

Research

Temperature and multiple parasites combine to alter host community structure

Olwyn Friesen, Robert Poulin and Clément Lagrue

O. Friesen (<https://orcid.org/0000-0003-4274-3868>) ✉ (olwynfriesen@gmail.com), R. Poulin (<https://orcid.org/0000-0003-1390-1206>) and C. Lagrue (<https://orcid.org/0000-0003-3347-6497>), Dept of Zoology, Univ. of Otago, Dunedin, New Zealand.

Oikos

130: 1500–1511, 2021

doi: 10.1111/oik.07813

Subject Editor and

Editor-in-Chief: Pedro Peres-Neto

Accepted 24 May 2021

Predicting the effects of climate change requires understanding complex interactions among multiple abiotic and biotic factors. By influencing key interactions among host species, parasites can affect community and ecosystem structuring. Yet, our understanding of how multiple parasites and abiotic factors interact to alter ecosystem structure remains limited. To empirically test the role of temperature variation and parasites in shaping communities, we used a multigenerational mesocosm experiment composed of four sympatric freshwater crustacean species (isopods and amphipods) that share up to four parasite species. Mesocosms were assigned to one of four different treatments with contrasting seasonal temperatures (normal and elevated) and parasite exposure levels (continuous and arrested (presence or absence of parasite larvae in mesocosm)). We found that parasite exposure and water temperature had interactive effects on the host community. Continuous exposure to parasites altered the community structure and differences in water temperature altered species abundance. The abundance of the amphipod *Paracalliope fluviatilis* decreased substantially when experiencing continuous parasite exposure and elevated water temperatures. Elevated temperatures also led to parasite-induced mortality in another amphipod host, *Paracorophium excavatum*. Contrastingly, isopod hosts were affected much less, suggesting increasing temperatures in conjunction with higher parasite exposure might increase their relative abundance in the community. Changes in invertebrate host populations have implications for other species such as fish and birds that consume crustaceans as well as having impacts on ecosystem processes, such as aquatic primary production and nutrient cycling. In light of climate change predictions, parasite exposure and rise in average temperatures may have substantial impacts on communities and ecosystems, altering ecosystem structure and dynamics.

Keywords: community dynamics, competition, multiple stressors, seasonal shift, parasites, temperature, thermal stress

Introduction

Climate, including seasonal variation, plays an important role in determining the abundance and diversity of parasites and hosts, which subsequently affect host–parasite dynamics (Harvell et al. 2002, Kutz et al. 2009, Macnab and Barber 2012, Marcogliese

2016, Labaude et al. 2017). As parasites impact species distributions, biodiversity, trophic interactions and community structure in many ecosystems (Price et al. 1986, Kiesecker and Blaustein 1999, Thomas et al. 1999, Wood et al. 2007, Friesen et al. 2019), any alterations in parasite dynamics may have cascading effects on the whole ecosystem (Mitchell et al. 2003, Epstein 2010, Marcogliese 2016, Auld and Brand 2017, Labaude et al. 2017, Friesen et al. 2020). Therefore, it is essential to assess how these factors may interact in order to better understand seasonal dynamics within the community and predict the impacts of climate change.

Physiological tolerance and thermal optimum vary widely among ectotherms, and as such, external temperature is particularly crucial to host–parasite interactions (Quinn et al. 1994, Kirk et al. 2018). Changes in the external environment affect the host's internal environment and that of its parasites (Quinn et al. 1994, Marcogliese 2001, 2016, Barber et al. 2016, Kirk et al. 2018). Increases in temperature accelerate biochemical reactions and metabolic rates of ectothermic hosts and parasites (Quinn et al. 1994, Helmuth and Hofmann 2001). These changes can affect host–parasite interactions, accelerate parasite development, shorten generation time, impact transmission of non-feeding, free-living stages of parasites (like trematode cercariae), increase host susceptibility by weakening host defences, and modify parasite-mediated competition (Blanford et al. 2003, Larsen et al. 2011, Macnab and Barber 2012, Sheath et al. 2016, Mouritsen et al. 2018). This in turn may affect the outcome of competition among hosts that share the same species of parasites (Esch et al. 1975, Studer et al. 2010, Macnab and Barber 2012). Further, multispecies infections in hosts are common (Pedersen and Fenton 2007, Lagrue and Poulin 2008a, Alizon et al. 2013, Friesen et al. 2017). The effects of these diverse within-host parasite assemblages can be very difficult to predict, particularly in systems experiencing substantial changes in abiotic conditions, such as temperature (de Roode et al. 2004, Balmer et al. 2009, Alizon 2013, Lange et al. 2014). Further, as temperatures are expected to continue to increase in many ecosystems, understanding potential impacts on parasites and their hosts is essential for predicting community dynamics in years to come.

Although we have a growing understanding of some of the direct consequences of changing temperatures on both hosts and parasites (Studer et al. 2010, Larsen et al. 2011, Labaude et al. 2017, Molnár et al. 2017, Mouritsen et al. 2018, Klemme et al. 2021), most studies have used small scale, single generation experiments, focusing on two host species with one shared parasite. Communities are shaped by parasite-mediated interactions (Friesen et al. 2020), and natural communities comprise multiple parasite species infecting a variety of hosts that interact with each other. Although modeling studies and literature reviews have explored the role of changing temperatures on parasites and host–parasite interactions (Poulin 2005, Marcogliese 2008, Altizer et al. 2013, Molnár et al. 2013), empirical tests of the impacts of multiple parasite species on community dynamics in the context of temperature changes, due to seasonal shifts or climate

change, are still lacking, at least to the best of our knowledge. In contrast to the very few earlier studies considering temperature–parasitism interactions at a host community level (Mouritsen et al. 2018), our study includes the effects of more than one parasite species across more than one host generation and a broader taxonomic range of hosts (both amphipods and isopods), to closer replicate interactions occurring in natural systems. The interplay of biotic and abiotic factors, such as temperature, competition and predation, may lead to outcomes other than those predicted by studying each impact alone. Therefore, to make reliable long-term predictions on how shifts in temperature, such as between seasons, may affect parasites and their effects on community dynamics, there is a strong need for controlled, multigenerational experiments.

Here, we examined how differences in temperature and parasite exposure affected community structure and dynamics by using mesocosms comprising four freshwater crustacean host species, two amphipods, *Paracalliope fluviatilis* and *Paracorophium excavatum*, and two isopods, *Austridotea annectens* and *A. lacustris*, that share a range of macroparasites (Friesen et al. 2020). We manipulated host exposure to the infective larval form of two of the most common shared parasite species, the trematodes *Coitocaecum parvum* and *Maritrema poulini* (Friesen et al. 2017). We used two biologically relevant temperatures, representing the average and high end of the natural temperature range and seasonal variation of our focal sample site (Schallenberg and Burns 2003). Although these species are exposed to the higher temperature, they only experience these temperatures for short periods, and can use refuges within their habitat to find lower temperatures (Schallenberg and Burns 2003). We are expanding on previous work that demonstrated that parasites shape their host community, impacting recruitment and relative abundance of hosts and non-host species (Friesen et al. 2020). If temperature modulates parasite-mediation in this community, we expect to see differences in parasite abundance, host survival, fecundity and abundance among treatments that vary in parasite exposure and temperature.

