specimens between 3.5 and 4.5 mm body length (reproductive adults) were haphazardly selected and dissected under a light microscope. Trematode metacercariae were counted and classed as immature (size: 150 ± 100 μm) or mature (single or double walled cyst, size: 300 ± 50 μm) (see Keeney et al. 2007 for pictures of all stages of metacercariae). Melanized metacercariae were also counted (Bryan-Walker et al. 2007). *Maritrema novaezealandensis* was the only trematode found infecting amphipods.

Transect 5 was excluded from analyses of field survey data because of consistently low natural infection prevalence, and this locality was consequently selected for deployment of experiments (*Zeacumantus subcarinatus* was rarely observed along transect 5 which was isolated on both sides by gravel/sand bars). To test for spatial and temporal variability in infection intensity, a repeated measures two-way ANOVA compared square-root-transformed intensity values among the three shore heights sampled from November to March (followed by Tukey HSD multiple comparisons tests).

We tested for a positive linear relationship (null hypothesis) between mean intensity of immature metacercariae (represents infection intensity at time = x) and mean intensity of mature metacercariae 1 month later (represents metacercariae at time = $x + 1$ that have successfully developed from previous months). In addition, we tested alternative functions of best fit in the relationship between immature and mature metacercarial intensity. To do so, we pooled data across transects, at the low-, mid- and high-shore locations to yield 90 data points (6 transects × 3 shore heights × 5 months: November immature metacercarial mean intensity versus December mature metacercarial mean intensity, etc.). Because our goal was to test for best fit rather than to infer causality, we selected parametric regression statistics (although the data are not independent: i.e., subsets of data represent different shore heights and months).

To determine if the number of metacercariae harbored by an individual amphipod is positively related to the number of melanized metacercariae present, we pooled infection data for all of the amphipods collected for each monthly survey (15 amphipods × 3 shore heights × 6 transects × 6 months). We then calculated the mean number of melanized metacercariae for each metacercarial count with >3 representative amphipods to conduct a regression analysis (means were selected because data was non-normally distributed). In total, 1,604 amphipods were included in the analysis with relatively higher numbers of amphipods falling into the lower metacercarial counts.

Experiments

Transplant experiments were conducted at sites in Otago Harbour in January and March of 2009 by caging amphipods at locations with varying prevalence of *Maritrema novaezealandensis*. Shore heights were estimated by extrapolation from tide height predictions for the standard port, Port Chalmers (MetService, New Zealand) (ESM).

Transplant cages Cages were constructed from 250-ml square sample jars (LABSERV: Polypropylene) by cutting panels from the bottom and 4 sides of each jar and then replacing the panels with 1.0-mm mesh (Nitex). Mesh was affixed to the jar using hot glue (Bee Creative: Model No. BC0027S). An approximately 30 cm length of wire was then used as a staple inserted into the sample jar lid to secure the cage, upside down, to the substratum. Pilot studies verified both the high retention and survivorship of amphipods in cages in flow-through laboratory aquaria (over 24 h), and the ability of cercariae to cross through the mesh.

Animal collection Amphipods were collected from different sites (see below) using a 1.0-mm mesh net (which retained animals > 3.5 mm), transported to the laboratory in 10-L buckets filled with seawater, and haphazardly dispensed into each cage using a Pasteur pipette. Sea lettuce (*Ulva* spp.: ∼5 × 5 cm) was provided for shelter and food. Cages were then placed in flow-through aquaria overnight and deployed in the field the following morning as described below.

Survivorship, infection intensity and shore height To determine if host survivorship relates to the intensity of new infections, amphipods were caged ($n = 100$ per cage; collected from a naive population: Hooper's Inlet; ESM) in intertidal pools at high (0.8 m above MLW) and low (0.2 m above MLW) shore positions on January 16 near transect 5. Fifteen cages at each shore height were divided evenly among three treatments to achieve infection levels representing a continuum between 0 and 95 metacercariae amphipod^{-1} by adding the trematode's first intermediate host, *Zeacumantus subcarinatus* (snail), to the cages: (1) high infection intensity: 12–20 infected snails out of 25 (collected near transect 6: infection status was determined after the experiment); (2) moderate infection intensity: 3–6 infected snails out of 25 (collected near transect 3); and (3) low infection intensity: no snails. At the completion of the experiment (duration was 100 h), the number of amphipods remaining in each cage was counted. Fifteen amphipods (>3.0 mm in length) were then haphazardly selected from each cage for dissection to estimate metacercarial intensity. ANCOVA compared amphipod survivorship between the high and low shore transplant locations using mean number of metacercariae amphipod^{-1} as a covariate on square-root transformed data.

