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ORIGINAL PAPER

Temperature effects on the impact of two invasive parasitic copepods on the survival, growth, condition, and reproduction of native mussels

E. Rosa Jolma · Ana Born-Torrijos · Marc Y. Engelsma · Hans Heesterbeek · Anieke van Leeuwen · Robert H. Twijnstra · K. Mathias Wegner · David W. Thieltges

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Abstract An increase in temperature due to climate change may affect the geographic ranges of invasive parasites and alter their impact on native hosts. Our goal was to determine if the effects of infection by two species of invasive endoparasitic copepods on native blue mussel hosts (*Mytilus edulis*) change with increasing temperatures. We investigated this with a laboratory experiment using temperatures that represent annual mean and mean summer water temperatures of past observations and future predictions for the study area, the European Wadden Sea (10–26 °C). Over a period of 8–20 weeks, infection with *Mytilicola intestinalis* lowered mussel condition and infection with *Mytilicola orientalis* decreased mussel shell growth. High temperatures decreased mussel growth and condition in general, but only at low temperatures (10–14 °C) the parasite-induced loss of condition was evident compared to uninfected mussels. Mussel mortality and reproductive activity were not affected by parasite infection, although both were impacted by temperature: the highest temperature (26 °C) increased mussel mortality, and gamete ripening only occurred at lower temperatures (10–18 °C). Taken together, these results suggest that both infection and high temperatures have independent negative effects. However, an increase in temperature does not worsen the effect of infection on individual mussel hosts, and neither does infection decrease host tolerance for long-term exposure to high temperatures. These findings add to our understanding of the interplay between increasing temperature and the interaction between invasive parasites and native hosts, and help predicting host and parasite dynamics in systems affected by species invasions and climate change.

Keywords Climate change · Infection effect · Invasive parasite · *Mytilus edulis* · *Mytilicola intestinalis* · *Mytilicola orientalis*

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E. R. Jolma (✉) · A. Born-Torrijos · A. van Leeuwen · R. H. Twijnstra · D. W. Thieltges
Department of Coastal Systems, NIOZ Royal Netherlands Institute for Sea Research, Den Burg, Texel, The Netherlands
e-mail: rosa.jolma@nioz.nl

E. R. Jolma · H. Heesterbeek
Department of Population Health Sciences, Utrecht University, Utrecht, The Netherlands

M. Y. Engelsma
Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, The Netherlands

K. M. Wegner
Alfred Wegener Institute – Helmholtz Centre for Polar and Marine Research, Coastal Ecology, Wadden Sea Station Sylt, List, Germany

D. W. Thieltges
Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands

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Introduction

Invasive parasites, ranging from viruses to metazoans, can affect newly acquired native host species in the invaded range by reducing host survival or compromising the host's immune system, reproduction, and behaviour (Daszak et al. 2000; Hatcher et al. 2015; Thieltges and Goedknegt 2023). These individual-level effects can lead to changes in host populations, which, together with changes in host functional traits, can affect the populations of interacting species, ultimately leading to ecosystem level repercussions (Hatcher et al. 2015; Thieltges and Goedknegt 2023). Concurrently, climate change is affecting individuals, populations, and ecosystems (Walther 2010). Climate change impacts on individuals and populations range from direct effects, such as an increase in mortality due to acute heat stress, to indirect effects, for example via shifts in (the timing of) food availability and predation pressure (Walther 2010). There are numerous ways in which climate change can affect the success and outcome of parasite invasions: an increase in temperature can lead to range expansions of parasites, vectors and potential host species and accelerate the growth and reproduction speed of parasites and ectothermic hosts leading to changes in invasion success and infection pressure (Barber et al. 2016; Bojko et al. 2023).

Less is known about how changes in temperature affect the outcome of infections of native hosts with invasive parasites. An increase in temperature may alter the function of the host's immune system through temperature dependence of all metabolic processes in ectothermic organisms, or heat stress that affects also endothermic species (Barber et al. 2016; Hing et al. 2016), possibly affecting host tolerance and resistance to infection. Conversely, infections may affect the temperature tolerance of hosts, potentially exacerbating the effects of climate change (Barber et al. 2016). A recent review revealed how sparse studies are on this combined effect on individual hosts (Bojko et al. 2023). Deeper system-specific understanding will be essential to unravel the fundamentals of this interaction between temperature and invasive parasites regarding their effects on hosts.

In this study, we investigated temperature effects on the impact of invasive parasites on native hosts in a well-studied parasite-host system in the Wadden Sea, a temperate coastal marine ecosystem that is undergoing rapid warming (van Aken 2008; Royal Netherlands Institute for Sea Research 2024). From 1900–1999 to 2000–2020, the annual mean water temperature has already increased from 10.2 °C to 11.6 °C, with a further 1.3–3.0 °C increase predicted by the end of the century (assuming a total 2.7–4.4 °C increase from the baseline with SSP2-4.5 and SSP5-8.5 predictions, see; van Aken 2008; Pörtner et al. 2022; Royal Netherlands Institute for Sea Research, 2024; Jolma et al. 2024). Aside of abiotic changes, the Wadden Sea also experiences the establishment of >2 new non-indigenous species on average per year (Reise et al. 2023). One of these was the parasitic copepod *Mytilicola intestinalis* Steuer, 1902, that invaded the system in the 1930s and was originally associated with high mortality in native blue mussel hosts (*Mytilus edulis*) (Korringa 1950; Feis et al. 2016). The parasite is still present with up to 60% prevalence but appears to have ceased to cause mortality, with its current impact rather restricted to a lowered condition of infected mussels at 18 °C (Feis et al. 2016, 2022). Infection with *M. intestinalis* may also alter mussel immune responses and predispose them to infection with *Vibrio* bacteria (Demann and Wegner 2019). Another closely related invasive parasitic copepod, *Mytilicola orientalis* Mori, 1935, arrived in the Wadden Sea with its original host, the Pacific oyster (*Magallana gigas*), on the first decade of the twenty-first century (Feis et al. 2019). Like *M. intestinalis*, also *M. orientalis* lowers mussel condition at 18 °C but it has not been reported to cause mortality in native mussels (Goedknegt et al. 2018). Mussel condition is a proxy for reproduction, because in preparation for spawning up to 59% of adult mussel soft tissue weight consists of reproductive tissue (Duinker et al. 2008), and it can also be considered as a proxy for energy reserves in general. Since mussel reproductive output is size-dependent, possible effects of parasite infection on host growth could have negative effects on fitness (Sprung 1983). Climate change affects blue mussel populations in at least two ways: mild winters lead to poor mussel recruitment, possibly through the early arrival of predatory crabs, and repeated summer heat waves cause mass mortality (Beukema & Dekker 2014; Seuront et al. 2019). High temperature also affects mussel metabolism since both the filtering activity and shell growth decline in temperatures above 22 °C (Vajedsamiei et al. 2021). With the existing information on the

