International Journal for Parasitology: Parasites and Wildlife 21 (2023) 296–304

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Filtration and respiration responses of mussels (*Mytilus edulis*) to trematode parasite infections (*Renicola roscovita*) and transient heat exposure

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ARTICLE INFO

Keywords:
Bivalve
Metacercariae
Metabolic cost
Temperature fluctuation
Host manipulation

ABSTRACT

The mussel *Mytilus edulis*, a host to various trematode species, experiences performance decrements due to these infections. Yet, the impact magnitude and potential interactions with environmental stressors remain largely unexplored. This study scrutinizes the effect of *Renicola roscovita* infections on mussel filtration and respiration. We first assessed performance in both uninfected and lab-infected mussels at a mild temperature (16 °C), following an acute heat ramp to 30.5 °C and subsequent cooling. The experiment revealed neither a significant direct impact of the infection on the mussels' performance, nor any significant interplay between the infection and temperature variations. To account for possible infection effects obscured by low sample sizes or mussel size disparities, we conducted a reassessment at 16 °C using both small and large mussels. Infection notably hampered filtration in large mussels, with a marginal impact on smaller ones. A positive correlation was found between infection intensity and mussel filtration capacity, though the infection had no discernible impact on respiration. Our consistent finding of an 11-12 % infection effect size across all experiments indicates a slight reduction in mussel filtration due to trematode infections. While the exacerbating effect of transient heat stress on the infection's impact on filtration was not statistically significant, future investigations should explore potential interactions with prolonged heat stress. Our findings underscore the nuanced ways in which parasitic infections can influence marine bivalve physiology, emphasizing the need for more comprehensive studies that incorporate environmental stressors, such as heat stress, to fully elucidate the impact of parasitism on marine ecosystem health and resilience.

1. Introduction

The bivalve mussel *Mytilus edulis* sensu lato is a species complex that forms epibenthic mussel bed ecosystems in shallow waters of the Northern Atlantic Ocean and the Baltic Sea (Stuckas et al., 2017; Larsson et al., 2017). Mussels are of high commercial importance as their aquaculture contributes billions of Euros to global food and non-food services each year (Seed and Suchanek 1992; Schatte Olivier et al., 2020; Avdelas et al., 2021). Furthermore, they are considered ecosystem engineers due to the high level of biodiversity that they support by providing habitat in the form of a mussel bed matrix (Borthagaray and Carranza 2007; Buschbaum et al., 2009; Zippay and Helmuth 2012). In addition, mussels provide important ecosystem functions and services via their extensive filtration activity, which is not only essential for nutrient and energy cycling but also for trapping suspended particulate organic matter and contaminants and controlling the community structure of micro-planktonic producers and pathogens (Gili and Coma 1998; Widdows et al., 1998; Burge et al., 2016).

The filtration activity of mussels can be affected by various factors, among which temperature plays a pivotal role (Vajedsamiei et al. 2021a, 2021b). In particular, heatwaves, which are amplified by ocean warming (Lima and Wethey 2012; Boyd et al., 2016), can have detrimental impacts on mussel performance (Vajedsamiei et al. 2021a, 2021b). In response to high critical temperatures, mussels suppress their metabolic rates and enter a 'metabolic depression' phase to control the heat-induced increase in ATP synthesis and consumption and protect the

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https://doi.org/10.1016/j.ijppaw.2023.07.007
Received 29 May 2023; Received in revised form 25 July 2023; Accepted 25 July 2023
Available online 27 July 2023

organism's energy reserves at high critical temperatures (McMahon et al., 1995; Sokolova et al., 2012a). This phenomenon results in the oscillation of filtration (feeding) and respiration rates of mussels during daily temperature fluctuations, including transient exposures to high critical temperatures in shallow habitats (Guppy and Withers 1999; Hui et al., 2020). The continuation of metabolic depression periods, which can occur during heatwaves, might be a more common phenomenon in the course of climate warming, resulting in lower growth rates of mussels. Prolonged heat waves with baseline temperatures of 27–28 °C are known to be lethal for *M. edulis* (Jones et al., 2009; Vajedsamiei et al., 2021b).

Heat stress responses of mussels may further be exacerbated by infections with parasites and pathogens. Mussels serve as hosts for a variety of metazoan parasitic taxa, including polychaetes, copepods, and trematodes (Lauckner 1983). One of these species is the trematode *Renicola roscovita* which represents one of the most abundant parasites of *Mytilus edulis* in northern Europe (Lauckner 1983; Goedknegt et al., 2019; Bommarito et al., 2021). In its complex life cycle, *R. roscovita* infects a first intermediate host, the marine gastropod *Littorina littorea,* in which it asexually produces thousands of cercariae (Werding 1969). For this asexual reproduction, the parasite invades a substantial part of the host tissue, provoking host castration, inducing high metabolic costs, and elevating mortality risk (Mouritsen et al., 1999; Sorensen and Minchella 2001; Mouritsen and Poulin 2002; Macleod and Poulin 2016). Cercariae then emerge from the snail into the external environment and enter their second intermediate mussel host through the mussel's inhalant siphon. Here, the parasite uses penetration glands along with a specialized penetration apparatus (stylet) to puncture the epidermis of the mussel and preferably encyst in gills and labial palps as metacercaria—a transitional stage between dispersive larval stages and adult stages in the definitive bird hosts. In general, metacercarial stages often negatively affect second intermediate hosts less than the first intermediate host (Lauckner 1983; Bower et al., 1994). However, as the labial palps and gills where *R. roscovita* metacercariae encyst are critical functional structures of mussels, infections can result in lower growth rates and condition indices ultimately causing a reduced filtration capacity, with more potent effects in larger mussels (Thieltges 2006; Stier et al., 2015). This reduced performance of infected mussels is likely to exacerbate some heat stress responses, but the potential synergistic effects of the two stressors on mussel performance have not been investigated to date.