Material and methods

Study system and animal collection

We collected amphipods, isopods and New Zealand mudsnails *Potamopyrgus antipodarum*, from the littoral zone of Lake Waihola (46°01'14 S, 170°05'05 E) in May 2017. Lake Waihola water temperatures reach 20°C during the summer months (December–January, Schallenberg and Burns 2003); sustained periods at these temperatures, and above, are likely to become more common. One temperature logger (HOBO Tidbit ver. 2) was placed in Lake Waihola in July 2017 as part of a longer-term monitoring project. The logger was placed at a depth of 1 m and recorded temperature every hour.

All four host species, *P. fluviatilis*, *Paracorophium excavatum*, *Austridotea annectens* and *A. lacustris*, compete over

resources and habitat, and may face intra-guild predation, (Holton 1984, Lagrue and Poulin 2007, Friesen et al. 2017, 2018). They also fill important functional roles in the ecosystem, as primary consumers of algae and as important food sources for a number of fish and birds. Host species in this system also have relatively short life spans, providing an opportunity to examine the role of both temperature and parasites across more than one generation.

Maritrema poulini infects the New Zealand mudsnail (first intermediate host), crustaceans (second intermediate hosts) and waterfowl (definitive hosts; Presswell et al. 2014). The trematode *C. parvum* shares a similar life cycle but uses fish as definitive hosts (Lagrue and Poulin 2007). Both amphipod species are host to these trematode species. The isopod, *A. annectens* is also commonly infected by *M. poulini*. *Maritrema poulini* is also found in *A. lacustris* but at extremely low prevalence (Presswell et al. 2014, Friesen et al. 2018, 2020, Goellner et al. 2018). As both *M. poulini* and *C. parvum* can successfully complete transmission between first and second intermediate hosts in a mesocosm setting and parasite exposure rate can be controlled, these trematodes were selected as focal parasites and used to create continuous exposure (infective larval stages of both species present in the mesocosm through infected first intermediate host snail) and arrested exposure (no additional exposure to infective parasite larval stages of either species in mesocosm) treatments. The overall structure of this study system is outlined in Friesen et al. (2020).

Amphipods and *A. annectens* isopods were captured using dip-nets and sieved to select larger animals. No amphipod or isopod smaller than 2 mm in total body length were collected. *Austriodotea lacustris* individuals were collected by hand underneath large rocks near the shoreline. The mudsnail was collected from macrophytes, sediment and stones in Lake Waihola. Isopods, amphipods and snails were transported and maintained separately by species in 10 l tanks containing aerated lake water and aquatic plants, *Myriophyllum triphyllum* and *Elodea canadensis*, for food. All tanks were kept in the same room under a controlled photoperiod (12 h dark and light) at ambient temperatures ($14^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$). Animals were maintained for two days prior to experimentation.

Snail infections

Snails were separated into 12-well plates with ten individuals per well in approximately 2 ml of filtered lake water to determine their infection status. They were then incubated for at least 3 h at 20°C under constant light to trigger the emergence of cercariae (Hay et al. 2005). Wells were screened for the presence of parasite larval stages and cercariae were identified using morphological features (Hechinger 2012, Presswell et al. 2014). Snails in wells containing cercariae were further separated and screened again individually. All snails shedding *M. poulini* or *C. parvum* cercariae were subsequently isolated by infection status and maintained in 250 ml plastic containers filled with aerated water and food until needed in mesocosm experiments. No parasite species

co-infections were found in these snails. A subsample of uninfected snails was also haphazardly selected for use in arrested exposure mesocosms. Snails that were considered uninfected individuals were screened once more pre-experiment to confirm their status and then dissected post-experiment for further verification and to ensure no false negatives were present. All snails presumed uninfected were confirmed to be uninfected post-experiment.

Mesocosm experiment

We manipulated the exposure of two parasite species and water temperature in a 2×2 factorial mesocosm experiment to explore their impacts on the composition of communities with four species of crustaceans. All mesocosms were set up in identical 14 l aquaria (315 wide \times 185.5 deep \times 245 mm high, glass sides with plastic base and lid). Further details about the set-up of mesocosms can be found in the Supporting information. Each tank in the elevated temperature treatment included a water heater (25 W glass aquarium heater). Heaters were turned on at the beginning of the experiment after the addition of the animals, to ensure a gradual increase in temperature. Thermometers were placed in an equal number of tanks of each treatment type and monitored daily.

Mesocosms contained 20 *P. fluviatilis* (10 females, 10 males), 15 *P. excavatum* (sex unknown as it is impossible to differentiate sexes without dissection), 10 *A. annectens* (near identical mixture of large and small individuals in each replicate to include a range of age and sex), 5 *A. lacustris* (including one pair to ensure at least one mature female and male per replicate) and two snails (above). The initial numbers of individual crustaceans reflect approximately the relative abundance of each species in the field (Lagrue et al. 2015).

Arrested exposure tanks contained naturally infected crustacean hosts and two uninfected snails, whereas continuous exposure tanks contained naturally infected crustaceans, one snail infected with *M. poulini* and one snail infected with *C. parvum*. All study species were collected from natural settings where parasites are present, and thus may have had some pre-existing parasite burden (e.g. infected individuals in the arrested parasite treatments). Normal temperature tanks were kept at ambient room temperature throughout the experiment (water temperature, $11^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and elevated temperature tanks were heated constantly (water temperature, $19.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$). Selected temperatures were within the natural range ($4\text{--}24^{\circ}\text{C}$), with the elevated temperature treatment being in the higher range of temperatures recorded at this location (Schallenberg and Burns 2003, Lagrue and Poulin 2008b).

Mesocosms ran for three weeks, with six replicates per treatment. Each mesocosm was supplied ad libitum with aquatic plants (*M. triphyllum* and *E. canadensis*) for food. Plants were weighed to ensure an equal amount of food was added to each mesocosm. All plants were cleaned with water and then frozen for 24 hours to ensure that no additional animals or parasite infective stages were transferred.