effects of the two invasive parasites at current ambient temperatures and climate change impacts on blue mussels at elevated temperatures, we set out to investigate their combined effect with a laboratory experiment. Using a range of observed and predicted future water temperatures in the Wadden Sea, we measured the effects of parasite infections on 1) survival; 2) growth; 3) condition; and 4) reproduction of mussels to specifically investigate how the effect of infection with either invasive parasitic copepod changes with temperature.

Methods

Experimental design

Parasite collection and experimental setup are described in detail in Jolma et al. (2024). In short: 150 blue mussels of 35–40 mm shell length were collected from a parasite-free wild population (Sint Maartenszee, 52.791559N, 4.669481E) in July 2022. The mussels were acclimated to five temperatures (10 °C, 14 °C, 18 °C, 22 °C and 26 °C) for 31–48 days before parasite exposure (for practical reasons starting all replicates took 17 days). The infectious copepodites originated from parasite eggs collected from southern Wadden Sea mussels and incubated at room temperature (20–23 °C). Experimental mussels were individually incubated with 24 infectious copepodites of *M. orientalis* or *M. intestinalis* for 24 h to guarantee a high probability of copepodite ingestion (Gee and Davey 1986); control mussels underwent the same treatment without parasites. This resulted in 10 mussels within each parasite exposure and temperature treatment. The maximal shell length of every mussel was recorded on the day of exposure. Mussels were kept in individual jars with flow-through systems within temperature-controlled water baths until dissection and they were fed with $40*10^6$ cells of phytoplankton concentrate per mussel/day mixed into the incoming water (Microalgae Mix, Acuinuga, A Coruña, Spain, for other details see Figure S1 in Jolma et al. 2024). Mussels were checked daily on weekdays and mortality was recorded.

Mussels were dissected at five time points spanning 8–20 weeks (Fig. 1). At every time point 30 mussels were dissected: six mussels per temperature; two controls, two exposed to *M. orientalis* and two exposed to *M. intestinalis* (Fig. 1). On the assigned dissection day, the maximal shell length of each dissected mussel was measured again to assess growth. Approximately 10×5 mm pieces of the mantle and the gonad of each mussel were collected in 10% sea water formalin and 24 h later the samples were transferred to 70% ethanol. Mussel intestinal tracts were dissected and the number of *Mytilicola* spp. parasites were counted under a stereo microscope with 10× magnification (ZEISS SteREO Discovery V8) to determine the infection status and parasite load

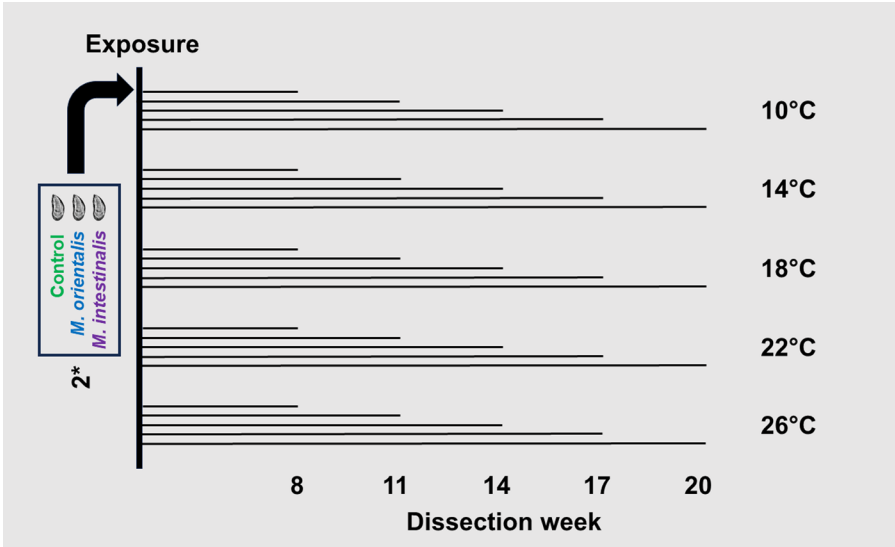

Fig. 1 Experimental design: Each line represents six mussels: two controls, two exposed to *Mytilicola orientalis* and two exposed to *Mytilicola intestinalis*. The mussels were exposed to five different temperature treatments and dissected at five time points. The total number of experimental mussels was 150 (6 mussels *5 temperatures *5 time points)

(number/mussel). Samples of muscle, intestine and intestinal content were collected for a separate study. The remaining mussel soft tissues were freeze-dried, and the dry weight was recorded. Because of the tissue sample collection before drying and dry weight recording, the dry weight measurements were likely to be marginally decreased. The size of the samples was kept small (< 5% of all soft tissues) and consistent across all mussels to minimise this effect and avoid bias.

