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Combined effects of salinity and trematode infections on the filtration capacity, growth and condition of mussels

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ABSTRACT: The blue mussel (*Mytilus* species complex) is an important ecosystem engineer, and salinity can be a major abiotic driver of mussel functioning in coastal ecosystems. However, little is known about the interactive effects of abiotic drivers and trematode infection. This study investigated the combined effects of salinity and *Himasthla elongata* and *Renicola roscovita* metacercarial infections on the filtration capacity, growth, and condition of *M. edulis* from the Baltic Sea. In a laboratory experiment, groups of infected and uninfected mussels were exposed to a wide range of salinities (6–30, in steps of 3) for 1 mo. Shell growth was found to be positively correlated with salinity and optimal at 18–24 at the end of the experiment, imposed by constraints in shell calcification under lower salinities. Mussel shell growth was not affected by *H. elongata* infection. While salinity had only a minor effect on tissue dry weight, infected mussels had a significantly lower tissue dry weight than uninfected mussels. Most interestingly, the combination of salinity and trematode infections negatively affected the mussels' condition indices at lower salinity levels (6 and 9), suggesting that trematode infections are more detrimental to mussels when combined with freshening. A significant positive effect of salinity on mussel filtration was found, with an initial optimum at salinity 18 shifting to 18–24 by the end of the experiment. These findings indicate that salinity and parasite infections act as synergistic stressors for mussels, and enhance the understanding of potential future ecosystem shifts under climate change-induced freshening in coastal waters.

KEY WORDS: Condition index · Filtration · Freshening · Growth · Parasite · Salinity · *Himasthla elongata* · *Mytilus*

1. INTRODUCTION

Marine shallow-water habitats are subject to rapid changes in abiotic parameters, such as peaks in temperature or rapid changes in salinity (Cloern et al. 2016). Organisms inhabiting this zone can be well-adapted to these stressors; however, with rapid global change, the effects of abiotic stressors on these organisms might be intensely amplified (Kennish 2021). In particular, severe environmental impact on species that structure communities (foundation species) can majorly alter community composition, food webs, and ecosystem services (Seitz et al. 2014, Griffiths et al. 2017).

Blue mussels (*Mytilus* species complex; Vendrami et al. 2020) are foundation species of mussel bed ecosystems in temperate seas. They are essential ecosystem engineers, forming epibenthic mussel beds that

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facilitate the presence of other species and therefore contribute to the functional diversity in shallow-water habitats (Ragnarsson & Raffaelli 1999, Beadman et al. 2004, Norling & Kautsky 2008). One of the main abiotic drivers of mussel functioning is salinity (Riisgård et al. 2012, Noor et al. 2021), in particular in coastal ecosystems with steep salinity gradients such as the Baltic Sea, where salinity ranges from 27 psu in proximity to the North Sea to <10 psu in proximity to the Baltic Proper (Hansson & Gustafsson 2011). Generally, mussels show optimal growth rates at a salinity of about 25 psu in laboratory experiments (Hiebenthal et al. 2012) and reduced shell growth with increased freshening along the Baltic Sea's salinity gradient (Westerbom et al. 2002). Energetically costly deposition of calcium carbonate shell material at lower salinities as well as calcium concentrations and high variation in carbonate chemistry in estuarine habitats have been proposed to be among the underlying mechanisms (Thomsen et al. 2015). In addition, negative effects of freshening on mussel growth are probably also linked to reduced filtration (feeding) activity, particularly when salinity drops below 5 psu (Riisgård et al. 2013). With a projected seawater freshening of 1.5–2 psu by 2100 for the region (Gräwe et al. 2013), the various detrimental effects of lower salinities on mussel performance may become more pronounced.

In addition to salinity as a dominant abiotic driver in the Baltic Sea, the ecological performance of mussels can also be affected by biotic interactions such as parasitism. Mussels are often infected with trematodes that use them as second intermediate hosts in which the parasites form metacercariae that await being ingested by the final host (birds or fish, depending on the species; Lauckner 1983, Bommarito et al. 2021). Metacercarial infections can cause structural tissue damage and inflammation that can compromise the functioning of mussels. Two common species of trematodes infecting the common periwinkle *Littorina littorea* as a first intermediate host and mussels as a second intermediate host in the Baltic Sea are *Renicola roscovita* and *Himasthla elongata* (Werding 1969, Bommarito et al. 2021). Infections with *R. roscovita* can be found mainly in the labial palps and gill tissues where they can lead to a reduction in mussel filtration and growth rates (Thieltges 2006, Stier et al. 2015). In contrast to *R. roscovita*, *H. elongata* generally encysts as metacercariae in the mantle and foot of mussels, causing tissue damage and impairing the mussel's ability to form byssal threads used for substrate attachment (Lauckner 1983, 1984). *H. elongata* infection was also reported to reduce heart rates and growth in mussels (Bakhmet et al. 2017). However, it is unknown whether *H. elongata* infections also affect the filtration activity of mussels. More importantly, it is also unknown whether trematode infections can interact with salinity effects in altering mussel filtration and growth.