Twenty *P. fluviatilis* amphipods were added weekly (two additions over the duration of the experiment) to simulate natural migration of fresh individuals into the area (Friesen et al. 2020). Though all four host species live and interact in the littoral zone, *P. fluviatilis* lives amongst the aquatic macrophytes whereas the other three species (the isopods *A. lacustris* and *A. annectens*, and the tube-dwelling corophioid amphipod *P. excavatum*) spend most of their time in the benthic regions, often buried within the substrate (Friesen et al. 2018, 2020). Although the latter species may exhibit movement in search of resources or mates, they consistently re-settle on or very near their prior location (Marsden 2002, Friesen et al. 2018). The rates of migration we used in this experiment, 20 individuals per mesocosm per week, are based on our best estimates of the amount of migration occurring in natural conditions from years of field observations. Unfortunately, no specific published data on these rates are currently available. Additional information on how the migration rate was estimated can be found in the Supporting information.

Amphipods used to simulate natural migration were isolated in small containers (250 ml plastic containers with aged lake water; one per replicate) before transfer to mesocosms. Containers with individuals assigned to the elevated temperature treatment were gradually brought up to the same temperature as the mesocosms using a small incubator over 3 h, to ensure they were not heat-shocked (Quinn et al. 1994). No amphipods died during this transition process. Animals assigned to the normal temperature treatments were maintained at ambient room temperature for the same period of time. All mesocosms were maintained in the same room, with an even number of each treatment type on each shelf. Temperatures of normal and elevated water were recorded throughout the experiment.

At the end of the experiment, mesocosms were disassembled by carefully removing small aliquots of water and sand substrate that were subsequently screened by two observers and all live crustaceans were captured, until the tank was empty. We performed all amphipod dissections within 72 h. Isopods were dissected within one week. Each individual crustacean was measured, sexed and dissected to record the number of individual parasites of each species present. Further information on sampling and how individuals were sexed is included in the supporting information (Supporting information). Amphipods and *A. annectens* smaller than 2 mm were considered to have hatched in the mesocosm during the experiment as individuals selected at the beginning were over this size threshold; the same was applied to *Austridotea lacustris* smaller than 2.6 mm. Egg presence and numbers were also recorded for each gravid female. Precopulatory pairs, where a male is clasping a female, were also recorded (Sutcliffe 1992). We defined parasite prevalence as the percentage of infected hosts, abundance as the number of parasites per host including zeroes, and mean abundance as the mean number of parasites per host within a specific sample of hosts (Bush et al. 1997). For crustacean hosts, relative abundance was defined as the percentage of the total number of recovered crustacean

individuals in each mesocosm belonging to each of the four species.

Statistical analysis

We tested for the effects of temperature (elevated and normal) and parasite exposure (continuous and arrested), as well as their interaction, on the absolute and relative abundance of each host species at the end of the experiment using a model-based analysis of multivariate abundance data, with parasite exposure and temperature the main predictors, using the *mvabund* package (ver. 4.1.9, Wang et al. 2012; <www.r-project.org>). This model-based analysis uses a generalized linear model as its framework to analyze abundance data and, unlike other distance-matrix-based methods, this analysis does not confuse location with dispersion, which can lead to inflation of type 1 and type 2 errors (Wang et al. 2012, Warton et al. 2012). We used the *anova.manyglm* function in *mvabund* to test for significant effects of treatment on specific species, with a negative binomial distribution. We corrected for multiple tests using the *p.adjust* (*method* = "adjusted").

Differences in sex ratios among treatments at the end of the experiment were examined using nominal logistic analysis, again using temperature and parasite exposure levels (and their interactions) as main factors, as there are three categories of sex (female, male and juveniles). Similarly, differences in parasite abundance and the number of eggs per female were analyzed using a factorial ANOVA and Tukey–Kramer HSD post hoc tests to determine if the four treatments differed. The proportion of females carrying young, and the proportion of male–female pairs were analyzed using nominal logistic analysis. Parasite prevalence was compared among treatments using a nominal logistic analysis.

We examined community dissimilarity between the mesocosms of each treatment at the end of the experiment, using non-metric multidimensional scaling (nMDS) ordination analysis with Bray–Curtis distances based on host absolute abundance, using the *vegan* package (<www.r-project.org>, Oksanen et al. 2015). We ran a permutational multivariate analysis of variance (PERMANOVA) to test for differences among host absolute abundance between treatments based on Bray–Curtis distances. The relationship between parasite exposure and temperature variables with the NMDS axes was also examined with the *envfit* function. We produced a two-dimensional ordination plot, where samples that are grouped more closely together represent more similar host communities than samples spread further apart.

To test the hypothesis that treatment (parasite exposure and temperature) affects community host structure and abundance, we performed a confirmatory path analysis using the *piecewiseSEM* package (ver. 2.1.0, Lefcheck 2016, <www.r-project.org>). Specifically, we used piecewise structural equation modeling (SEM) to examine the effects of parasite exposure and temperature on host species absolute abundance, parasite abundance and host species interactions. Piecewise structural equation modeling can also be used to test the direct and indirect effects of treatments on

host abundance. We checked the data to ensure it met the assumptions of homoscedasticity and multinormality. To better compare response variables, we reduced the model by removing non-significant interactions (Halliday et al. 2019).

A relationship was considered significant if the p-value was ≤ 0.05 and considered a trend if the p-value was 0.05–0.10. Normality of the data was verified using the Shapiro–Wilk test and equal variance was checked with residual plots; all tests returned a p-value > 0.05 and the samples were independent, therefore all assumptions were met in each test. Statistical analyses were performed in JMP Pro 12 (SAS Inst.) and R statistical software (<www.r-project.org>).

Results

We found that parasite exposure and water temperature had interactive effects on the host community (Fig. 1–5). Continuous exposure to parasites altered the community structure and differences in water temperature altered host species abundance. Abundance of the amphipod *Paracalliope*

fluviatilis decreased substantially when experiencing continuous parasite exposure and elevated water temperatures. High temperatures also led to parasite-induced mortality in the other amphipod host, *Paracorophium excavatum*. Contrastingly, isopod hosts were affected much less, suggesting that predicted temperature rise in conjunction with higher parasite exposure might increase their relative abundance in the community.

Overall, when disassembling the mesocosms, we recovered 826 *P. fluviatilis*, 218 *P. excavatum*, 151 *Austridotea annectens* and 213 *A. lacustris*. Five species of parasites, *Maritrema poulini*, *Coitocaeum parvum*, *H. spinigera*, *A. galaxii* and an unidentified cestode (Family Hymenolepididae) were found in the crustacean hosts. The highest number of individual parasites per host was 42 for *M. poulini* (in *A. annectens*), 5 for *C. parvum* (in *P. excavatum*) and 1 for both *A. galaxii* (in *P. fluviatilis*) and *H. spinigera* (in *P. excavatum*). Infection patterns of the experimental host communities prior to the experiment, derived from other studies, are described in more detail in the supporting information (Supporting information).