Mussel growth was measured as the difference in maximal shell length between the start and the dissection day. Condition was calculated as dry weight/maximal shell length^3 (mg/cm^3, Riisgård et al. 2014). The reproduction index was evaluated based on mantle histology using a grading from zero (no signs of reproduction) to three (fully ripe for spawning), following Duinker et al. (2008) and using samples from Dutch aquaculture mussels submitted to Wageningen Bioveterinary Research as a reference.

Data analysis

All 150 mussels were included in survival analyses. For the other variables (growth, condition and reproduction), we included only mussels that survived until their assigned dissection day and that were representative of their infection status (117/150; i.e. only uninfected control mussels and infected exposed mussels). Not finding parasites in the intestine of an exposed mussel at the dissection day could have been the result of failed infection or of parasites dying at some point between the initial infection and dissection. Therefore, it was not possible to determine if these mussels had experienced an infection and they were excluded from the analysis. 10/117 of growth observations were below zero (range from −0.4 to −0.1 mm); they were included in the analysis because the same amount of measurement error was present in all observations and excluding these would have resulted in a bias against low values. This was verified with a histogram where growth observations followed a normal distribution that extended slightly below zero. For condition analyses, two outliers with unrealistically high condition values (> 10 mg/cm^3) were removed because those were likely to be due to problems in the drying process (remaining moisture) resulting in N = 115. All analyses were done in R (v2023.12.1 + 402, R Core Team 2023) with package *survival* for survival analysis (v3.3-1, Therneau 2020), *lme4* for generalised linear mixed effect models and negative binomial models (v1.1-31, Bates et al. 2015), *dplyr* for data transformation (Wickham et al. 2023), and *ggplot2* (v3.4.0, Wickham 2016) with *ggpubr* (v0.5.0, Kassambara 2023) for data visualisation.

Mussel survival

Most dead mussels were found in an advanced stage of autolysis and therefore it was not possible to detect parasites inside of them. Consequently, due to the potential bias towards survivors, parasite load could not be included as a predictor. Because of this, we used exposure to infectious stages as a predictor in survival models instead of infection status. The impact of exposure to the two parasites on mussel survival was explored with Kaplan–Meier survival curves, followed by Cox proportional hazards regression analyses that used parasite exposure and temperature as predictors for survival. Mussel dissections that occurred according to the predetermined experimental schedule resulted in censoring that was uniform across treatment groups (temperature and parasite exposure, Fig. 1). An interaction term between temperature and parasite exposure could not be included because almost all mortality was clustered in one temperature resulting in covariance of temperature and parasite exposure at the two temperatures with only two mussel deaths each. Hence, we decided to investigate the effect of parasite exposure separately at the temperature in which most mussel mortality occurred (18/22 of all deaths occurred at 26 °C).

Mussel growth, condition and reproduction

The initial analysis of the effects of the predictor variables on mussel growth, condition, and reproduction was done with generalised linear mixed effect models that included ‘treatment tank’ as a random effect because each temperature treatment consisted of two treatment tanks (for details see Figure S1 in Jolma et al. 2024). Since including the random effect did not improve the fit of any model based on Akaike’s information criterion (AIC) and limited our ability to include interactions between predictor variables, simpler models without tank as random effect were

used for the final analysis. The effects of temperature and infection status on mussel growth and condition were analysed with linear regression models and the effects on mussel reproduction with negative binomial models (because the distribution of reproduction index values was negative binomial). The predictor variables of all initial models were temperature, infection, parasite load, and time since the beginning of the experiment. All initial models included interaction terms between all predictor variables and the final model was chosen with backward elimination of predictor variables and interactions to optimise AIC values.

Results

Mussel survival

Out of 150 mussels, 128 survived until their assigned dissection day. Temperature had a significant effect on mussel survival but exposure to either parasite species did not have an effect in the Cox proportional hazards models (Table 1). Mussel survival was the lowest at 26 °C with 60% (18/30) mortality that occurred continuously from the beginning of the experiment (Fig. 2c). At 14 °C two mussels exposed to *M. intestinalis* died (on days 29 and 90), and at 18 °C one mussel exposed to *M. intestinalis* (day 102) and one exposed to *M. orientalis* (day 39) died (Fig. 2a-b). No mortality occurred at 10 °C and 22 °C. At 26 °C the survival curve of mussels exposed to *M. intestinalis* was below that of control mussels until day 110 (halfway between the dissections in week 14 and week 17) where the lines crossed (Fig. 2), possibly as a sign of the proportional hazards assumption not being completely met (i.e. the effect of parasite exposure might not have been the same throughout the follow-up time). Also, the survival curve of *M. orientalis* exposed mussels remained below that of controls for most of the follow-up time but it met with the control curve three times before crossing it (Fig. 2).

Parasite load in mussels

The number of parasites per host differed by temperature and parasite species, with most of the high loads (> 10 parasites/mussel) occurring in mussels infected with *M. intestinalis* at 18 °C and 22 °C (Online Resource 1a). Still, including parasite load as a fixed effect did not improve the fit of any model based on AIC values and it did not influence mussel growth, condition or reproduction. Thus, parasite load was not included in the final models.