The present study aimed to understand the combined effects of salinity and trematode infection on filtration capacity, growth (shell length, dry weight), and condition (condition index, CI) of blue mussels in the Western Baltic Sea. Assuming that trematode infections are energetically demanding for mussels, we expected the infection to limit the mussels' ability to cope with osmotic stress and elevated energy demands at low salinity levels, leading to stronger decreases in filtration and growth with declines in salinity for infected versus uninfected mussels.

To test our hypotheses, we compared the responses of uninfected and laboratory-infected blue mussels to a salinity gradient in a 1 mo experiment. We initially infected mussels with *H. elongata* and *R. roscovita*, as these 2 species often co-infect blue mussels (authors' unpubl. data). Nonetheless, because *R. roscovita* infection was largely unsuccessful, we turned our focus towards explaining the impact of *H. elongata* infection on mussels, while briefly discussing possible reasons for *R. roscovita* infection failure.

2. MATERIALS AND METHODS

2.1. Experimental design, setup, and the salinity treatment

The fully crossed experimental design comprised (1) a salinity treatment, with 9 levels ranging from 6–30 psu (in steps of 3 psu) and (2) an infection status treatment with 2 levels: infected versus uninfected mussels. The setup consisted of 9 mesocosm tanks (Pansch & Hiebenthal 2019), in which 4 Plexiglas cylinders (4 l each), containing 4 mussels each, were deployed. Two cylinders contained infected and the other 2 contained uninfected mussels. To obtain the different salinity levels, seawater, deionised water, and salt deprived of calcium and bicarbonate (Seequasal-Salt; Seequasal) were used. Two tanks (600 l), located in the same climate chamber where the experiment was run, were used as water sources with salinities of 6 and 30 psu. The other salinity levels were obtained employing mixing proportions of source water in 10 l buckets prior to water exchange. Calcium and bicarbonate were added to each source tank following the equations by Beldowski et al.

(2010) and Anderson & Dyrssen (1981). Calcium bicarbonate concentration was measured in each source-tank twice per week; the ammonium was measured twice per week in each cylinder. Mussels were fed every 6 h with *Rhodomonas salina* (7000 cells ml^{-1}) through peristaltic pumps. *R. salina* (cultured at 16°C by the Kiel Marine Organism Culture Centre at GEOMAR) is a cryptophyte and has been used as a food source for filter feeders in several experiments (Riisgård et al. 2013, Sanders et al. 2018). Before every filling, the algal culture's cell concentration was measured to calculate the volume needed to obtain a standard concentration of around 7000 cells ml^{-1}. The complete experimental design is depicted in Fig. 1.

2.2. Host collection and acclimation

Infected *Littorina littorea* were used as cercariae donors to infect mussels. Around 500 were collected from Årøsund (Denmark) (55.25° N, 9.70° E) in November 2017 (see Table 1). From previous samplings, this site is known to contain a good prevalence of infected individuals (around 30 and 25% of individual snails are infected with *Himasthla elongata* and *Renicola roscovita* respectively in this area; Bommarito et al. 2021). To determine their infection status, *L. littorea* individuals were placed in 6-well plates (1 snail well^{-1}) filled with aerated filtered seawater and exposed to heating lamps at 25–27°C for 3–4 h to induce cercariae shedding. Subsequently, the wells were checked under the dissection microscope, and infected *L. littorea* were identified based on released cercariae. *L. littorea* individuals were identified as infected by *H. elongata* and *R. roscovita* taxonomically following Werding et al. (1969) and based on the information gathered from a previous field study investigating prevalence and abundance of these trematode species in the same sampling location (Bommarito et al. 2021). Individuals infected by *H. elongata* and *R. roscovita* were kept in a net located in a 400 l tank provided with a flow-through system and located in a climate chamber at around 10°C for 3 mo. *L. littorea* individuals were fed ad libitum with the brown macroalga *Fucus vesiculosus*, known to be a food source for herbivores such as *L. littorea* based on previous studies (Karez et al. 2000, Rohde et al. 2004). In March 2018, 4 wk before the start of the experiment, *L. littorea* individuals were acclimatised to 14°C to trigger the production of cercariae by sporocysts and rediae and the consequent release.