Parasite infection patterns

Prevalence of *M. poulini* in *P. fluviatilis* differed as a function of parasite exposure (Supporting information), being higher under continuous parasite exposure regardless of temperature. Mean *M. poulini* abundance varied similarly among treatments and was higher in continuous exposure tanks but did not differ between temperature treatments (Table 1, Fig. 1a). Prevalence and mean abundance of *C. parvum* in *P. fluviatilis* showed a similar pattern, although the abundance of *C. parvum* also tended to be higher in normal temperature treatments (Table 1, Supporting information).

In contrast, prevalence of *M. poulini* in *P. excavatum* did not differ among treatments (Supporting information). However, mean abundance of *M. poulini* varied with both parasite exposure and temperature, being lower in the continuous exposure elevated temperature treatment (Table 1, Fig. 1a); the interaction was not significant. Together, temperature and parasite exposure appeared to have a synergistically negative effect on the prevalence of *C. parvum* in *P. excavatum* (Supporting information). The mean abundance of *C. parvum* in *P. excavatum* was impacted by temperature, but not by parasite exposure (Table 1, Fig. 1b). Mean abundance was highest in the normal temperature treatments (Fig. 1b).

The prevalence and mean abundance of *M. poulini* in *A. annectens* did not differ among treatments (Table 1, Fig. 1a, Supporting information). No parasites were found in *A. lacustris* in any of the treatments. Differences in the non-focal parasites between treatments can be found in the Supporting information.

Multispecies infections

Both species of amphipods had multispecies infections; *C. parvum* and *M. poulini* co-infected 0.85% of *P. fluviatilis*

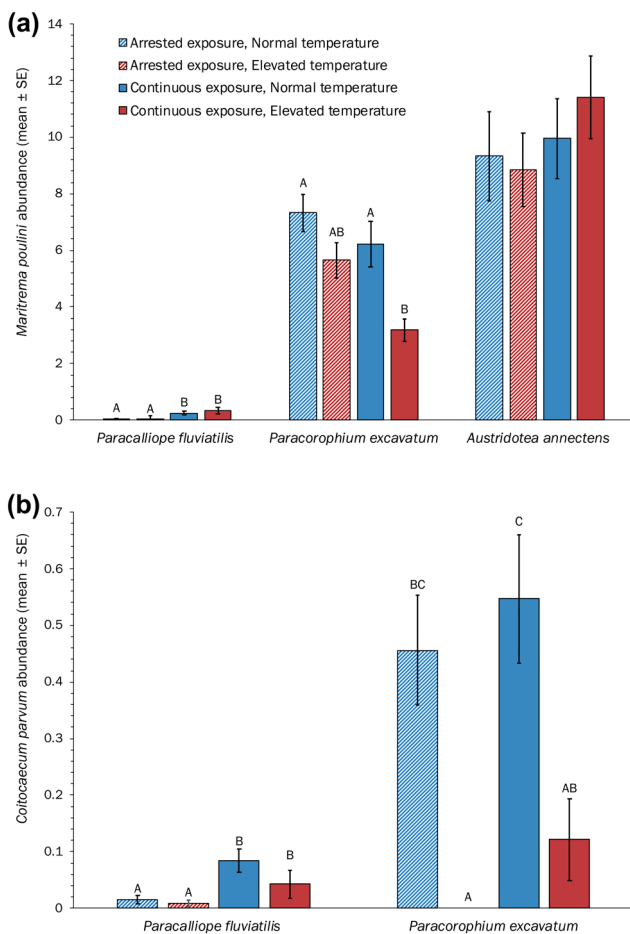


Figure 1. Comparison of (a) *Maritrema poulini* and (b) *Coitocaeum parvum* parasite species abundance per treatment group (mean \pm SE) in all four treatment types. Tukey–Kramer HSD post hoc test results are reported between all treatment groups per species.