Survivorship, infection intensity and exposure history To determine if amphipod survivorship and susceptibility to new infections relate to prior exposure to *Maritrema novaezealandensis*, we conducted an additional experiment using the same cages described above but without snails. To achieve a reciprocal transplant design, we collected amphipods from Lower Portobello Bay near locations with high (transect 6: >90%) and low (transect 5: <5%) *M. novaezealandensis* infection prevalence. To assess difference in survival and susceptibility related to prior exposure to the trematode, we also included amphipods from a naive population: Hooper's Inlet (ESM). The snail first intermediate host of *M. novaezealandensis* is absent from Hooper's Inlet; thus, although huge numbers of amphipods have been examined from this site in recent years, infected individuals have never been found (Bryan-Walker et al. 2007; Fredensborg et al. 2004). Five groups of three cages ($n = 100$ amphipods per cage; each cage housed amphipods from a different collection locality) were placed in intertidal pools separated by 5–10 m along a horizontal transect at 0.7 m (shore height). We conducted this experiment once at each of two localities in Lower Portobello Bay (adjacent to transects 5 and 6) on March 2 and 16 (experiment duration was 100 h in both cases). Amphipod survivorship and metacercarial intensity were quantified as described above. An ANCOVA tested for significant differences in amphipod survivorship with metacercarial intensity as a covariate among the three experimental

populations of amphipods, between the two replicate experiments, and between the two transplant locations. A paired *t* test was used to test for differences in infection susceptibility between naive amphipods and those that were exposed to low levels of trematode infection.

Statistical analyses

All analyses were conducted in R (2.9.1) (alpha = 0.05). Assumptions of normality (Shapiro–Wilk Test) and homogeneity of variances (Bartlett's Test) were tested prior to analyses as required. When transformation did not meet assumptions for parametric tests, alpha was reduced to 0.01 to avoid the probability of making a Type I error. Covariance matrix circularity (Mauchly test statistic) was checked for repeated measures analyses and data were log-transformed if necessary.

Results

Field surveys

Prevalence (% individuals that harboured metacercariae per sample) was highly variable in both space and time and within any given month ranged from 0 to 100%. The greatest change in prevalence from 1 month to the next at a fixed sampling site occurred at transect 3 (high shore): prevalence dropped from 100% in February to 13% in March. The number of metacercariae per infected amphipod was also highly variable and ranged from 1 to a maximum of 54 (January, transect 5, high-shore). Infection intensity was significantly higher in high- versus low-shore, while mid-shore localities displayed intermediate intensities (Fig. 1). Infection intensity was also significantly higher in January and February (9 metacercariae amphipod^{-1}) in comparison to other months (repeated measures two-way ANOVA on square-root transformed data, shore height effect: $F_{2,15} = 14.06$, $P = 0.0013$; month effect: $F_{5,75} = 5.95$, $P = 0.00094$; followed by Tukey HSD multiple comparisons tests).