Mussel growth, condition and reproduction

With the mean of 0.0035 mm/day (95% confidence intervals [CI] 0.0027–0.0043 mm/day, Fig. 3) across all treatments, our mussel growth was slightly lower than expect based on the growth rates of mussels with high food availability reported in a previous study (Kamermans and Saurel, (2022). In mussel growth models, including interactions between any

Table 1 The impact of parasite exposure and temperature on mussel survival. Output from two Cox proportional hazards models. The full model includes all temperatures. The effect of infection on survival was assessed also separately at 26 °C because most mussel deaths occurred at 26 °C and the number of observations precluded an interaction between temperature and infection in the full model

	Full model		26 °C	
	Coefficient	Standard error	Coefficient	Standard error
Mytilicola orientalis	0.17	0.56	−0.02	0.98
Mytilicola intestinalis	0.58	0.53	0.23	1.26
Temperature	0.36***	0.08	NA	NA
N	150		30	
Number of events	22		18	
Degrees of freedom	3		2	
Concordance index	0.86		0.53	

Significance levels: ***$p < .001$

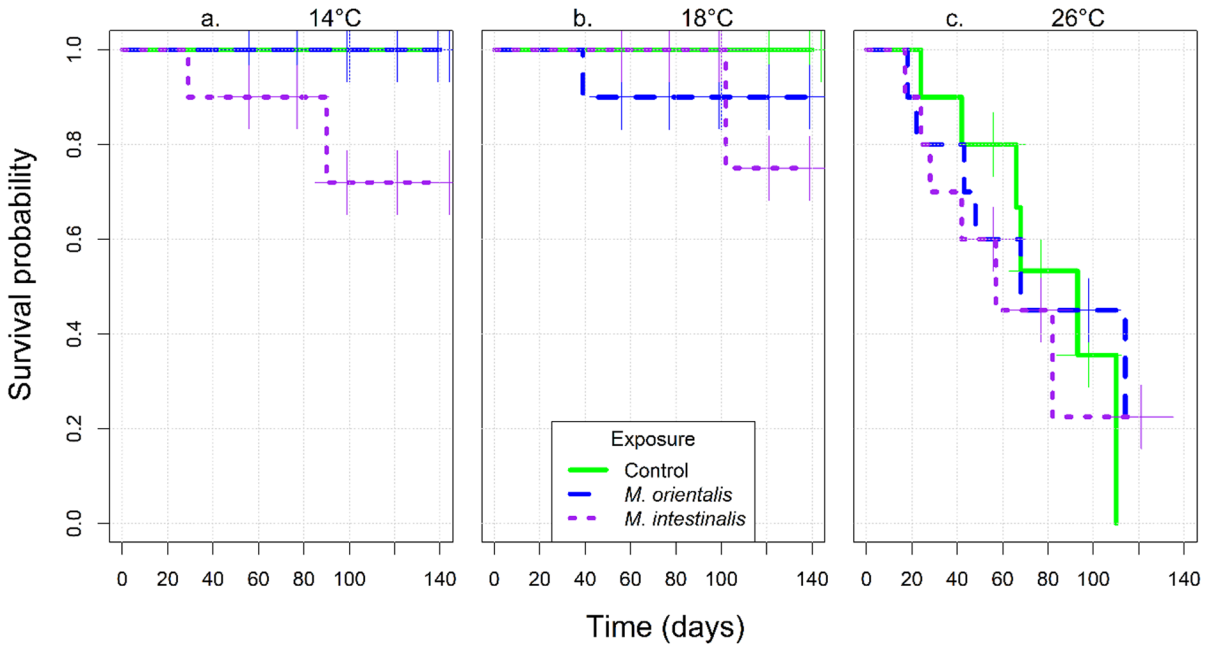

Fig. 2 Kaplan–Meier survival for mussels over time at different temperatures. Survival curves are displayed for 14 °C, 18 °C and 26 °C only, because no mortalities occurred at 10 °C and 22 °C. Crosses represent censoring events (dissection time points in Fig. 1)

predictor variables did not improve model fit (AIC without interactions 616.6, whereas with interactions 617.4–627.1) and no significant interactions occurred. Thus, the final model included only time, temperature and infection as fixed effects and no interactions (Table 2). Time had a positive effect, and both temperature and infection with *M. orientalis* had negative effects on mussel shell growth (Fig. 3, Table 2). Across all temperatures, the mean growth of control mussels was 0.0047 mm/day (95% CI 0.0030–0.0063) and of *M. intestinalis* infected mussels 0.0033 mm/day (95% CI 0.00195–0.0047), whereas *M. orientalis* infected mussels grew only 0.0024 mm/day (95% CI 0.0013–0.0034, Fig. 3).

The condition of most mussels at 22 °C and 26 °C was low (< 4 mg/cm^3), whereas most mussels at all lower temperatures had medium condition (4–6 mg/cm^3), except for control mussels at 10 °C most of which had high condition (> 6 mg/cm^3, Fig. 4, classification based on Riisgård et al. 2014). Including an interaction between temperature and infection slightly improved the fit of condition models (AIC 372.7 vs. 373.4). The final model included time, temperature and infection as fixed effects and an interaction term between infection and temperature. Time, temperature and infection with *M. intestinalis* had negative effects on mussel condition (Fig. 4, Table 2), but the interaction between temperature and infection with either parasite was not significant with a tendency for higher temperatures to mitigate the effects of *M. intestinalis* infection (Table 2).

All mussels at 26 °C, and all except for one at 22 °C, were in a reproductively inactive state (index 0, Fig. 5). At 10 °C, 75% of control mussels had a high level of reproductive activity (index 2–3) and 75% of mussels in both infection groups had some level of reproductive activity (index 1–3, Fig. 5). The reproductive activity of mussels at 14 °C and 18 °C was intermediate with indices ranging from 0 to 2 in most treatments (Fig. 5). Including interactions between predictor variables did not improve model fit (AIC without interactions 232.3, whereas with interactions 232.4–242.3) and no significant interactions occurred. Thus, the final model included only time, temperature and infection as predictor variables without interactions (Table 2). Time had a positive effect and temperature a negative effect on mussel reproduction (Table 2), while infection with either parasite species did not have a significant effect (Table 2).