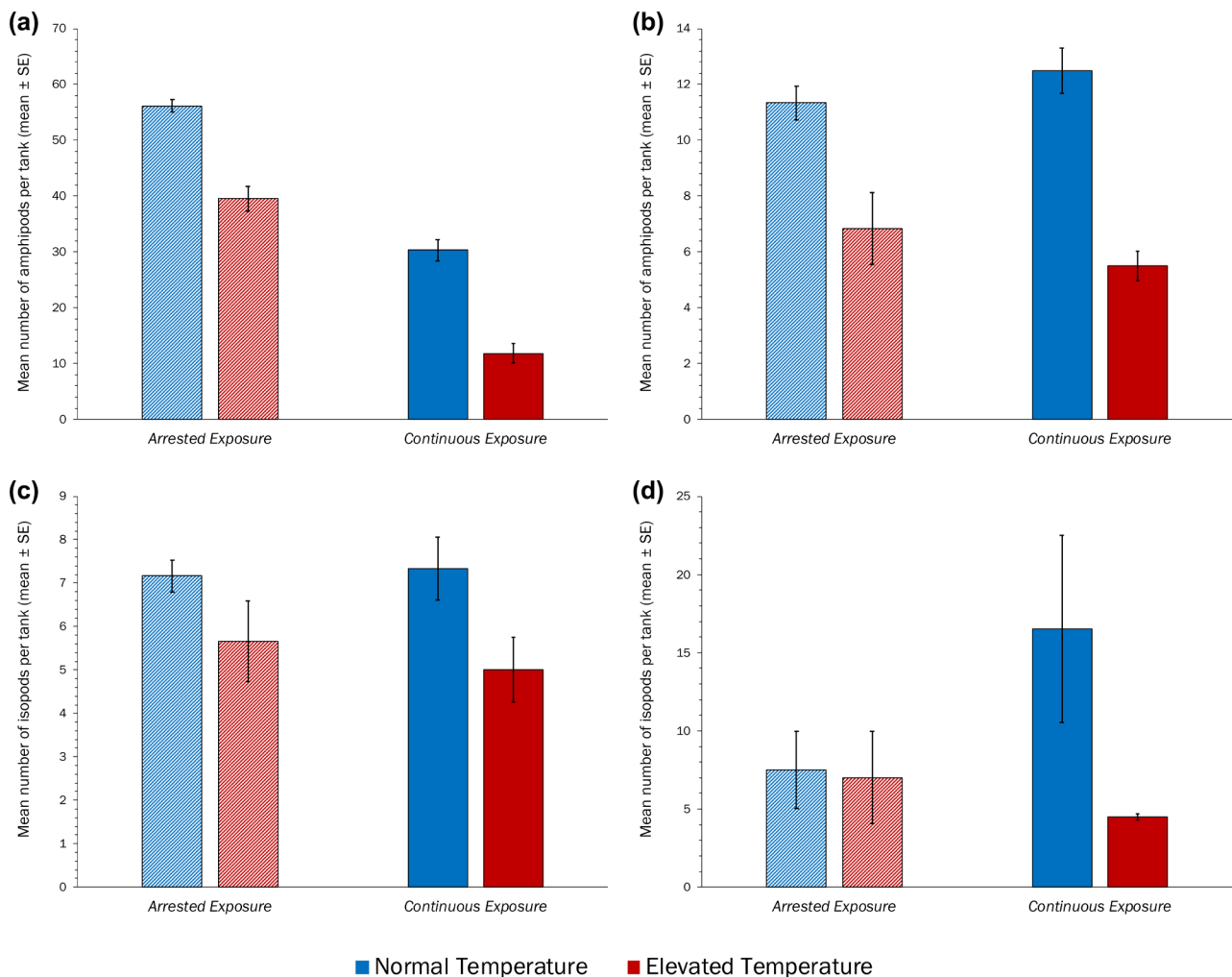


Figure 2. Comparison of (a) *Paracalliope fluviatilis*, (b) *Paracorophium excavatum*, (c) *Austridotea annectens* and (d) *A. lacustris* species abundance per treatment group (mean number of individuals per tank \pm SE) in all four treatment types, arrested parasite exposure with normal or elevated temperatures and continuous parasite exposure with normal or elevated temperatures.

and 22% of *P. excavatum*. The proportion of co-infections by *M. poulini* and *C. parvum* in *P. fluviatilis* was affected by temperature but not parasite exposure (Supporting information). Co-infections in *P. fluviatilis* did not occur in elevated temperature treatments. The prevalence of co-infections in *P. excavatum* varied both with parasite exposure and temperature, with a significant interaction effect (Supporting information). Co-infections were most frequent in normal temperature treatments (Supporting information). Interestingly, *C. parvum* in *P. excavatum* was only found in individuals that were also infected with *M. poulini*.

Host population dynamics

Absolute abundance of the four host species differed among all treatments, between the continuous and arrested exposure treatments as well as normal and elevated temperatures, and there was an interaction between temperature and parasite exposure (model-based analysis of multivariate

abundance data (*mvabund*), parasite exposure: Dev=20, $p=0.006$, temperature: Dev=55, $p=0.001$, interaction: Dev=18, $p=0.003$; Fig. 2, summarized in the Supporting information).

Abundance of the amphipod *P. fluviatilis* varied among all four treatments; it was much higher in mesocosms with arrested parasite exposure and normal temperature and lower under continuous parasite exposure and elevated temperature (*mvabund*, parasite exposure: Dev=18.9, $p=0.001$; temperature: Dev=28, $p=0.001$; interaction, Dev=13, $p=0.002$, Fig. 2a). Elevated temperature had an interactive effect with parasite exposure, decreasing the absolute abundance of this species. The number of juveniles that hatched within the mesocosm also differed among treatments, with a negative relationship between temperature and the number of juveniles present (Supporting information). Interestingly, there was no difference in juvenile numbers due to parasite exposure (Supporting information). Abundance of *P. excavatum* was lower in elevated temperature treatments but did

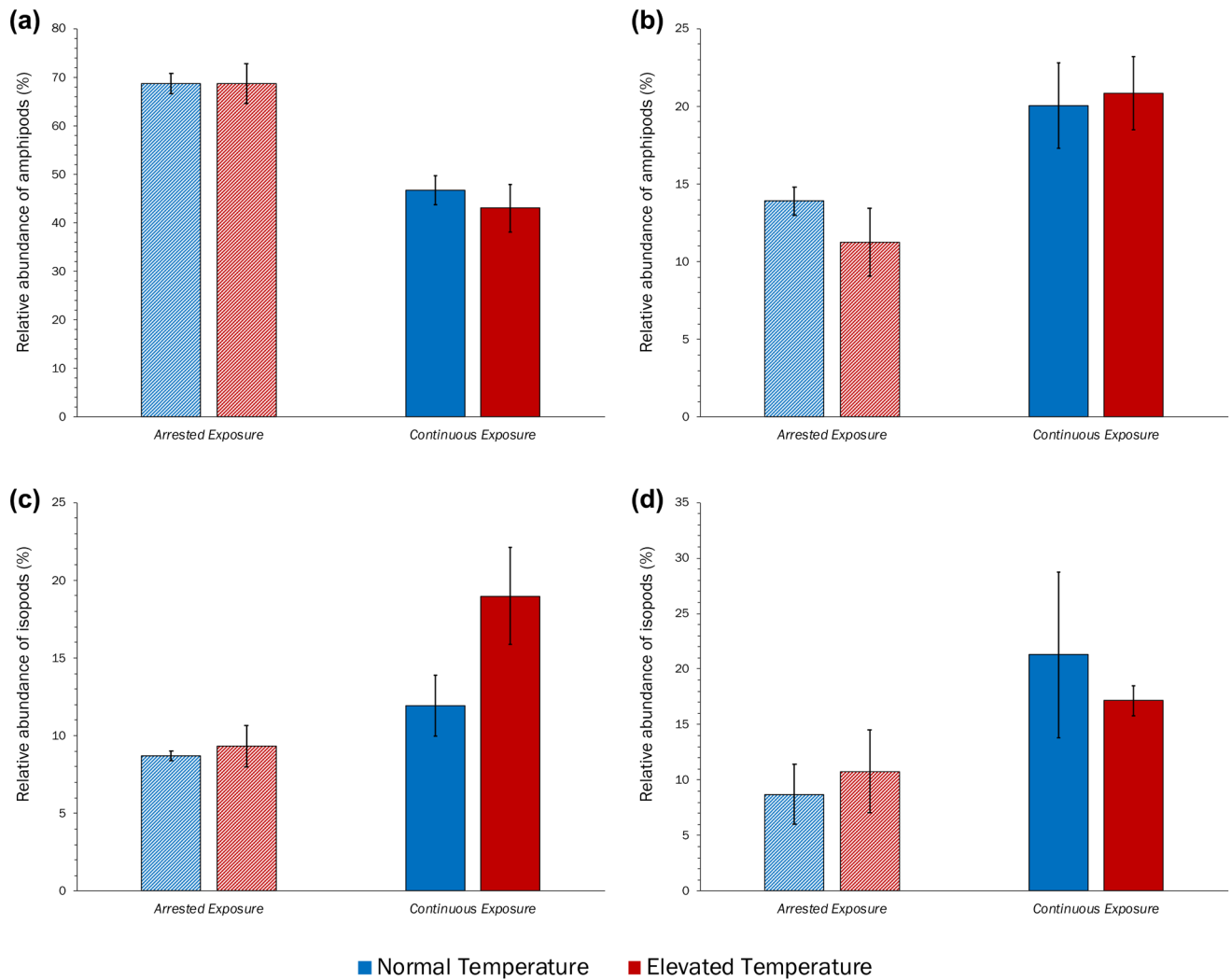


Figure 3. Comparison of relative species abundance (as a percentage of the sample community, mean \pm SE) of (a) *Paracalliope fluviatilis*, (b) *Paracorophium excavatum*, (c) *Austridotea annectens* and (d) *A. lacustris* in all four treatment types, arrested parasite exposure with normal or elevated temperatures and continuous parasite exposure with normal or elevated temperatures.