The present study aimed to determine whether *R. roscovita* metacercarial infections affect the filtration and/or respiration and the acute heat sensitivity of the mussel *M. edulis*. We first recorded the responses of laboratory-infected and uninfected mussels under a constant optimum temperature followed by a 24-h thermal fluctuation to test the following hypotheses: (*i*) *R. roscovita* infection impacts *M. edulis* filtration and/or respiration, and (*ii*) the effect is exacerbated under temperature fluctuations that impose brief critically-warm exposures. As we observed statistically insignificant impacts of infections on mussel filtration rates due to high inter-individual variability in filtration, we conducted two more experiments to retest the first hypothesis, focused on the sole effect of parasites. For this, we used two mussel sizes of 20 ± 2 mm and 40 ± 2 mm to maintain consistency and comparability with earlier published research. We could then compare average impacts on filtration (effect sizes non-standardized by variance) between experiments. In addition to the two main hypotheses, the relationship between filtration rate and infection intensity were evaluated for infected mussels.

2. Materials and methods

The hypotheses of this study were tested in three separate experiments. In Experiment 1, we tested whether the infection by the parasite *Renicola roscovita* impacted *Mytilus edulis* filtration and/or respiration responses during a 5-h constant mild temperature followed by a 24-h fluctuation between mild and critically high temperatures. In Experiment 2 and 3, we performed additional tests with larger sample sizes to strictly assess the responses of infected versus uninfected mussels of two size classes, large and small, to constant mild temperature exposures lasting around 1 or 2 h.

2.1. Parasite and host sources

Specimens of the first intermediate host *Littorina littorea* snail were collected from a coastal site (54°21′32.3532″ N 10°8′38.7168″ E; area ca. 2 km^2 and depth ca. 0.5 m) located in the Kiel fjord in the southwestern Baltic Sea. Approximately 1000 snails were collected in October 2018 for Experiment 1 and 1500 snails in June 2020 for Experiment 2 and 3. Snails were brought to the laboratory in GEOMAR (Helmholtz Center for Ocean Research Kiel, Germany) and kept in a constant-temperature room at 15 °C inside mesh bags (10 L) submerged within a 100 L tank supplied by a flow-through of sand-filtered seawater from the Kiel Fjord. Snails were fed *ad libitum* with *Ulva* sp. and *Fucus vesiculosus*. On the following days, snails were incubated individually under warm illumination to induce cercariae emergence following (Thieltges and Rick 2006). Afterward, the water in each beaker (snail-surrounding water) was screened with a stereomicroscope (Nikon, SMZ1000 body, C–PS160 stand) to detect the presence of cercariae. Morphological identification of emerged cercariae were done based on Werding (1969). From 1000 to 1500 snails initially screened for Experiment 1 and 2, respectively, a total of 15 and 35 snails released *R. roscovita* cercariae. These snails infected with *R. roscovita* were kept in a separate holding tank from snails that did not shed cercariae, and both were returned to the flow-through system at 15 °C until controlled infections of the second intermediate host, *M. edulis*, began.

Mussels were collected from the mussel farm "Kieler Meeresfarm" in the Kiel fjord (54° 22′ 59.1″ N 10° 09′ 41.8″ E) at the same time as snails for each experiment (in October 2018 for Experiment 1 at 13.5 °C, and in June 2020 for Experiment 2 and 3 at 16.0 °C), and 30 individuals (randomly selected with different sizes) were inspected with a stereomicroscope as whole soft body squash preparations to ensure that they were free of metacercariae. For all experiments, mussels were acclimated to laboratory conditions in one or two 5-litter containers (for Experiment 1 and Experiment 2 and 3 respectively) supplied with seawater from a flow-through system at 15 °C and feed *ad libitum* with *Rhodomonas salina* for 4 weeks prior to "control infection" step. Then small or large mussels were individually kept for 2–3 days at 15 °C in a 100 mL or a 250 mL seawater-filled container, respectively. Mussels acclimated for Experiment 1 were of 20 ± 2 mm shell length, and those of Experiment 2 and 3 were 20 ± 2 mm and 40 ± 2 mm (details specified below). Once a day, the water of all containers was changed, and small and large mussels were fed with 2 and 5 mL of a living *R. salina* solution (2 million cells mL^{-1}) provided by Kiel Marine Organism Culture Centre at GEOMAR, KIMOCC.

2.2. Controlled infections

Experiment 1 and 2 were conducted in November–December 2018 and July–August 2020, respectively. Before experiments, *R. roscovita* infected and uninfected *L. littorea* were individually distributed among 50 mL beakers and kept for 2 h under constant warm illumination. Cercariae less than 4 h old were considered viable and infective since Thieltges and Rick (2006) suggests that the functional longevity of *R. roscovita* cercariae (the time when cercariae are no longer able to infect a host despite still being alive) is between 8 and 16 h, a time two to four times longer than the used in this study. Furthermore, laboratory infections with 4 h cercariae were successful in a study previously performed on *Himasthla elongata* (a trematode species with the same second-intermediate host and similar swimming behaviour) (Bommarito et al., 2020). All water containing viable and infective cercariae of *R. roscovita* was pooled together and well-mixed to generate a genetically mixed array of cercariae, then added to half of the mussel

containers (Studer and Poulin 2013). The other half of the mussel containers received water from uninfected snails. For Experiment 1, each mussel received 60 mL of snail water, and for Experiment 2 and 3, depending on the recipient mussel size, 30 mL or 80 mL of snail water was added to the mussel container. Immediately afterward, mussels were subjected to one dose of *R. salina* food suspension to induce their filtration activities and valve-opening response to increase the chance of cercarial entrance to mussels (Riisgård et al., 2011).

For Experiment 1, 24 mussels were used, and 12 out of 24 were successfully infected in a one-time infection endeavour using 750 mL of snail water containing ca. 35000 cercariae (the density of cercariae was determined by counting cercariae in a subsample of 50 ml and estimating the total number of cercariae in the water sample). For Experiment 2 and 3, 10 out of 20 small and 18 out of 36 large mussels were infected three times due to a shortage of emerged cercariae (ca. 1500 cercariae in 1750 mL snail water). After controlled infection, all the mussels were kept in a constant temperature room under the above-explained conditions for 7 days to ensure the full encystment of metacercariae.