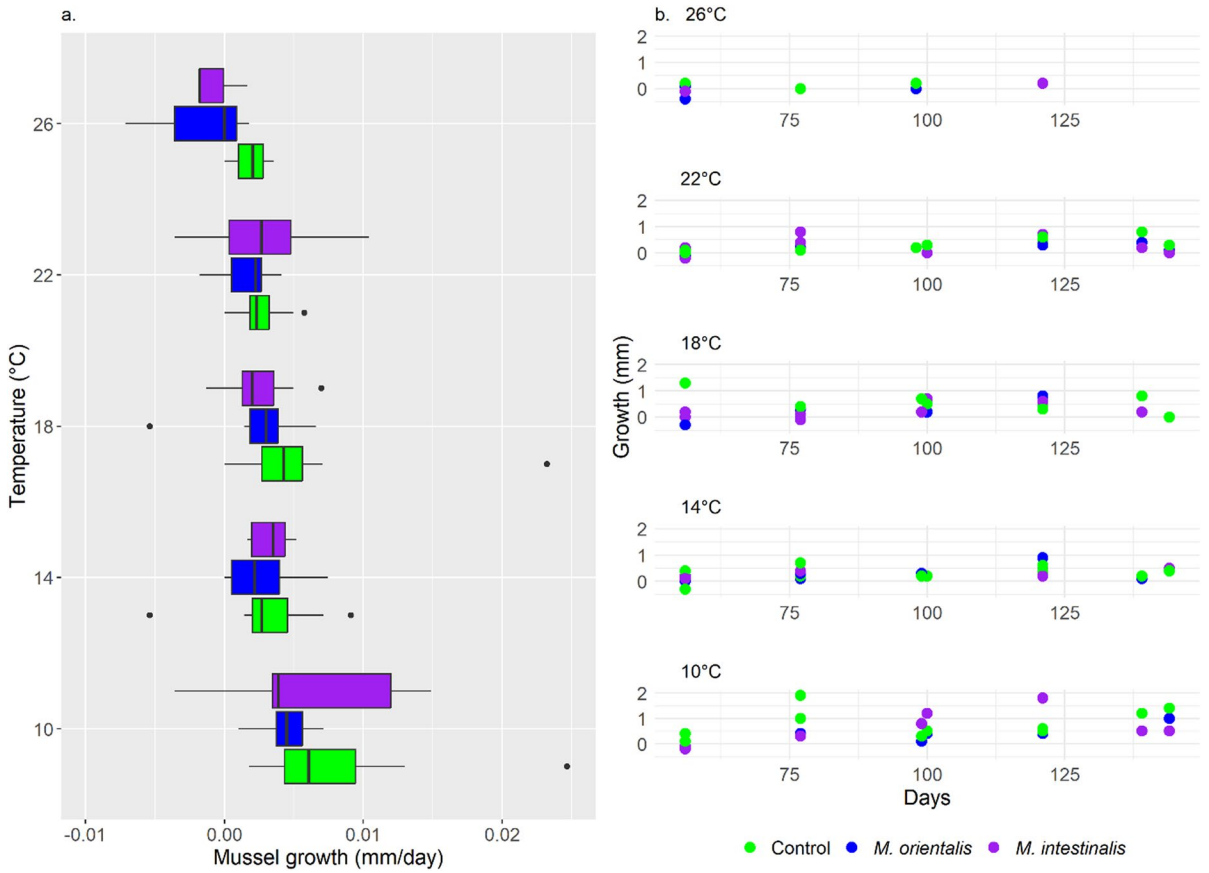

Fig. 3 Mussel growth by infection, temperature and time. **a**. with all time points combined (mm/day), and b. by time and infection at each temperature (mm). Time had a significant positive effect on growth, while temperature and infection with *M. orientalis* affected it negatively. No interactions between temperature and infection with either parasite occurred (Table 2)

Table 2 The impact of time, infection and temperature on three host variables: growth, condition and reproduction. This table contains the output of best models chosen based on AIC values, including an interaction term improved model fit only for mussel condition. Growth and condition were assessed with linear regression and reproduction with negative binomial regression. An extra decimal place was included for the standard errors that were < 0.005 for the sake of clarity

	Growth		Condition		Reproduction	
	Coefficient	Standard error	Coefficient	Standard error	Coefficient	Standard error
Intercept	5.19***	1.54	9.01***	0.74	1.26*	0.48
Time (days)	0.04***	0.01	−0.01**	0.004	0.01***	0.003
Temperature	−0.28***	0.06	−0.18***	0.04	−0.19***	0.02
M. orientalis	−1.66*	0.75	−0.27	0.93	0.02	0.23
M. intestinalis	−0.82	0.73	−1.94*	0.91	−0.05	0.23
Temperature **M. orientalis*	NA	NA	0.01	0.05	NA	NA
Temperature **M. intestinalis*	NA	NA	0.10†	0.05	NA	NA