Overall, the number of mature metacercariae was generally low (<1 amphipod^{-1}) when amphipods contained either few or many immature metacercariae. Mature metacercariae accumulated in the amphipod population while infection intensity of immature metacercariae remained low (<5 amphipod^{-1}), evidenced by a positive linear relationship (regression line slope = 0.77; Fig. 2). However, with increasing infection intensity characteristic of mid- and high-shore locations, the number of mature metacercariae displayed a negative linear relationship with immature metacercarial intensity (regression line slope = −0.22; Fig. 2). The fit for two piecewise
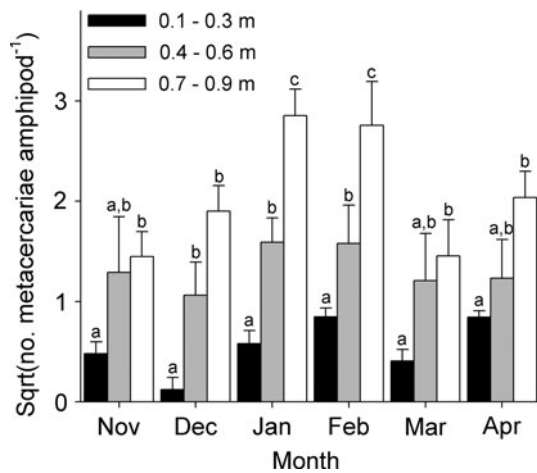
Fig. 1 Number of metacercariae per *Paracalliope novizealandiae* amphipod (mean square root + 1SE, $n = 15$ amphipods per sample) in 6 months along fixed transects ($n = 6$) at high- (0.7–0.9 m, *black*), mid- (0.4–0.6 m, *gray*) and low- (0.1–0.3 m, *white*) shore locations near Dunedin, New Zealand during October 2008–April 2009. Different *letters* above *bars* represent statistically significant differences at $P < 0.05$ determined by repeated measures ANOVA. Summer months are January and February
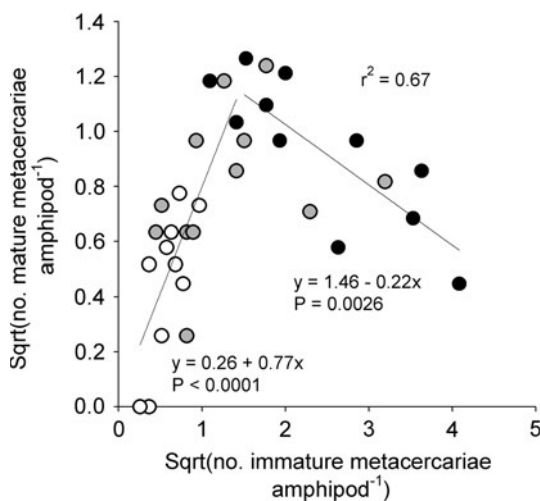

Fig. 2 Number of immature metacercariae hosted by *Paracalliope novizealandiae* in 1 month (mean pooled for November–March sampling intervals) plotted against the number of mature metacercariae per amphipod in the next month (November–April) at fixed sampling locations in the low- (0.1–0.3 m: *white*), mid- (0.4–0.6 m: *gray*) and high-shore (0.7–0.9 m: *black*). Each data point ($n = 90$) is a mean of 15 amphipods and is square-root transformed. Ascending and descending *regression lines* are from a two-line piecewise analysis with the breakpoint equal to the *x*-value that corresponds to the maximum *y*-value. P = significance level of the slope

regression lines was significantly better than a single linear regression on square-root transformed mature versus immature metacercariae intensity (ANOVA: $F_{1,86} = 26.06$, $P < 0.0001$). In contrast, the mean number of melanized metacercariae per amphipod did not change with infection intensity (Fig. 3).
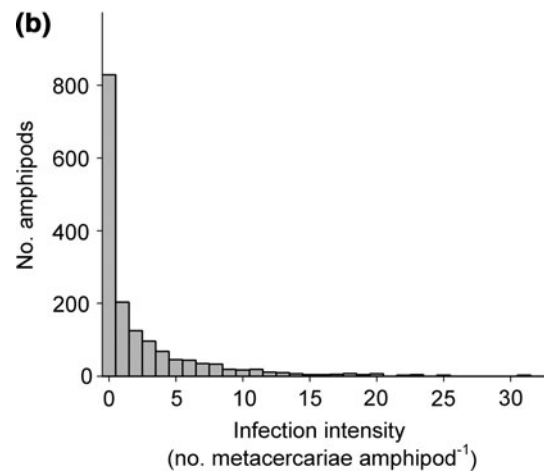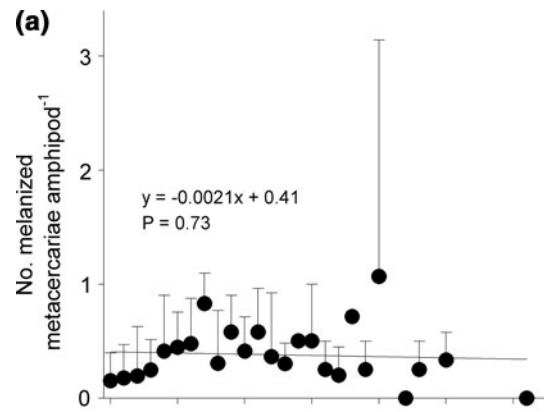