2.3. Experiment 1: mussel filtration and respiration in response to parasite and heat stress

Experiment 1 tested the combined effect of infection and temperature on mussels' filtration rate and respiration (oxygen consumption) and was composed of 8 temporally replicated trials with different mussels assigned to each trial using simple randomization in December 2018. In each trial, filtration and respiration rates of 3 different mussels, randomly selected from infected and uninfected mussels, were simultaneously recorded in 3 separated containers during a pre-heat stress phase (at 16 °C; on the time interval 14:00–5:00) followed by a 24 h temperature fluctuation phase (linear heating 16–30.5 °C on 5:00–17:00 and subsequent cooling 30.5–16 °C on 17:00–5:00) and a post-heat stress phase (16 °C; 5:00–8:00). The temperatures 16 and 30.5 °C represent a mild present-day and an end-of-century extreme summer temperature, respectively (Gräwe et al., 2013; Franz et al., 2019).

All trials were performed using the Fluorometer- and Oximeter-equipped Flow-through Setup (FOFS) based on the protocol described by Vajedsamiei et al. (2021c). In FOFS, a phytoplanktonic (*R. salina*) food suspension was continuously pumped into 4 paths. Along each path, the food suspension passed from an oximetry (or incubation) cylinder and, subsequently, a fluorometry chamber. The filtration or respiration rate of 3 mussels placed in 3 incubation chambers at each time point was calculated based on the difference between chlorophyll (Chl) or dissolved oxygen concentrations taken from three flow-through paths, each containing one mussel, and the measurement taken from one mussel-free flow-through path of FOFS every 5 min (data cropped to 32-h long measurements for each mussel). The food concentration in the ambient of mussels was always kept within the optimal range (1000–7000 cells mL^{-1}) for filtration activity (Supplementary Table 1) (Riisgård et al., 2012).

2.4. Experiment 2 and 3: filtration and oxygen consumption rates of small and large mussels under constant temperature

Experiment 2 and 3 focused on testing the effect of infection on filtration and oxygen consumption rates of mussels from two size classes. These experiments were conducted in August 2020 and consisted of two sets of 7 and 12 trials using small and large mussels, respectively. In each experimental trial, filtration and respiration rates of 3 different mussel specimens were simultaneously recorded every minute for 2 h (for small mussels in the afternoon interim) and for 1 h (for large mussels on morning and afternoon time intervals) at the constant mild temperature of 17 °C using FOFS. The collected data was then cropped to 96 or 40 min for small and large mussels, respectively.

2.5. Post-experimental dissections

After Experiment 1 or 2, the length of mussels was measured with a digital Vernier calliper (0.01 mm). Subsequently, mussels were dissected, the whole body was flattened using a glass compressorium, and the number of metacercariae was counted with a stereomicroscope. The same procedure was used for uninfected mussels to confirm the uninfected status.

2.6. Data analyses

Initial data processing was done using Python (Python Software Foundation) based on the scripts and the protocol described by Vajedsamiei et al. (2021c). Raw Chlorophyll (Chl) and oxygen measurements from each experimental trial were denoised using a robust estimation method, temperature corrected, and converted to units of interest. The delay in the Chl measurement, caused by the Chl sensor being post-positioned relative to the oxygen sensor in each path of FOFS, was addressed using linear differential modelling. The revised time series were then used to calculate filtration and respiration rates. Not all the trial's outputs were used because time series were lost or affected by technical problems (i.e., trapped air bubble covering fluorometer sensor), broken magnetic stirrer causing lack of mixture in oximetry chamber. From 24 mussels used in Experiment 1, data for 8 infected and 11 uninfected mussels were analysed. From 20 small and 36 large mussels used in Experiment 2 and 3, measurements of 9 infected and 9 uninfected small mussels and measurements of 18 infected, and 16 uninfected large mussels were analysed separately for two different size groups.

Data analyses were conducted using R (version 4.1.0) and RStudio© 1.4.1717 (Team, 2021). Using the *bam* function from the *mgcv* package (Wood et al., 2016; Wood 2017), we defined Generalized Additive Mixed Models (GAMMs) or Linear Mixed Model (LMM) separately for filtration and respiration response variables as functions of *time* and *infection status* or *infection intensity* (details as follows).

For Experiment 1, (*i*) filtration and respiration rates as functions of *time* and *infection status* were modelled as GAMMs, whereas the responses over *time* were highly nonlinear due to temperature fluctuation. In each GAMM, *time* was a smooth-effect predictor that could have an effect with some degree of nonlinearity. *Infection status* was an ordered factor allowing the intercept (or the mean) and the degree of nonlinearity (effective degrees of freedom, *edf*) of the reference level smoother (*uninfected*) to be compared to zero and a straight intercept line, respectively. The treatment level smoother (*infected*) was compared to the reference level smoother. (*ii*) To determine whether *infection intensity* influenced the heat recovery potential of mussels, we used only post-fluctuation phase (times >1700 min) filtration or respiration rate data from each infected mussel time series, scaled the values (dividing to the mean filtration or respiration before heat ramp (times <300 min)), and defined GAMMs using the scaled time series of infected mussels. Additionally, the difference in recovery potential during the post-warming period was measured by modelling the scaled filtration rate for both infected and uninfected mussels using GAMMs. The rationale behind scaling was that the level of recovery depends on the original level of response. In all GAMMs, the number of basis functions (knots) was chosen to optimize the k-index while balancing the non-linearity (degree of freedom) and goodness of fit of the models (Wood 2017).

For Experiment 2 and 3, (*i*) filtration and respiration rates as functions of *time* and *infection status* were models as LMMs, whereas the responses were highly consistent over time. Besides, (*ii*) GAMMs were defined to test whether filtration and respiration were affected by *infection intensity and time*, separately examined for small and large size mussels.