Significance levels: †$p < 0.1$, *$p < .05$, **$p < .01$, ***$p < .001$

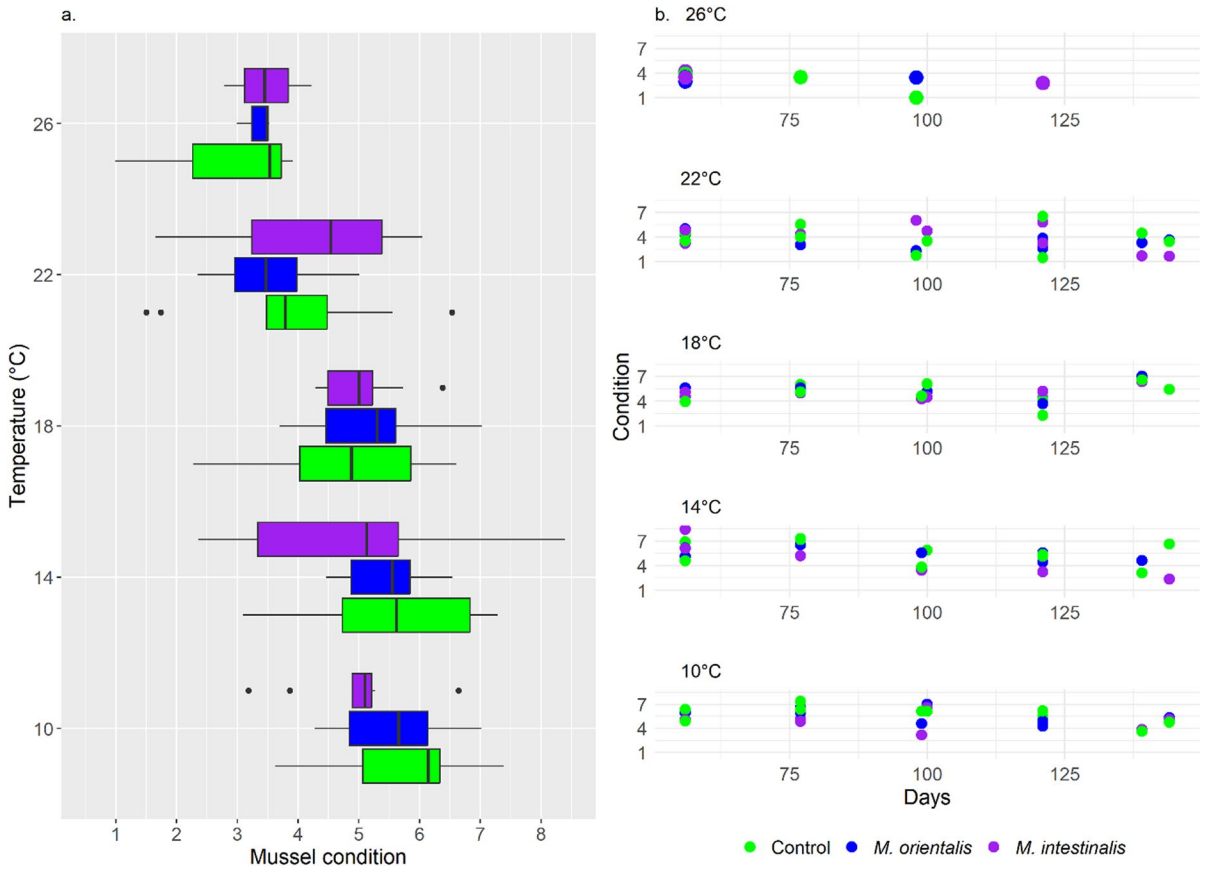

Fig. 4 Mussel condition by infection, temperature and time. **a**. with all time points combined, and b. by time and infection at each temperature. Time, temperature and infection with *M. intestinalis* had a significant negative effect on condition. Including an interaction between temperature and infection improved model fit with the *p*-value of temperature * *M. intestinalis* being close to significant (*p*-value = 0.053, Table 2)

Discussion

Temperature influenced all measured mussel traits: survival, growth, condition, and reproduction. In contrast, infection effects were less prominent; infection with *M. orientalis* had a slight negative effect on mussel growth and infection with *M. intestinalis* a slight negative effect on mussel condition. The only case where temperature interacted with the impact of infection was the negative effect of infection with *M. intestinalis* on mussel condition, which occurred mostly at the two lowest temperatures (10–14 °C). The relatively weak way temperature modulates *Mytilicola* spp. inflicted damage in individual mussels indicate that climate change may increase the relative burden on mussels more as a direct effect of increasing temperatures than as an indirect effect of parasite infection.

Exposure to parasites did not lower mussel survival, whereas exposure to the highest temperature (26 °C) did. This increase in mortality at the highest temperature was expected because a repeated exposure to high temperatures increases mussel mortality, and mussel feeding declines at temperatures above 22 °C, indicative of severe heat stress (Seuront et al. 2019; Vajedsamiei et al. 2021). The low condition of the few mussels at 26 °C that survived until dissection also points to near starvation at that temperature. Our mortality findings fit with earlier observations of Demann and Wegner (2019) who suggested that in an experimental setting mussel mortality was higher at an elevated temperature (19.4–23.3 °C) compared