Fig. 3 a Number of melanized metacercariae (mean +1SD) versus infection intensity in *Paracalliope novizealandiae* pooled for collections over 6 months at three shore heights. *Line* best-fit regression. **b** Total number of amphipods in each metacercarial count; minimum is 3

Experiments

Survivorship, infection intensity and shore height. The number of amphipods that survived the 4-day January experiment ranged from 6 to 97 out of approximately 100 that were initially placed each cage. The maximum number of metacercariae harboured by an amphipod was 122. There was a significant negative relationship between survivorship and the intensity of new infections (no. of metacercariae amphipod^{-1}) at both high- and low-shore heights (ANCOVA on square-root transformed data: infection intensity effect, $F_{1,26} = 202.51$, $P < 0.0001$) (Fig. 4). While the slopes were similar between the two shore heights (shore height by infection intensity effect, $F_{1,26} = 0.79$, $P = 0.38$), their elevations differed (shore height effect, $F_{1,26} = 11.40$, $P = 0.0023$) indicating that, at similar infection intensities, high shore individuals display survivorship values that were 10–30% lower than amphipods from low shore locations (Fig. 4).
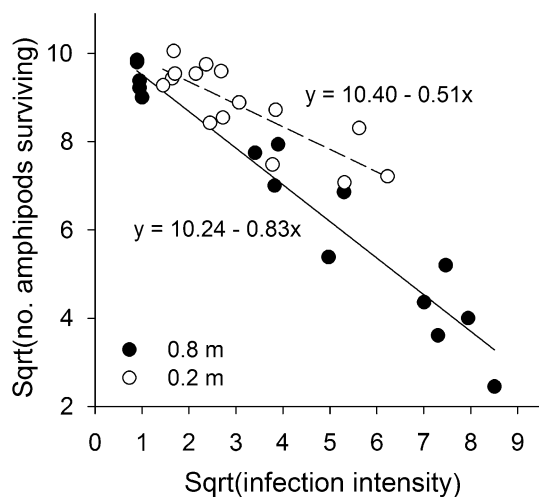
Fig. 4 Survivorship of *Paracalliope novizealandiae* (square root of number of amphipods surviving) versus infection intensity in an experiment where ~100 amphipods were caged with varying numbers of infected snails at high (0.8 m) and low (0.2 m) shore heights in a low infection prevalence locality adjacent to transect 5. For each data point, infection intensity is a mean of 15 amphipods. Descending lines are from a best-fit regression
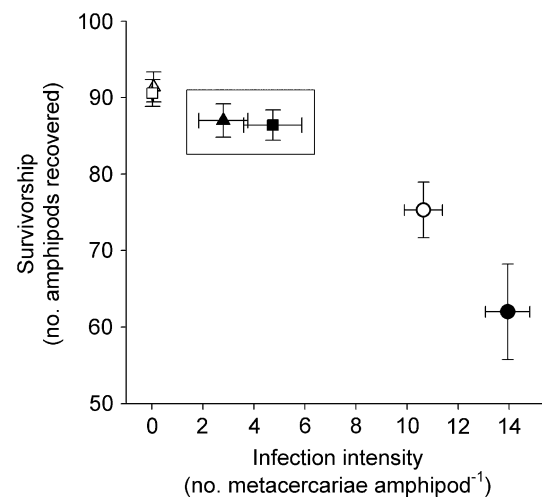

Fig. 5 Survivorship of *Paracalliope novizealandiae* versus infection intensity based on exposed amphipods collected from localities with high infection prevalence (>90%, *circles*), low infection prevalence (<5%, *triangles*,) and naive specimens (no infection, *squares*) and transplanted to high-shore tidepools adjacent to transects in high infection locality (transect 6, *filled symbols*) or low infection locality (transect 5, *open symbols*). Naive amphipods were infected with significantly higher numbers of metacercariae than exposed amphipods when transplanted into high-infection area (*gray box*). Data are the mean ± 1SE of ten replicate cages pooled from two experiments (see text for further information)