In all GAMMs and LMMs, mussel identity (*replicate)* was a random effect factor, and Restricted Maximum Likelihood (REML) was used for unbiased estimation of variance components (Wood et al., 2016).

Temporal autocorrelation of residuals was assessed for each model using *check_resid* function from *itsadug* package (Wood 2017) and the lag-one autoregressive term was considered in the models (Wood 2017). The models' scripts can be found in the supplementary material. After testing for the significance of main and interactive effects, modelling assumptions regarding states of residuals were checked.

Our setup's limited capacity for simultaneous recording of the response of only three mussels at each trial imposed a sample size limitation (explained above). Thus, power analyses were performed using the *powerCurve* function from the *simr* package with 200 simulations, to determine sample sizes required for observing statistically significant effects of the infection-status on filtration rates ($P \leq 0.05$). Data from the pre- and post-fluctuation phases of Experiment 1 (times $\leq$355 and $\geq$ 1700 min) and small-size mussel data of Experiment 2 were used in three separate power analyses. For the power analyses, LMMs were defined (using *lmer* from *lme4* package) with the same model designs as previous LMMs but without autocorrelation term.

3. Results

Post-experimental dissections demonstrated that the laboratory mussel infections were successful. An infection intensity of 3030 ± 1510 SD metacercariae mussel^{-1} (n = 8) was attained for mussels of Experiment 1. Lab-exposed infections of Experiment 2 and 3 resulted in 166 ± 46 SD metacercariae mussel^{-1} (n = 9) and 584 ± 312 SD metacercariae mussel^{-1} (n = 18), for small mussels and large mussels, respectively. Mean relative parasite intensity was 143 ± 72 SD metacercariae mussel^{-1} mm-shell-length^{-1} (n = 8) for mussels in Experiment 1 and 8 ± 2 SD metacercariae mussel^{-1} mm-shell-length^{-1} (n = 9) for small and 15 ± 7 SD metacercariae mussel^{-1} mm-shell-length^{-1} (n = 18) for large mussels in Experiment 2 and 3. None of the mussels were infected with other macro-parasites (i.e., polychaetes, copepods, or trematodes) and no *R. roscovita* metacercariae were found in uninfected mussels.

3.1. Experiment 1: mussel filtration and respiration in response to parasite and temperature fluctuation

The deviance explained by the Generalized Additive Mixed Model (GAMM) was 86 % and 73 % for filtration and respiration rates, respectively. Considering the whole duration of Experiment 1, *R. roscovita* metacercariae infection did not significantly impact the filtration or respiration rate of the host *M. edulis* mussels, evaluated both in terms of the main and interactive effects (Fig. 1 A, B; Table 1). The distance between smoothers that modelled filtration or respiration time series of uninfected versus infected mussels was not significant during the pre-fluctuation phase when the temperature was constant and mild (16 °C), neither in the subsequent heating and cooling phases of the 24 h fluctuation, nor during the post-fluctuation phase (constant 16 °C) (see Fig. 1 A, B).

Both infected and uninfected mussels suppressed filtration to zero during the late-warming phase at > 25 °C, and filtration rate did not resume until lower temperatures were reached (Fig. 1A). The filtration rate recovered to a maximum 50 % of its initial level, whereas the respiration recovered to almost 90 % of its initial rate.

When analysing the data of pre- and post-fluctuations phases separately using Linear Mixed Models (LMMs), we again found that the effect of infection on filtration or respiration was not significant ($p > 0.05$). Despite being non-significant, regarding the effect size non-standardised by variance, filtration rates of infected mussels were, on average 9 % lower than uninfected ones in the pre-fluctuation phase, and this effect size became 37 % (albeit still non-significant) in the post-fluctuation-phase (Fig. 1A; Table S4). In the pre-fluctuation phase of Experiment 1, the power analysis suggested that approximately 85 replicates per infection status were required to detect a statistically significant ($p \leq 0.05$) infection impact on filtration with approximately 80 % test power (Fig. S3). In the post-fluctuation phase, the required sample size was estimated to be approximately 40 replicates per infection status (Fig. S3).

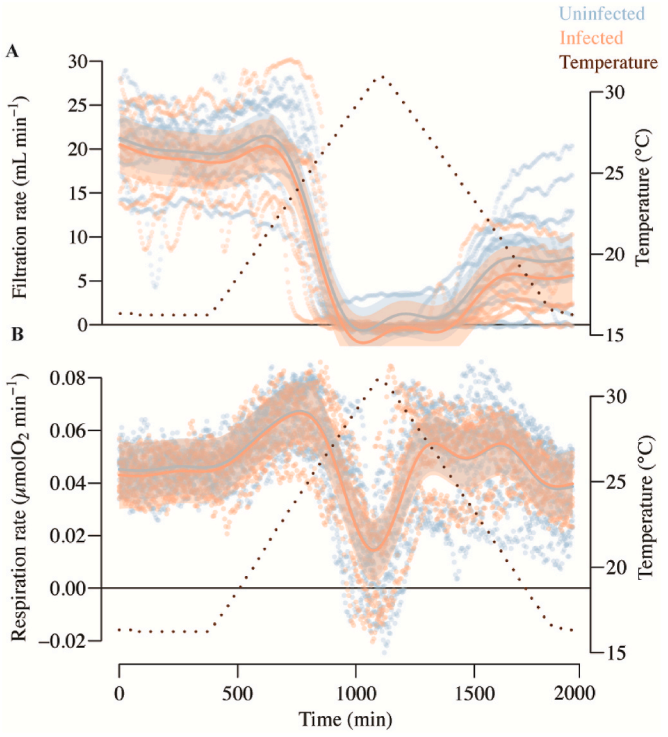

Fig. 1. Mussel filtration and respiration responses during Experiment 1. Generalized Additive Mixed Models (GAMMs) of responses of small size mussels uninfected and infected with *Renicola roscovita* during exposure to a constant mild temperature (for 5 h) followed by a 24-h thermal fluctuation. Each point represents filtration or respiration measurement per 5 min (shaded areas represent 95 % CIs). Sample size for each group was 8 and 11 for infected and uninfected, respectively. The negative values recorded during the metabolic depression phase are due to extra random variation in the measurement, variability between individuals and the white noise of oximeter device.