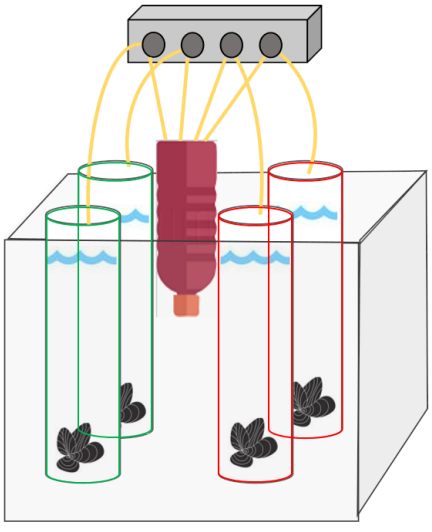

Fig. 1. Experimental setup: it consisted of 9 main tanks (like the one shown in the figure) with 9 different salinity levels (6, 9, 12, 15, 18, 21, 24, 27, 30 psu). Each tank included 4 cylinders containing 4 individual mussels. In each main tank, 2 of the cylinders (in red) contained 4 infected and 2 cylinders (in green) contained 4 uninfected *Mytilus* individuals. The mussels were fed every 6 h with *Rhodomonas salina* from plastic bottles (in pink) using peristaltic pump dosers. Each dose of food included ca. 28 million *R. salina* cells needed to create 7000 cells ml^{-1} in each 4 l mussel cylinder

Mussel hosts were collected in April 2018 (see Table 1) from the Kieler Meeresfarm, a marine aquaculture facility in the inner Kiel Fjord (54.36° N, 10.16° E) known to be uninfected by trematodes (Bommarito et al. 2020). The genome of *Mytilus* species complex in the Western Baltic Sea (Kiel Fjord) was recently found to be predominantly *M. edulis*, with small fractions of *M. trossulus* and *M. galloprovincialis* due to introgressive hybridization (Vendrami et al. 2020). In total, 144 mussels of 18–21.5 mm shell length were used for the experiment. Mussels were transferred to a climate chamber and acclimatised in 2 aerated 600 l flow-through tanks filled with filtered seawater at a temperature of 16°C. During this time, mussels were fed with *R. salina* twice per day.

2.3. Infection of mussels and the experiment

Out of around 500 *L. littorea* collected, 65 were found to be infected with *H. elongata* and 35 with *R. roscovita*. We decided to use those different numbers considering that the cercarial output from a host individual might not be predictable, depending on abiotic (i.e. temperature or night duration) and biotic factors (i.e. host activity or cercariae production by rediae) (Hawking 1975, de Montaudouin et al. 2016).

Table 1. Timeline of the different experimental phases: sampling, infection of *Mytilus edulis* individuals with cercariae of *Himasthla elongata* and *Renicola roscovita*, acclimation of *Mytilus edulis* to the different salinity levels, filtration rate measurements, and measurements of sizes at the end of the experiment

Sampling	Infection	Acclimation	Filtration rate measurements	Size measurements
Littorina littorea: 5 mo before the start of the experiment	1st infection event: 8 d before the start of the experiment	4 d before the start of the experiment (lasted 4 d, salinity increased/decreased by 4 each day)	Start measurement: 4 d after the start of the experiment	4 wk after the start of the experiment (end)
M. edulis: 9 d before the start of the experiment	2nd infection event: 7 d before the start of the experiment		End measurement: 27 d after the start of the experiment	
	3rd infection event: 6 d before the start of the experiment			
	4th infection event: 5 d before the start of the experiment			