Survivorship, infection intensity and exposure history. In March, 36–96 amphipods survived out of ~100 amphipods placed in each cage at the start of the experiment. As observed in January, there was a significant negative relationship between survivorship and overall infection intensity (note that metacercariae from amphipods collected from the high prevalence locality near transect 6 were comprised of both new infections and previously acquired metacercariae at various stages of development) (ANCOVA: $F_{1,47} = 7.99$, $P = 0.0069$) (Fig. 5). For a given infection intensity, amphipod survivorship did not differ among the three experimental populations of amphipods, between the two replicate experiments, or between the two transplant locations, nor were interactions among each possible subset of factors detected (ANCOVA: in all cases $F < 0.23$, $P > 0.67$) (Fig. 5). Although survivorship did not vary spatially, infection intensity did. Overall, the intensity of immature metacercariae per amphipod was higher in cages transplanted to the high prevalence locality adjacent to transect 6 (two-way ANOVA: effect of transplant location: $F_{1,54} = 33.24$, $P < 0.00001$) (Fig. 5). Thus, we focused on these cages to compare infection intensity between paired samples of the naive population (Hooper's Inlet, 0% prevalence), and amphipods collected from the low prevalence population (near transect 5, <5% prevalence). Naive specimens were more susceptible to new infections than the exposed population: mean (±1 SE) metacercariae intensity amphipod^{-1} = 4.7 ± 1.2 and 2.8 ± 1.0, respectively (paired t test: $t = -6.29$, $df = 9$, $P = 0.00014$) (Fig. 5).

Discussion

Parasite-induced mortality is often assumed to occur in nature, based on either theory or laboratory findings, but it is rarely quantified. Ours is one of the rare studies to complement field data on natural patterns with data from in situ experiments demonstrating parasite-induced mortality. Field patterns suggest that *Paracalliope novizealandiae*, a second intermediate host of the microphallid trematode, *Maritrema novaezealandensis*, suffers decreased survival when infected. Specifically, amphipods sampled from fixed locations with low or high intensities of new infections in 1 month did not harbor high intensities of mature metacercariae in the next month (Fig. 2). In comparison, amphipods at locations with moderate infection intensities displayed the highest numbers of mature metacercariae. These data imply that a total of five immature metacercariae is a threshold above which mortality is manifested. Fredensborg et al. (2004) also suggested that the most heavily infected individuals were being removed from the population, based on laboratory observations that there was a decrease in the variance-to-mean ratio in intensity values for the largest size classes only. Larger animals tend to acquire more parasites and thus incur decreased survival. However, alternate hypotheses can explain why amphipods with mature metacercariae drop out of our dataset when the intensity of immature metacercariae infections increase.

For instance, it is possible that amphipods with heavier parasite loads may display enhanced immune efficiency (melanization ability). Even so, the mean number of melanized metacercariae observed did not change significantly with increasing metacercarial intensity (Fig. 3). Amphipods with high numbers of mature metacercariae might also display different behaviors than uninfected individuals, such as being more likely to migrate from the area. However, this possibility can be ruled out because the entire intertidal region was sampled and laboratory studies show reduced activity in infected amphipods (Leung and Poulin 2006).

Our field experiments documented intensity-dependent mortality in *Paracalliope novizealandiae* that otherwise would have survived the 4-day transplant duration in weather conditions that were generally sunny and warm (January: max. air temperature ranged from 21 to 24°C, 0% cloud cover) or cloudy and cool (March: 16–18°C, 50–100% cloud cover) (Figs. 4 and 5). Several pieces of evidence support the view that initial infection by the trematode is pathological in nature. Penetration of the cuticle causes heamolymph loss that may result in osmotic stress and acute anaemia, as well as increased vulnerability to microbial infections (Jensen et al. 1998; Mouritsen and Jensen 1997; Meyers 1990). Furthermore, migration of metacercariae occurred within the first 2 days of infection (Fredensborg et al. 2004) and the resulting tissue damage may be harmful. *P. novizealandiae* also displayed reduced levels of activity upon infection, likely a pathological rather than behavioral response (Leung and Poulin 2006). In addition, our field data are consistent with the results of laboratory infection experiments conducted by Fredensborg et al. (2004) who observed significant and rapid declines in the survivorship of amphipods newly infected by moderate (25) and high (100), but not low (<5), numbers of cercariae. Thus, bursts of cercarial output from snail first intermediate hosts (as occur in response to temperature increases and high light intensity) will decrease host survival and may drive mass mortalities of amphipods in summer months, as is known from other amphipod hosts infected by microphallid trematodes (e.g., Mouritsen et al. 2005; Jensen and Mouritsen 1992).