During the cooling period, mussels started to partially recover filtration capacity when the temperature was lowered to <20 °C after 1700 min (Fig. 1A). The capacity for post-heat recovery of filtration (or scaled filtration recovery) followed a dome shape pattern in relation to the infection intensity (Fig. 2A) while it was linear for the respiration rate (Fig. 2B). GAMMs explained 96.8 % and 87.3 % of filtration and respiration variance, respectively. Nevertheless, the effect of parasite intensity was not significant neither for filtration (edf = 1.666, F-value = 1.207, P = 0.389) nor for respiration (edf = 1.006, F-value = 0.06, P = 0.808) (Fig. 2).

3.2. Experiment 2 and 3: R. roscovita effects on filtration and respiration of mussels from two size classes

The LMMs explained 83 % and 97 % of the variance in filtration responses of small and large mussels, respectively. For the respiration response, the LMM explained 94 % of the variation in both small and large mussels. For large mussels (40 mm), the variation in filtration rate was significantly explained by infection status, as both the intercept (*uninfected-infected*) and fixed term (*infected)* were significant (P = 0.011; Table 2; Fig. 3 B). Infected large mussels filtered approximately 12 % less than uninfected mussels. For small mussels (20 mm), the infection effect on filtration was marginally insignificant (P = 0.055; Table 2; Fig. 3 A), with a non-standardised effect size of ca. 11 %. According to the power analysis, 25 replicates were needed to detect a statistically significant effect of infection on the filtration rate of small mussels. Moreover, the infection effect on respiration rate was

Table 1
Renicola roscovita metacercarial infection effects on mussel filtration and respiration rates over the whole experiment tested using Generalized Additive Mixed Models (GAMMs). The intercept (or the average) and the degree of nonlinearity (effective degrees of freedom, *edf*) of the reference level smoothers (*Uninfected*) are compared to zero and straight intercept lines, respectively. The treatment level smoothers (*Infected*) are compared to the reference level smoother. Parametric coefficients' estimates are intercept values or differences. Besides, the effects of random variance in time series intercept are tested as *s(replicate)*. The significant impact is considered for *p-value* ≤ 0.05.

Filtration rate	A. parametric coefficients	Estimate	Std. Error	t-value	p-value
	Intercept (Uninfected)	10.5609	0.8256	12.7913	<0.0001
	Infected – Uninfected	−1.3624	1.2579	−1.0831	0.2788
	B. smooth terms	**edf**	**Ref.df**	**F-value**	**p-value**
	s(Time) : Uninfected	9.9409	9.9993	195.2528	<0.0001
	s(Time) : Infected	1.0281	1.0558	0.6242	0.4240
	s(replicate)	11.8251	17.000	2.2851	<0.0001
Respiration rate	**A. parametric coefficients**	**Estimate**	**Std. Error**	**t-value**	**p-value**
	Intercept (Uninfected)	0.0468	0.0031	15.3024	<0.0001
	Infected – Uninfected	−0.0005	0.0047	−0.1143	0.9090
	B. smooth terms	**edf**	**Ref.df**	**F-value**	**p-value**
	s(Time) : Uninfected	11.7837	11.9918	50.3226	<0.0001
	s(Time) : Infected	1.0181	1.0360	0.4693	0.4908
	s(Replicate)	15.2636	17.0000	8.7906	<0.0001

insignificant for both small and large mussels (P > 0.05; Table 2; Fig. 3 C and D).

A positive relationship between filtration rate and infection intensity was detected for both small and large mussels (Fig. 4). The intercept and the smooth effect of the number of parasites and time were significant for both the small and large mussels (P < 0.001). For small mussels: the smoother *number of parasites* was nearly a second-degree curve (edf = 1.9366, F-value = 102.5735), and so was the smoother of *time* (edf = 1.9686, F-value = 26.1321). For large mussels: the smoother number of parasites was slightly less curvilinear (edf = 1.6102, F-value = 36.1847), while the smoother of time was closer to a second-degree curve (edf = 1.9994, F-value = 65.6676). However, there was no significant correlation between infection intensity and respiration rate for both small and large mussels (P = 0.108, P = 0.57 for small and large mussels, respectively) (Fig. 2 and Table 3 in Supplementary).

4. Discussion

This study analysed the effects of metacercarial infections of *Renicola roscovita*, a trematode common in the north-east Atlantic and Baltic Sea, on the filtration and respiration processes of *Mytilus* mussels and on their acute heat sensitivity. The infections consistently reduced filtration by a 9-11 % absolute effect size across three different experiments. Although non-significant, we found the effect size enlarging to 37 % at mussels' recovery from transient heat exposure. Additionally, size-related differences emerged as the larger mussels displayed a statistically significant drop-in filtration rate due to infection. However, these alterations did not affect the overall respiration rates. Interestingly, we observed a correlation between higher infection intensities and elevated filtration capacities in mussels. Our findings hint towards a subtle interplay between infection, physiological processes, and individual mussel characteristics. We further discuss these results, explore the study's limitations, and propose avenues for future research in this fascinating intersection of parasitology, physiology, and environmental stressors.

We initially expected *R. roscovita* infections to worsen the filtration and respiration responses of mussels to acute heat stress. The rationale behind our prior expectation was that: (*i*) metacercarial cysts (measuring ca. 150 μm in diameter) could have interfered with the beating of lateral cilia on gill filaments impacting their particle capture and water pump function (Werding 1969; Galaktionov and Dobrovokskij 2003); and (*ii*) the infection could have imposed an energic toll due to immunological reactions of mussels and haemolymph loss after cercarial injury. Notably, haemolymph loss was expected to be greater in the early stages of infection (first few hours) when hundreds of cercariae were penetrating mussel tissue with the stylet (i.e., a sword-like apparatus in the cercariae used for puncturing host tissue) (Werding 1969). In general, if a major allocation of ATP to immunological and repair responses were occurring, infected mussels would have initiated their metabolic (feeding and respiration) depression responses at lower temperature limits.