In addition, a few host individuals could release from a few to thousands of cercariae. From previous studies, we knew that the cercariae emergences of *H. elongata* and *R. roscovita* could differ greatly. While the cercarial emergence from one snail of the former could be around 700, the latter could reach around 2000 (Prokofiev et al. 2016, C. Bommarito & M. Khosravi pers. obs.). Therefore, it seemed reasonable to use twice the number of individuals infected with *H. elongata* as those infected with *R. roscovita*. To infect mussels, *L. littorea* infected with *R. roscovita* and *H. elongata* were located separately in two 2 l jars and exposed to heating lamps at 25–27°C to allow cercariae shedding. After 3 h, 40 ml of cercariae stock (20 ml from *H. elongata* stock + 20 ml from *R. roscovita* stock) were individually added to 72 beakers of 50 ml. The cercariae stock was stirred 3 times clockwise and 3 times counterclockwise every 2 samplings to avoid parasite aggregation at the bottom of the tanks. To better estimate the distributed dose, 4 subsamples of 20 ml were taken from the stock, and cercariae were counted under a stereomicroscope (SMZ1000; Nikon). After administering the stock, a single mussel was immediately added to each beaker. Then, 10 ml of filtered seawater was added to each beaker, together with around 40 µl of *R. salina*, to enhance filtration of cercariae by mussels. The beakers containing mussels were then left in the climate chamber (16°C) to ensure cercariae infection and metacercariae encystation. After 12 h, the water in the beakers was changed and the mussel infection event was repeated. There were 4 infection events in total, performed over 4 d to ensure the highest number of metacercariae encystation, following Poulin (2010) and Liddell et al. (2017), who detected higher infection rates when hosts were repeatedly exposed to small doses of cercariae. The total number of cercariae administered to each mussel, based on the estimated distribution dose, was approximately 200 *H. elongata* and 50 *R. roscovita.* For a detailed timeline, see Table 1.

Infected (total of 72) and uninfected (total of 72) mussels were deployed in the cylinders (4 mussels per cylinder) and acclimatised to the respective salinity levels by increasing or decreasing salinity by 4 units per day (according to Riisgård et al. 2013). During the acclimation and the entire experimental period, the cylinders were aerated to maintain constant air supply and constant mixing of the water column. After 4 d of acclimation, the experiment lasted for 1 mo.

2.4. Size and CI measurements

Mussel length was measured with analog callipers at the beginning and end of the experiment, when the individual was dissected, carefully removing all tissue from the shell. The tissue of each infected mussel was squeezed between 2 glass slides (compressor) to verify the presence and count the number of metacercariae. All uninfected mussels were also squeezed between the 2 glass slides to confirm the absence of metacercariae and to avoid any bias in tissue loss of the infected mussels, leading to lower tissue weight. After counting the number of metacercariae in each mussel individual, the tissue was

carefully poured into pre-weighted aluminum pots, dried at 80°C for 24 h, and then weighed. The CI of each mussel was calculated using the equation by Dare (1976), where CI = W/L^3, where W is the dry weight (mg) and L is length (mm), following Riisgård et al. (2001) and Stier et al. (2015).

2.5. Filtration rate measurements

During the entire period, filtration rate (FR) was measured twice: once 4 d after the start of the experiment (first week) and once after 27 d (last week) (see Table 1). Each filtration measurement was preceded by a water change of all cylinders. Mussel FR was calculated as the volume of water cleared of suspended particles per unit of time (Riisgård 2001). The concentration of *R. salina* was determined by taking samples 15 min after the morning feeding episode by the peristaltic pumps (which were shut off after feeding) and then every 30 min for the next 2 h, counting the algal cells with a Z2 Coulter particle count and size analyser (Beckman Coulter). The second sampling consisted only of 2 measurements since a peak of decrement of algae concentration was already observed between the first and second measurements. For each withdrawal, syringes of 14 ml each were used, equipped with filters of 20 µm to avoid the entrance of particles larger than *R. salina*. After the withdrawal, the subsamples were examined with the Coulter counter. FR was determined after Coughlan (1969) as the decrease in algal concentration over time.

For the start measurement of FR, which included 4 measurements in total (one taken after 15 min and the others 60, 100, and 145 min after feeding), the 4 data points of the measurements of each replicate were plotted in a linear regression with a semi-log scale (algal concentration against time) to verify that all points were fitting well to the line (with $R^2 > 0.9$). When $R^2 < 0.9$, the last measurement was removed, and the 3 remaining measurements were plotted again to obtain an $R^2 > 0.9$. If the data points not fitting the line were in the middle of the linear regression (second or third measurement), the entire replicate was removed from the data set (see plots 1, 9, and 34 of Fig. S1 in Supplement at www.int-res.com/articles/suppl/m699p033_supp.pdf). This procedure was not applied to the second sampling, since only 2 samples were taken. Here, just start and end measurements were used to calculate FRs. Then, FR was calculated as: $FR = V \times [(\ln C_0 - \ln C_1)/t_1 - t_0)]$, where V (ml) is the volume of the experimental unit, $\ln C_0$ is natural logarithm of the cell concentration in the first measurement and $\ln C_1$ is that of the last measurement, t_1 is the time passed between the feeding and the last measurement (min), and t_0 is the time passed between the feeding and the initial measurement (min). The equation $F = 0.0024L^{2.01}$, from Pleissner et al. (2013), where F = filtration rate, was applied to predict FRs (l h^{-1}) based on mussel shell length (mm).