not differ between parasite exposure treatments (*mvabund*, parasite exposure: Dev=0.003, $p=0.97$; temperature: Dev=20, $p=0.001$; interaction: Dev=1.2, $p=0.31$; Fig. 2b). Abundance of *A. annectens* was not impacted by temperature or parasite exposure (*mvabund*, parasite exposure: Dev=0.06, $p=0.94$; temperature: Dev=3.5, $p=0.17$; interaction: Dev=0.2, $p=0.57$; Fig. 2c). Abundance of *A. lacustris* did not differ among treatments (*mvabund*, parasite exposure: Dev=1.1, $p=0.65$; temperature: Dev=3.7, $p=0.17$; interaction: Dev=3.5, $p=0.19$; Fig. 2d).

Host community structure

Host communities differed among treatments (PERMANOVA, $df=3, 23$, model $F=19.032$, $R^2=0.74$, $p < 0.0001$, Fig. 4a). The nMDS showed that *A. annectens*, *P. fluviatilis* and *P. excavatum* were impacted by temperature.

Paracalliope fluviatilis was also strongly impacted by parasite exposure (Fig. 4b). Parasite exposure was associated with the nMDS axis 1 ($R^2=0.73$, $p=0.001$) and temperature was associated with the nMDS axis 2 ($R^2=0.46$, $p=0.004$), indicating both are related to the differences host communities between treatments.

The relative abundance of both amphipod species and the isopod *A. annectens* in the invertebrate community differed between parasite exposure treatments but did not significantly differ due to temperature (*mvabund*, parasite exposure: Dev=51, $p=0.001$; temperature: Dev=4.3, $p=0.41$; interaction: Dev=4, $p=0.48$; Fig. 3, summarized in the Supporting information). Further statistical analysis of the differences in relative abundance can be found in the supporting information (Supporting information).

Structural equation modeling was used to explore the effects of parasite exposure (arrested versus continuous

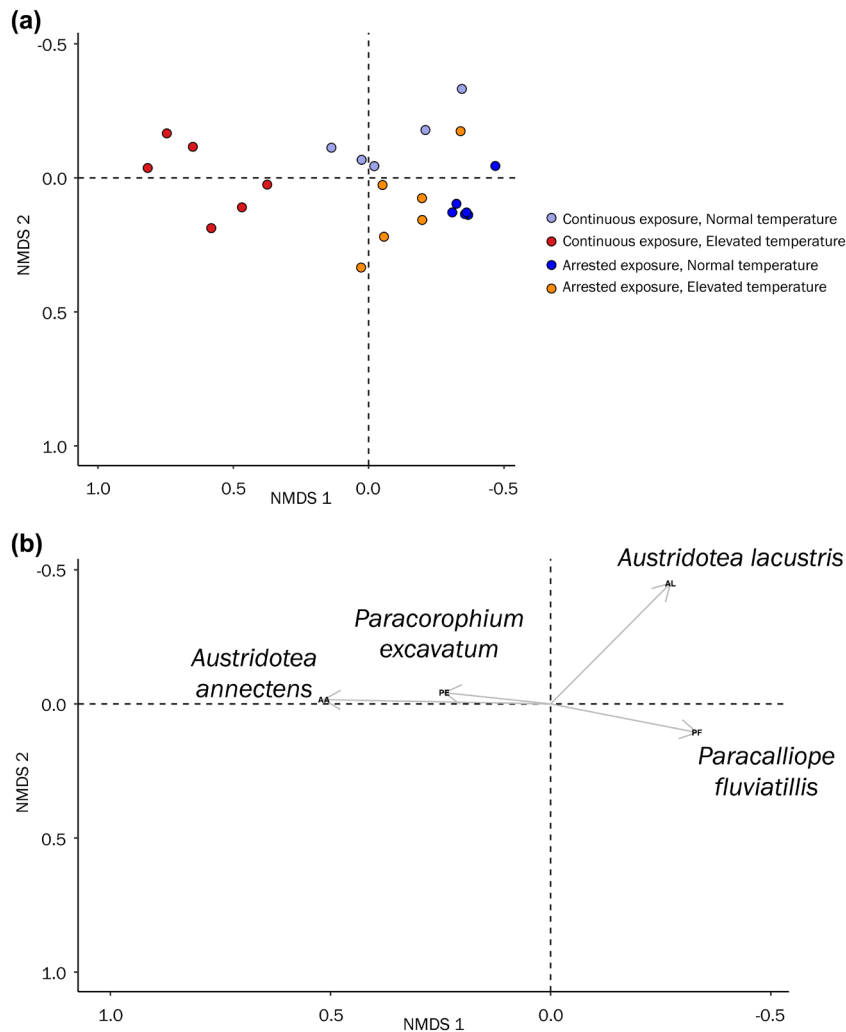


Figure 4. Community dissimilarity between the host communities (host absolute abundance in each mesocosm) of each treatment, using non-metric multidimensional scaling (nMDS) ordination analysis with Bray–Curtis distances based on host absolute abundance. (a) Host community overlap separated by treatment, where samples that are grouped more closely together represent more similar communities than samples spread farther apart. (b) Loading vectors of each host species. Stress was 0.075. Four treatment types, arrested parasite exposure with normal or elevated temperatures and continuous parasite exposure with normal or elevated temperatures.

exposure to *M. poulini* and *C. parvum*) and temperature (normal versus elevated temperature) on mesocosm host and parasite community structure (Fig. 5). The data were well fit by this model (Fisher's $C=26.6$, Robust χ^2 $p=0.65$). As we predicted, additional exposure to parasites (both *C. parvum* and *M. poulini*) negatively altered the absolute abundance of the smaller amphipod, *P. fluviatilis* ($p < 0.0001$, Fig. 5). However, parasite exposure had no direct effect on the abundance of *P. excavatum* and *A. annectens* ($p=0.30$, $p=0.88$, Fig. 5). Temperature also had a direct negative effect on the absolute abundance of *P. fluviatilis* and *P. excavatum* ($p < 0.0001$, $p=0.05$, Fig. 5). Temperature negatively affected *C. parvum* abundance in *P. excavatum* ($p=0.0004$, Fig. 5). Abundance of the isopod *A. annectens* also had a positive effect on that of *P. excavatum* ($p=0.034$, Fig. 5) and the abundance of the isopod *A. lacustris* had a positive impact on *P. fluviatilis* ($p=0.048$, Fig. 5).