Based on the findings in Experiment 1 (response to transient hours-long heat exposure), *R. roscovita* metacercarial main impact and its interactive effect with temperature (time) on mussel filtration or respiration were statistically not significant. Although not statistically significant, the enlarging trend of the absolute size of the infection effect on filtration was interesting: The difference in filtration between infected and uninfected mussels became more prominent during the post-heat (recovery) phase (from 9 to 37 %, with infected mussels having lower respiration rate than uninfected mussels). This can be explained by the 'metabolic depression' phase that *Mytilus* mussels enter to regulate heat-induced rises in ATP synthesis and consumption (McMahon et al., 1995; Sokolova et al., 2012b). This phase is typically followed by diminished

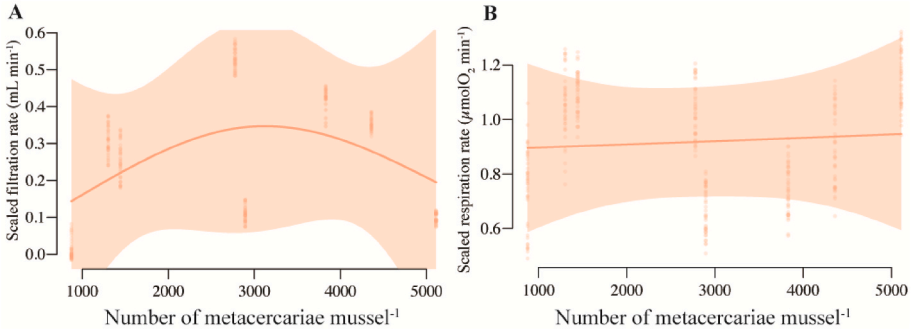

Fig. 2. Post-warming scaled mussel filtration (A) and respiration (B) in relation to infection intensity. Generalized Additive Mixed Models (GAMMs) predictions (lines) and 95 % CIs (shaded area) are conditioned on the average post-warming time points. Individual points represent filtration or respiration measured every 5 min and each stratum represents measurements of one mussel.

Table 2
Renicola. roscovita metacercarial infection effects on mussel filtration and respiration rates over Experiment 2 and 3 tested using linear mixed models. The intercept and the slope (over *Time)* of the reference level line (*Uninfected*) are compared to zero and the straight intercept line, respectively. The treatment level line (*Infected*) is compared to the reference level line. Parametric coefficients' estimates are intercept and slope values or the differences. Besides, the effects of random variance in time series intercept are tested as *s(replicate)*. The significant impact is considered for *p-value* ≤ 0.05.

		Small mussels				Large mussels			
Filtration rate	**A. parametric coefficients**	**Estimate**	**Std. Error**	**t-value**	**p-value**	**Estimate**	**Std. Error**	**t-value**	**p-value**
	Intercept (Uninfected)	24.183557	0.998530	24.219	<0.0001	66.507585	2.339536	28.428	<0.0001
	Intercept (Infected – Uninfected)	−2.707140	1.412135	−1.917	0.0554	−8.143140	3.215385	−2.533	<0.0114
	Uninfected : Time	−0.012703	0.002792	−4.549	<0.0001	0.137867	0.008742	15.770	<0.0001
	Infected : Time	0.025056	0.003949	6.345	<0.0001	0.016603	0.012015	1.382	0.1673
	B. smooth terms	**edf**	**Ref.df**	**F-value**	**p-value**	**edf**	**Ref.df**	**F-value**	**p-value**
	s(Replicate)	15.65	16	45.18	<0.0001	31.9	32	324.2	<0.0001
Respiration rate	**A. parametric coefficients**	**Estimate**	**Std. Error**	**t-value**	**p-value**	**Estimate**	**Std. Error**	**t-value**	**p-value**
	Intercept (Uninfected)	5.019e-02	2.551e-03	19.676	<0.0001	1.848e-01	9.944e-03	18.581	<0.0001
	Intercept (Infected – Uninfected)	−2.076e-03	3.607e-03	−0.576	0.565	−1.411e-02	1.367e-02	−1.032	0.302
	Uninfected : Time	−9.671e-06	6.927e-06	−1.396	0.163	5.168e-04	6.176e-05	8.368	<0.0001
	Infected : Time	1.085e-05	9.796e-06	1.108	0.268	8.230e-05	8.488e-05	0.970	0.332
	B. smooth terms	**edf**	**Ref.df**	**F-value**	**p-value**	**edf**	**Ref.df**	**F-value**	**p-value**
	s(Replicate)	15.85	16	104.8	<0.0001	31.61	32	81.45	<0.0001