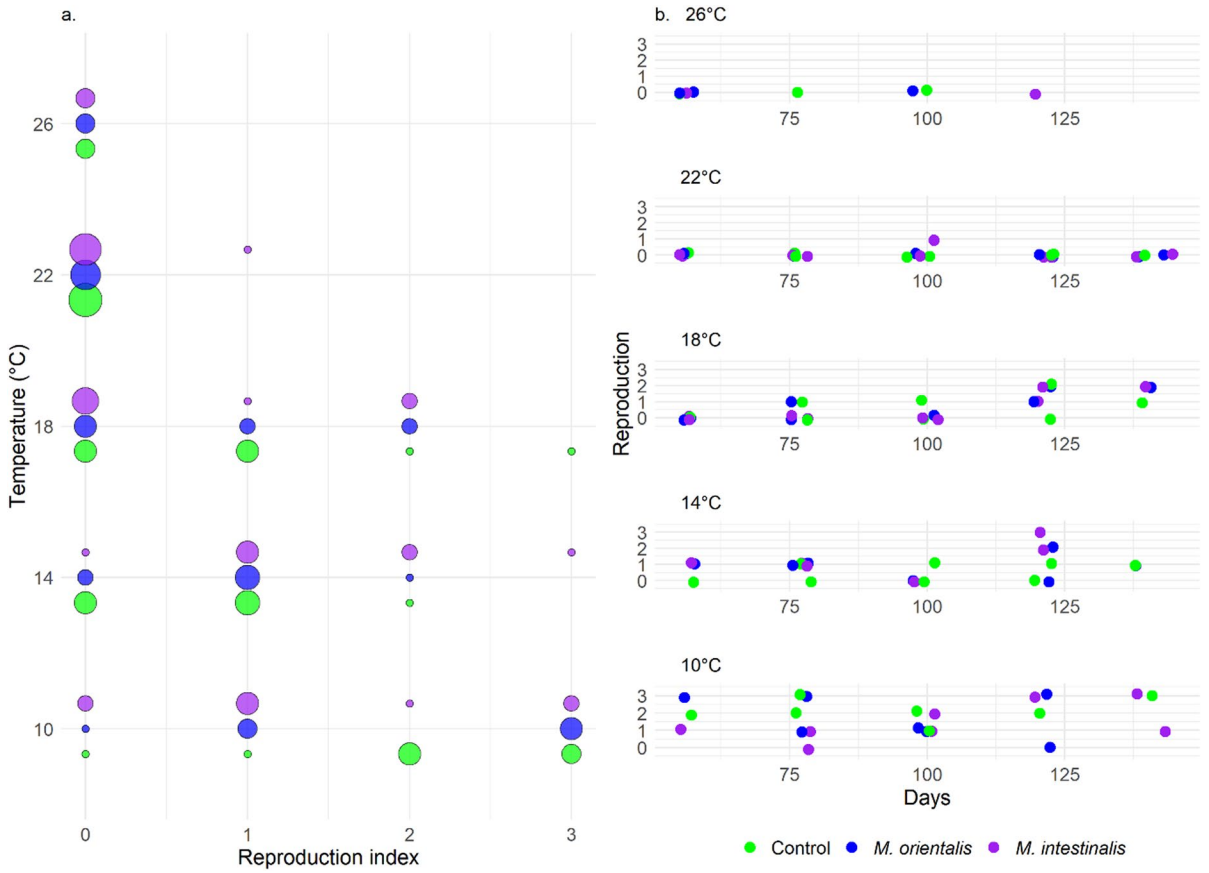

Fig. 5 Mussel reproduction by infection, temperature and time. **a**. with all time points combined and the size of the dot expressing the number of observations with (the largest = 10, the smallest = 1). **b**. by time and infection at each temperature. The location of dots in b. is slightly offset to enable the visualisation of all colours in the same locations (jitter of 2 for x and 0.15 for y). Time had a significant positive effect on reproduction, while the effect of temperature was negative. Infection with either parasite did not affect reproduction and no interactions between temperature and infection with either parasite occurred (Table 2)

to ambient temperature (15.8–16.6 °C). Interestingly, our only mussel mortalities at lower temperatures (14 °C and 18 °C) occurred in parasite exposure groups, one with *M. orientalis* and three with *M. intestinalis* exposure. That observation, combined with the survival curve of parasite exposed mussels staying below the control mussel curve at 26 °C until late in the experiment, could point to early stages of infection with juvenile parasites possibly compromising mussel survival, as a larger impact on hosts by *M. intestinalis* juveniles compared to adult parasites has been suggested before (reviewed by Goedknegt et al. 2018).

In general, infections with both parasite species had mild non-lethal effects on mussels. *Mytilicola orientalis* had a negative effect on mussel growth and *M. intestinalis* on mussel condition. In previous laboratory studies conducted at 18 °C, infections with both parasite species had negative effects on mussel condition and infection with *M. orientalis* did not affect mussel growth (Feis et al. 2016; Goedknegt et al. 2018). Interestingly, in our study neither of the two parasite species influenced mussel condition at 18 °C; the negative effect by *M. intestinalis* on mussel condition was clearest at 10–14 °C (Fig. 5), and *M. orientalis* affected only mussel growth across the whole range of temperatures. The likely explanation for our study to find a growth effect by *M. orientalis* unlike the observations in Goedknegt et al. (2018), was our longer follow-up time (which was

8–20 weeks, compared to 8–9 weeks in Goedknegt et al. (2018). Since growth is a slow response, this longer period may have allowed subtle differences between treatments to develop to a stage where they can be detected (Fig. 3).

Mussel condition and reproductive output are linked, and in peak spawning season up to 59% of soft tissue and 95% of mantle weight can consist of gonadal tissue because it replaces most of the storage tissue during gamete maturation (Duinker et al. 2008). Therefore, it was surprising that, while the reproduction index increased over time—as expected in the cooler range of temperatures where mussel reproduction happens (10–18 °C, Fig. 4) -, mussel condition had a decreasing trend, especially at 14 °C (Fig. 5). In our histology, gamete maturation occurred and the ratio of reproductive tissue to storage tissue in the mantle increased alongside it, but not to the extent seen in wild and cultivated Dutch mussels that we used as a reference. In the experimental mussels storage tissue was still abundant between reproductive acini even at high index values (acinus = a sac in which germ cells mature, reproduction index 2–3, Online Resource 1b). Based on this, the prolonged exposure to low temperatures drove our mussels to produce gametes, but the extent of storage tissue replacement with reproductive tissue did not appear as high as in wild mussels. This may indicate inadequate energy availability.