Host reproduction

Gravid females of all four crustacean species carry offspring in a brood pouch. This allows the comparison of the number of eggs per female. The paired individuals consisted of a male clasping a female in a precopulatory pair (Chadderton et al. 2003, Sutherland et al. 2007). Fecundity in *P. fluviatilis*, assessed as the mean number of eggs per gravid female in each treatment, was impacted by parasite exposure but not temperature (Supporting information). However, the presence of eggs (percent of females carrying eggs) was impacted by both parasite exposure and temperature, with a significant interaction between the main effects (Supporting information). The proportion of females with eggs was highest in the elevated temperatures with arrested parasite exposure, but they were completely absent in elevated temperatures with continuous parasite exposure (Supporting information). The

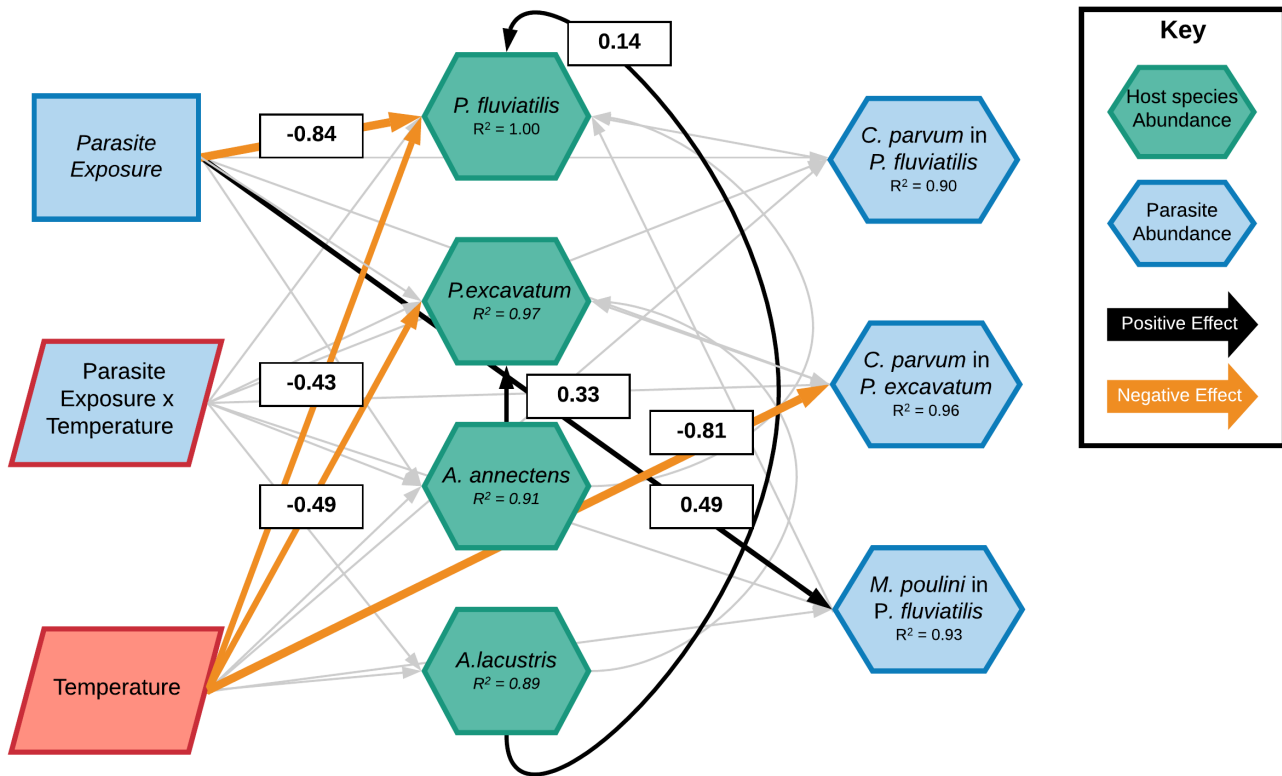


Figure 5. Structural equation model (SEM) of mesocosm experiencing different levels of parasite exposure and temperature. Arrows represent unidirectional relationships among variables. The black arrows denote positive relationships and orange negative ones. Arrows for non-significant paths ($p > 0.05$) are semitransparent. R^2 for component models are given in boxes of response variables. The thickness of the significant paths has been scaled based on the magnitude of the standardized regression coefficient.

mean number of *P. fluviatilis* juveniles was higher in normal temperature treatments.

Discussion

Assessing and potentially predicting the combined role of temperature and parasites in the structuring of communities, and ultimately ecosystems, is central to not only obtain a greater understanding of the functioning of ecosystems, but also to making accurate predictions on the potential consequences

of climate change on ecosystems. In contrast to the few earlier studies considering temperature–parasitism interactions at a host community level (Mouritsen et al. 2018), our study included the effects of more than one parasite species across more than one host generation. Our mesocosm experiment demonstrated that both temperature and parasites can alter host communities, strongly impacting community structure and abundance of host and non-host species, through effects on their survival and recruitment. Overall, parasite exposure affected community composition and structure, whereas temperature altered species abundance.

Table 1. Differences in parasite abundance among treatments and host species, with both main effects (parasite exposure and temperature) and interactions shown. Significant differences are shown in bold.

| Species | Test statistic | Parasite exposure | | Temperature | | Interaction | | |
|---------------------------------|---------------------|-------------------|------|-----------------|-------|-----------------|-------|------|
| | | F | p | F | p | F | p | |
| <i>Paracalliope fluviatilis</i> | | | | | | | | |
| | <i>A. galaxii</i> | $F_{3,822}$ | 0.83 | 0.36 | 0.026 | 0.87 | 0.026 | 0.87 |
| | <i>M. poulini</i> | $F_{3,822}$ | 45.8 | < 0.0001 | 0.63 | 0.43 | 2.1 | 0.15 |
| | <i>C. parvum</i> | $F_{3,822}$ | 13.3 | 0.0003 | 3.0 | *0.086 | 1.6 | 0.21 |
| <i>Paracorophium excavatum</i> | | | | | | | | |
| | <i>H. spinigera</i> | $F_{3,214}$ | 0.32 | 0.57 | 0.32 | 0.57 | 2.7 | 0.10 |
| | <i>M. poulini</i> | $F_{3,214}$ | 5.3 | 0.023 | 9.2 | 0.0028 | 0.75 | 0.39 |
| | <i>C. parvum</i> | $F_{3,214}$ | 0.98 | 0.32 | 17 | < 0.0001 | 0.02 | 0.89 |
| <i>Austridotea annectens</i> | | | | | | | | |
| | <i>M. poulini</i> | $F_{3,147}$ | 1.2 | 0.28 | 0.11 | 0.74 | 0.43 | 0.51 |