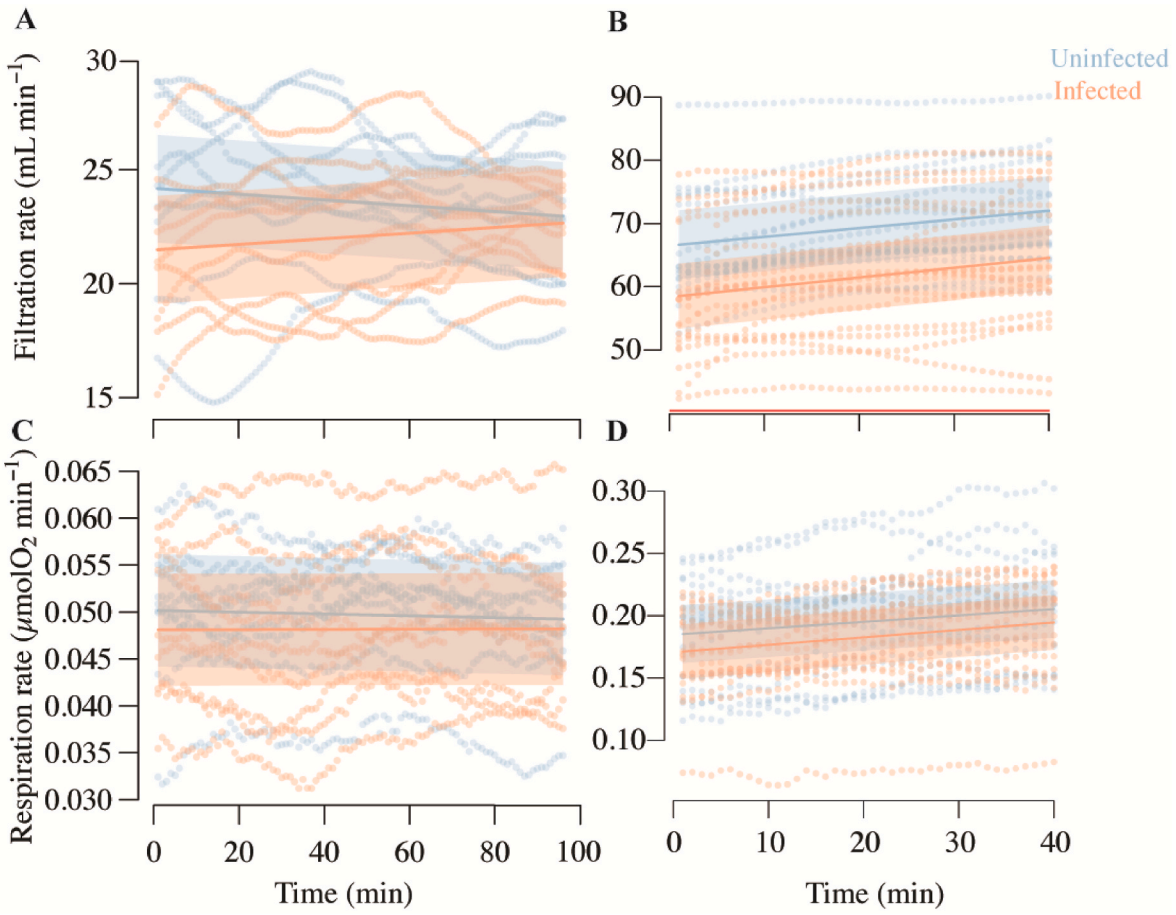

Fig. 3. Linear mixed models of filtration and respiration rates of small (A, C) and large (B, D) *M. edulis*, either uninfected (light blue) or infected (light pink) with *Renicola roscovita* under a constant temperature of 17 °C. The bottom red line in the subplot B indicates the interval of significant difference between smoothers. The shaded area represents 95 % CIs. Each point represents the filtration or respiration rate measured minutely. The sample size for each group of small or large mussels was 9–18 and 9–16 for infected and uninfected, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

feeding as a response to the oxygen debt induced by anaerobic metabolism (Pörtner & Knust, 2007; Collins et al., 2020). Consequently, due to the energy expenditures related to immunological responses to infection, infected mussels might undergo a lengthier depression period and experience an intensified heat-induced oxygen (and energy) debt. This, in turn, could impair their post-heat recovery of energy-demanding activities such as feeding.

In Experiments 2 and 3, we re-evaluated the harmful impact of *R. roscovita* metacercarial infection at a mild temperature in mussels of different sizes. Here, the infection impact on the filtration rate was statistically significant for larger mussels (P = 0.011) and marginally insignificant (P = 0.055) for small mussels. With regard to the

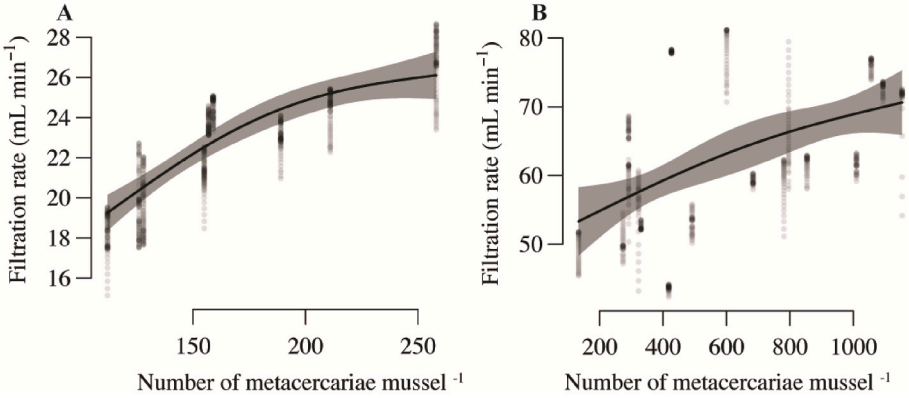

Fig. 4. Filtration rate in relation to infection intensity (metacercariae mussel^{-1}) for small (A) and large (B) mussels. Generalized Additive Mixed Models (GAMMs) predictions (lines) and 95 % CIs (shaded area) are conditioned on the average time points. Each point represents filtration measurement per minute and each stratum shows temporal filtration of one mussel.

marginally insignificant p-value for small mussels, we would like to highlight that p-values often neglect such small-size effects (Sullivan and Feinn 2012), and one must pay attention to the consistency of absolute sizes of infection effects on mussel filtration rates across our three experiments (ca. 9, 11, and 12 %). This suggests that *R. roscovita* metacercarial infections can slightly decrease the ability of mussels to feed. However, because filtration rates vary widely between individuals, detecting these effects within a single experiment can be challenging. These absolute effect sizes are in accordance with previous experimental results indicating that the growth of mussels in intertidal and shallow subtidal mussel beds decreased by 5–14 % due to *R. roscovita* infection (334 ± 270 metacercariae mussel^{-1}) (Thieltges 2006). Our findings were also partially in accordance with Stier et al. (2015), who found a negative impact of *R. roscovita* on filtration rates of mussels (small: 19–22 and large: 46–49 mm shell length) with a stronger impact in large compared to small mussels (71 versus 42 % reduced filtration rate, respectively). In the study of Stier et al. (2015), the average infection intensity was much higher than in our experimental infections (1559 versus 166 metacercariae mussel^{-1} and 3032 versus 584 metacercariae mussel^{-1} for small and large mussels, respectively) but still within the extremes found in natural systems (i.e., up to 6000 metacercariae mussel^{-1}) (Svärdh and Thulin 1985; Zens 1999; Buck et al., 2005).