The parasite was also detrimental at later infection stages. Hosts with multiple (mean ± 1SD: 10.6 ± 8.9) developing metacercariae suffered reduced survival in comparison to uninfected individuals, even in conditions where the rate of new infections was low (<0.25 metacercariae amphipod^{-1} day^{-1}). This may be because the marked growth of microphallid trematodes within their second intermediate host is rapid (Poulin and Latham 2003) and corresponds to a high demand for nutrients that could drain host reserves. However, the underlying pathology of the *Paracalliope novizealandiae* and

Maritrema novaezealandensis association at the various infection stages remains to be determined.

Several factors modulate the susceptibility of *Paracalliope novizealandiae* to infection by cercariae. Infection intensity varied spatially and temporally and was most intense in the high-shore during summer months. The impact of the parasite on amphipod survivorship was also greatest in the high-shore region: for a given infection intensity, amphipods caged in the high-shore suffered decreased survival in comparison to the low-shore. This pattern is probably related to one or a combination of physical conditions that are well known to vary in relation to shore height and season on sedimented shores, in particular temperature (reviewed in Raffaelli and Hawkins 1996). Yet, Meißner and Schaarschmidt (2000) demonstrated that trematode infection in the amphipod *Corophium volutator* did not influence the host's metabolic activity and tolerance to thermal stress. The infection status of *P. novizealandiae* is unrelated to survivorship at temperatures ranging from 15 to 34°C (A. Studer, unpublished data). However, high-shore shallow water pools are exposed to high temperatures and sunlight for longer durations than those in the low-shore; physical conditions that favor enhanced cercarial production and release from snails (e.g., Poulin 2006; Mouritsen and Jensen 1997; Mouritsen 2002). Pulses of cercarial output that are more intense may explain higher mortality rates in the high-shore cages.

For any given infection intensity, survivorship did not differ between *Paracalliope novizealandiae* collected from Lower Portobello Bay (exposed) and Hooper's Inlet (naive). However, the two populations differed in their susceptibility to the trematode. This finding is consistent with the hypothesis that exposure to parasites can select for differences among host populations. Naive amphipods were infected with significantly more parasites [on average, they harbored 1.9 ± 2.8 (mean ± SD) extra metacercariae] than those from the exposed population, despite being caged side by side. Our results corroborate laboratory infection studies conducted by Bryan-Walker et al. (2007), who reported that *P. novizealandiae* from Hooper's Inlet acquired almost two more metacercariae on average than Otago Harbor amphipods when challenged with ∼15 cercariae. Because we found the number of melanized metacercariae was very low in amphipods from both populations (<1%), the most parsimonious explanation is that Otago Harbour amphipods are better at avoiding infection. This is probably because, in the absence of the parasite, there has been less selection to maintain resistance against *Maritrema novaezealandensis* in Hooper's Inlet. For instance, behavioral defences (e.g., grooming; Fleischer et al. 1992) or morphological barriers (e.g., Rigby et al. 2002) such as a strong cuticle may allow exposed

amphipods to persist in areas with a high risk of infection. However, irrespective of the actual mechanism driving differences in the susceptibility of *P. novizealandiae* to the parasite, considerable variability in parasite effects at the population level are evident. Ultimately, because survivorship is related directly to infection intensity, naive animals that are exposed to the parasite will acquire higher infection intensities and will have correspondingly higher rates of mortality.

In summary, our results indicate that the microphallid trematode, *Maritrema novaezealandensis*, can severely reduce the abundance of its second intermediate host, *Paracalliope novizealandiae*, during summer months under natural conditions. Experiments further suggest that host mortality is most severe in the high intertidal because of an increased risk of infection and reduced survivorship at similar infection levels in the high- versus low-shore. Our field experimental results are also consistent with the hypothesis that exposure to *M. novaezealandensis* can drive the evolution of parasite resistance in amphipod populations and lead to inter-population variation in parasite effects. We found that a naive population was a particularly susceptible to parasite infection and would thus suffer considerably should the parasite invade their locality. Predicting the outcome of this host–parasite interaction under natural scenarios is difficult, and requires consideration of small-scale spatial variability, season, and co-evolutionary history. This study illustrates that the effect of parasitism on natural populations is far from homogeneous in space or time, and emphasizes the need for more realistic epidemiological models to forecast the impact of diseases.

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