Water temperature had species-specific effects on host survival and population dynamics. Amphipod survival was strongly affected by temperature. Both species had higher mortality rates in elevated temperature treatments, which is consistent with previous studies of amphipod-trematode systems (Jensen and Mouritsen 1992, Mouritsen and Jensen 1997, Studer et al. 2010). *Paracalliope fluviatilis* also appeared to be the most sensitive of the four hosts to both *Maritrema poulini* and *Coitocaecum parvum*. Combination of continuous parasite exposure with increases in temperature had highly contrasting effects on different host species. Recruitment of *P. fluviatilis* was also impacted by both temperature and parasite exposure, being lower in continuous exposure, elevated temperature treatments where no female carried eggs. Parasite exposure also appeared to be an important factor in reducing fecundity in *P. fluviatilis*; this fitness decline is corroborated by prior work in this system (Friesen et al. 2017). In the absence of simulated migration, used to replicate natural movement of *P. fluviatilis*, it is likely this species would have disappeared altogether. As survival is reduced in warmer environments, higher exposure to parasites may exacerbate this and lead to local population collapses of this species. Our results are consistent with other amphipod-trematode systems, where amphipod abundances decreased significantly when temperatures were elevated in the presence of parasites and the authors predicted the deterioration of amphipod coastal communities (Mouritsen et al. 2018).

Population decreases in the other amphipod species, *Paracorophium excavatum*, were also likely due to the combination of thermal stress and overall parasite infection intensity. *Coitocaecum parvum* was completely absent from this amphipod species in the arrested exposure elevated temperature treatment. The combination of temperature and parasite exposure had a negative, synergistic impact on the prevalence of *C. parvum* in this species. Our results suggest that the difference in the abundance of this parasite in *P. excavatum* is not due to temperature alone. Previous research has suggested that this amphipod does not suffer from parasite-induced mortality in the absence of other abiotic stressors (Friesen et al. 2017, 2020). The lack of infected individuals in the arrested exposure, elevated temperature treatment, where no parasite infective stages were added to the system, strongly suggests that mortality of naturally infected individuals increased because of thermal stress. Additionally, the abundance of *M. poulini* was negatively impacted by temperature, although the prevalence did not vary among treatments. These relationships are consistent with parasite-induced mortality in *P. excavatum*, particularly when also infected with *C. parvum*. Increased parasite-induced mortality due to elevated temperatures may have significant long-term consequences for this species if seasonally high temperatures are maintained for longer periods or if temperatures continue to rise, as predicted by climate change models.

Increased temperature and parasite exposure had different impacts on the isopods *Austridotea annectens* and *A. lacustris*, especially when compared to the dramatic differences seen in both amphipod species. Populations of *A. annectens*

were slightly decreased in elevated temperature treatments, although *A. lacustris* populations did not differ among any of the treatments nor did they appear to be directly impacted by temperature, suggesting they may have a much higher thermal tolerance. No parasite-induced mortality was apparent in this host. Highly variable parasite abundance and very high parasite prevalence are consistent with little parasite-induced mortality (Friesen et al. 2017).

Community structure was different between treatments, reflecting the unique impact of each combination of stressors. Although the amphipod *P. fluviatilis* had the highest abundance in all four treatments, its abundance was significantly lower under continuous parasite exposure and elevated temperature. Yet, abundance of the amphipod *P. excavatum* was strongly impacted by temperature variation. Overall, the asymmetrical impacts of temperature and parasite exposure on the different crustacean host species had consequences for the structuring of the entire community. Populations of isopods remained less affected by parasite exposure and temperature variations, suggesting that under increased thermal stress, beyond seasonal fluctuations as modeled here, both species may become more dominant in their communities. This is consistent with previous research that predicted the combination of thermal stress and parasitism may lead to shifts in community composition (Mouritsen et al. 2018). Increased temperatures may also cause host density to drop below the threshold for the minimum parasite transmission rates required for parasite persistence (Lafferty and Kuris 1999). The combination may then lead to localized extirpation events of both parasites and their hosts.

Worldwide, surface temperatures are expected to rise and heat waves to occur more frequently and last for longer periods (IPCC 2014). In 2018, New Zealand experienced the hottest summer on record, with summer temperatures in the study region 2.0°C above normal (Brandolino 2018). Temperature loggers at the study site indicated that 80% of summer days had mean water temperatures above 20°C. Based on our study, sustained temperatures at or above the seasonal high of 20°C can have substantial impacts on invertebrate populations. *Potamopyrgus antipodarum*, the first intermediate host of both *M. poulini* and *C. parvum*, has a relatively high upper thermal tolerance of 32.4°C at 96 hours (Quinn et al. 1994). It is highly likely that they will be able to survive these periods and maintain both trematode species, if the parasite species can persist in these abiotic conditions. Therefore, persistent heat waves, as seen in the 2018 summer, combined with enhanced parasite exposure will alter local communities.

Prolonged heat waves will likely induce significant changes in the species composition of communities, particularly if they also influence parasite exposure levels (Larsen and Mouritsen 2014, Mouritsen et al. 2018). Our results are consistent with previous research suggesting changes in temperature in combination with parasitism may alter community structure (Larsen et al. 2011, Goulson et al. 2015, Mouritsen et al. 2018). These changes may subsequently affect both top-down and bottom-up trophic interactions

within communities (Hunter and Price 1992). Changes in invertebrate host populations have implications for other species such as fish and birds that consume crustaceans but are also definitive hosts to many parasites. Differences in community structure and dynamics, as predicted by our results, will also have consequences on bottom-up interactions, such as aquatic primary production and nutrient cycling (Hunter and Price 1992). Here, we showed that both temperature and parasites can alter community structure in synergy, through the differential response of hosts and parasites to their environment. In light of climate change, we suggest that both parasite exposure and increasing average temperatures will have substantial long-term impacts on community structure and dynamics.

Acknowledgements – Field and lab assistance were provided by S. Goellner, B. Presswell, J. Bennett, C. Selbach and B. Ruehle. Special thanks to R. Grunberg, B. Ruehle, S. Bromagen, N. Chodkowsky, B. Joyner and K. O'Brien, for comments and advice on earlier drafts of this manuscript.

Funding – Financial support for this project was provided by the Dept of Zoology, Univ. of Otago.

Conflicts of interest – No conflict of interests.

Permits – No permits were required for this work.

Author contributions

Olwyn Friesen: Conceptualization (equal); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Writing – original draft (lead); Writing – review and editing (lead). **Robert Poulin:** Conceptualization (equal); Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting); Methodology (supporting); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (equal). **Clement Lagrue:** Conceptualization (equal); Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting); Methodology (supporting); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (equal).

Data accessibility

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.gtht76hmd>> (Friesen et al. 2021).

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