We aimed to avoid food limitation and therefore maintained a high level of feeding. It is possible though, that the bioavailability of the phytoplankton concentrate used was not as high as that of fresh phytoplankton and thus did not mimic natural conditions optimally. Another possible indication for limited energy intake was the overall slow growth that was fastest at the lowest temperature (10 °C Fig. 3) in which ectotherms use the least energy for basic metabolism. The highest growth rate at the lowest temperature was unexpected because without food limitation, mussel growth is expected to increase with temperature until approximately 20–22 °C before starting to decline with further temperature increase (Vajedsamiei et al. 2021). However, the temperature for maximal growth may differ by mussel population and in a previous study Dutch blue mussels had optimal growth at 8 °C with low food availability and at 8–15 °C with high food availability (Kamermans and Saurel 2022). Also, the condition of our experimental mussels was mostly in the medium category except for the two highest temperatures in which condition was low and temperature stress was likely to limit feeding (classification based on Riisgård et al. 2014). Thus, if food limitation occurred it was most likely not severe and based on an earlier study in which food limitation did not affect the impact of *M. orientalis* on mussels (Goedknegt et al. 2018) it should not have affected our main results.

While infection status (yes/no) had significant effects on mussel growth or condition, we did not detect an effect of the actual parasite load (i.e. numbers of copepods in infected mussels). This may be because all mussels were originally exposed to the same number (24) of infectious stages. Therefore, the number of parasites observed at the end of the experiment did not fully reflect the parasite load that was affecting each mussel throughout the experiment as some of the parasites were likely to have successfully infected the mussel but died before dissection. It is also possible that the mussels that had lower parasite loads at the end had invested more energy on immune function to get rid of them and thus the lower number was unlikely to reflect a lower cost. Especially at higher temperatures, the lifespan of both parasite species is shorter than the duration of the experiment: Korringa (1968) described the lifespan of *M. intestinalis* to be less than three months (12 weeks, approximately 84 days) in Galicia, Spain, and the lifespan of both parasites appeared shorter than 20 weeks (approximately 140 days) in our experiment, especially at the highest temperatures (Jolma et al. 2024). In earlier studies which also used the same number of infectious stages to infect each mussel, a high load of *M. intestinalis* had a negative effect on mussel condition, whereas a high parasite load of *M. orientalis* did not (Feis et al. 2016; Goedknegt et al. 2018). The follow-up time of both of those experiments was shorter than in our study and thus there was less time for the parasites to die before dissections. However, parasites were present at all temperatures throughout our experiment (Online resource 1a.), meaning that the shorter lifespan of parasites did not result in mussels losing all parasites before the last dissections even at the highest temperatures.

While the experiment revealed temperature and infection effects on mussel hosts, we acknowledge some limitations resulting from the specific experimental design. The dissections were divided over five

time points, resulting in a low number of observations at each time point, which increased the chance of type two errors. The reason for this design was to determine how temperature affects parasite growth which we have reported earlier (Jolma et al. 2024). Although this design was not originally optimised for detecting temperature effects on the impact of infection on hosts, adding the time component caused the study to run long enough to enable detecting parasite effects on mussel growth. In addition, it revealed the conflicting temporal trends between reproduction and condition, thus enabling us to estimate the success of our feeding regime. Another limitation was that we used mussels and parasites from only one area. Since the geographic ranges of the blue mussel and both parasite species are large and the parasite invasions have happened several decades ago, it is likely that local temperature adaptations have developed. It has already been shown that the effect of *M. intestinalis* on mussel condition occurs rather among sympatric mussels and parasites than in allopatric combinations (Feis et al. 2016). Therefore, it would be useful to repeat this study with mussels and parasites from a warmer region within the invaded range as well as with allopatric mussel-parasite combinations to assess whether the temperature effects we observed are general or system-specific.

In general, the individual level effects observed in our experiment can translate to the population level when considered together with the influence that temperature has on these parasite species and other interacting species in the ecosystem. The development speed of both parasite species increases with increasing temperature in the range 10–22 °C leading to shorter generation times, while their egg development and host entry success decline only at higher (26 °C) temperatures (Jolma et al. 2024). If temperature does not increase the mortality of free-living parasite life stages or infected hosts to the same extent, that can result in a higher concentration of infectious stages in the water column during the warmer than colder months due to shorter parasite generation times. We observed several generations of *M. intestinalis* occurring already during the experiment at 18 °C and 22 °C resulting in higher parasite loads per mussel (Online Resource 1a and Jolma et al. 2024). This explains why at the highest temperatures the parasite loads at the end of the experiment were not reduced compared to lower temperatures, even though parasites had shorter lifespan at higher temperatures. Multiple generations occurring rapidly at high temperatures may also cause higher infection pressure in warming environments and may result in higher infection prevalence in hosts. According to our results, this increased burden will have a negative effect either on mussel growth or condition depending on the parasite species. As larger mussels produce more offspring (Sprung 1983), and condition is linked to reproductive output (Duinker et al. 2008), an increase in the prevalence of either parasite species may affect the population-level reproductive output of blue mussels. This is relevant especially when climate change has a negative effect on mussel recruitment also through an increase in predation pressure following mild winters (Beukema and Dekker 2014). Because both parasites have a free-living planktonic life stage, an increase in temperature may also increase the predation on these stages because temperature affects the metabolic rate of predators (Barber et al. 2016). Therefore, to better understand the effects of climate change on this host parasite interaction the findings of this study need to be combined with other, interconnected effects of climate change on this ecosystem.

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Author contributions E. Rosa Jolma: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Writing—Original Draft Preparation, Writing—Review & Editing. Ana Born-Torrijos: Investigation, Writing – Review & Editing. Marc Engelsma: Investigation, Methodology, Writing—Review & Editing. Hans Heesterbeek: Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing—Review & Editing. Anieke van Leeuwen: Conceptualization, Supervision, Writing – Review & Editing. Robert H. Twijnstra: Investigation, Methodology, Resources, Writing—Review & Editing. K. Mathias Wegner: Conceptualization, Formal Analysis, Methodology,

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Data availability The data that support the findings of this study and R code used in the analysis are openly available in https://dataverse.nioz.nl at https://doi.org/10.25850/nioz/7b.b.fh

Declarations

Conflict of Interest The authors have no relevant financial or non-financial interests to disclose.

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