The effect of trematode infections on respiration rates was insignificant, with an absolute effect size of <5 % across all experiments. Disruption induced by metacercariae encystment, particularly on the gill and labial palp, may result in compromised filtration activity of gills, which might have no significant impacts on respiration since the organism takes up oxygen through diffusion (Jorgensen et al., 1986). Moreover, the feeding and digestion activities of *M. edulis* typically consume <20 % of the total mussel metabolic energy expenditure (Widdows and Hawkins 1989). Since, in our experiments, the absolute effect of parasites on filtration rate per se was small, its impact on respiration should have been minor. While the effect of infections on mussel respiration may be minor, one study suggests that this may not be a universal pattern for *R. roscovita* infections on bivalves. Magalhães et al. (2020) observed a significant ca. 40 % decrease in respiration rate of cockles infected by 10 versus 3 *R. roscovita* metacercariae. This surprising effect demand further studies resolving species-specific sensitivity of bivalve respiration to *R. roscovita* infections.

Finally, the results of Experiments 2 and 3 suggest that infection intensities in mussels can be higher in mussels with a higher filtration capacity, both among small and large size mussels. Such an evident correlation could not be found in Experiment 1, probably due to the higher availability of cercaria in the mussels' surrounding water during the lab-infection procedure. This overdose of cercariae might have forced the chances of successful infection regardless of the mussels' innate filtration rate. A positive relationship between infection intensity and filtration rate could result from two mechanisms. On the one hand, there is variation in filtration rates among mussel individuals (phenotypic variation) (Steeves et al., 2020). Therefore, a mussel with a phenotype characterized by a higher filtration rate might be innately prone to higher metacercarial infection intensity due to a higher "inflow" of cercariae. Alternatively, higher metacercarial infection intensities could result in an upregulation of filtration rates to compensate for the additional energetic costs of infections for mussels. Given that we did not observe a positive correlation between respiration rates and infection intensity, the first mechanism that mussels with higher innate filtration rates are in danger of acquiring higher infection levels seems more plausible. Recent research supports our claim, suggesting that mussels with a lower filtration rate are more adept at avoiding trematodes, resulting in a reduced infection intensity (Selbach et al., 2022). Although Mouritsen et al. (2022) showed that fear of parasite caused a reduction in filtration activity of *M. edulis* by 30 % to avoid *Himasthla elongata* infection, they also discovered a positive relationship between infection success and clearance rate, which is consistent with our findings. The existence of innate variation in mussel filtration rate is suggested as a possible explanation for the positive relationship between metacercariae intensity and filtration activity. This finding is also in accordance with (Nikolaev et al. (2006), who found that among *M. edulis* of the same age, higher infection intensities of the trematodes *H. elongata* and *Cercariae parvicaudata* occurred in larger individuals. Those larger mussels were most likely mussels with higher filtration rate phenotypes which usually also have a higher growth rate (Prieto et al., 2018). Notably, the positive relationship of infection intensity to filtration capacity found in our study provides a possible explanation for Thieltges (2006) not detecting a negative relationship between long-term growth and infection intensity of *R. roscovita* infected mussels, i.e., mussels with higher infection intensity might be of the phenotype of faster filtration and growth.

5. Conclusion

Overall, our results tentatively suggest that *R. roscovita* infections are benign in regard to mussel respiration, while they can slightly lower the ability of mussels to feed. Contrary to expectation, we did not observe significant interactions with short-term exposure to transient heat stress. Further series of experiments are required to investigate the effects of prolonged heat stress exposure. Also, measuring mussels' physiological performance before and after infection (e.g., using a before-after-control-impact design) must be considered in future studies using a setup that permits more replication. The power analysis results suggested that increasing the sample size from 8 to 40 mussels might have resulted in detecting a significant difference between infected and uninfected mussels' filtration recovery from heat exposure. Therefore, in

future experiments a larger sample size might be desirable. In addition, the role of mussel phenotypic variation in filtration capacity in driving infection levels and subsequent effects warrants further investigation to understand better the combined impacts of parasitism and heat stress on mussel performance and aquaculture in the context of ocean warming.

Authors' contributions

M.K., J.V., D.W.T. designed the study; M.K., J.V. ran the experiments, analysed data and wrote the first draft; D.M.D.M., C.B., and D.W.T. helped in writing and revising the manuscript. All co-authors discussed the results, reviewed and contributed to the final manuscript.

Ethical statement

To conduct this research: MK was funded by a PhD grant program from "Studienstiftung des Deutschen Volkes", DMDM and CB acknowledged support from BMBF/PTJ (Federal Ministry of Education and Research, Germany, grant 03F0821A, PI Wahl and grant 03F0821B, PI B Sures), JV received fund through Deutsche Forschungsgemeinschaft (DFG) project (Grant Number: PA 2643/2/348431475) and through GEOMAR. In this study, all applicable international, national, and/or institutional guidelines for the sampling, care and experimental use of animals were followed. Open Access funding enabled and organized by Projekt DEAL.

Declaration of competing interest

None.

Acknowledgments

We would like to extend our gratitude to professor Martin Wahl, GEOMAR Helmholtz Centre for Ocean Research, Kiel, for his generous support during the study. We gratefully acknowledge the help provided by Martin Sackmann during the Experiment 2 and 3. Additionally, we would like to express our appreciation to Dr. Tim Staufenberger and Nikolai Nissen of the "Kieler Meeresfarm" for supplying the mussels. We also thank Ulrike Panknin for providing the *Rhodomonas* culture.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.07.007.

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