2.6. Statistical analyses

Statistical analyses were performed with R v.4.0.2 and RStudio v.1.3.1073 (2009–2020 RStudio, PBC). Generalized linear models (GLMs) assuming a Gaussian residual distribution were fitted using the function 'glm' to test the main and interactive effects of salinity and infection status on mussel FR (at the start and end of the experiment). Mussel FR was used as the response variable, and salinity and infection status were used as fixed-effect predictors. Infection status was applied as a categorical predictor (factor), and salinity was used as a numerical predictor. A second-degree polynomial term was applied to consider the possibility of nonlinear effects of salinity on mussel FR. Goodness-of-fit of the model was verified by calculating R^2 through the function 'performance' (R package MuMIn v.1.46.0; Barton 2020).

Generalised linear mixed models (GLMMs; package 'lme4' v.1.1-29; Bates et al. 2015) assuming a Gaussian residual distribution were fitted using the function 'lmer' to test for the main and interactive effects of salinity and infection status on mussel size and CI. Mussel size (i.e. length, dry weight) was used as a response variable and salinity and infection status were used as fixed-effect predictors in each model. A second-degree polynomial term was also used in the model. Each unit containing 4 mussels was considered as a random-intercept factor (block). Goodness-of-fit of the model was verified by calculating the R^2 through the function 'performance' (package MuMIn). GLMs were also applied to test for the relation of metacercariae intensity (= number of metacercariae in the infected mussel) with mussel length, dry weight, or FR (start and end of the experiment). Length and dry weight of each mussel were used as response variables and the number of metacercariae in each mussel as the fixed-effect predictor. For the GLMs on FRs, the FR (start and end of the experiment) was used as the response variable, and the number of metacercariae of the 4 mussels located in each cylinder were averaged and used as

the fixed-effect predictor. Since little to no significant effects were found, we included these results in the Supplement (Figs. S2 & S3, Tables S1–S4). Residuals plots of all models were visually inspected for homogeneity of variances (residuals vs. predicted plot) and deviations from normality (*Q*–*Q* plot) using the 'DHARMa' package (Hartig 2020).

To verify an effective change in dry weight from the beginning to the end of the experiment, the mean dry weight of 20 freshly collected mussels (length interval of 18–21.5 mm) was compared to the mean final dry weight of the experimental mussel individuals by visually inspecting the differences through boxplots (Fig. S4).

3. RESULTS

Following the 4 infection events, with around 200 cercariae inoculated to each mussel individual, the average (±SD) number of *Himasthla elongata* metacercariae found in each mussel tissue was 23 ± 14.08 (See Fig. S5 for *H. elongata* distribution frequency). On the contrary, infection by *Renicola roscovita* was rather unsuccessful, with only 5 of 72 experimental mussels infected and all with extremely low metacercariae intensity (average metacercariae abundance in 72 infected mussels: 0.09 ± 0.38).

3.1. Shell length, tissue dry weight, and CI

The GLMM analysis revealed a significant positive correlation between mussel final shell length and salinity (GLMM, $p < 0.001$) but no significant correlation with infection status (Table S5), with the highest mussel length reached at 24 psu (Fig. 2a). The variance explained by the GLMM including the interactive effects of salinity and infection status was 47%.

The GLMM on tissue dry weight revealed a significant negative correlation with mussel infection status (GLMM, $p = 0.004$; Fig. 2b) but no significant correlation with salinity (Table S6). The variance explained by the model, however, was only 8%. When comparing the initial dry weight of 20 mussels to the final dry weight of the experimental mussels, a trend of uninfected mussels growing more than infected mussels was revealed. The infected mussel tissue increased only minimally in tissue biomass from the beginning to the end of the experiment (Fig. S4).

The analysis of CI showed a significant interaction between salinity and infection status (GLMM, $p = 0.009$; Table S7, Fig. 2c), with CI decreasing in infected mussels at low salinities, as well as an additive negative effect of salinity (GLMM, $p < 0.001$; Table S7, Fig. 2c) and infection status (GLMM, $p = 0.003$; Table S7, Fig. 2c). The variance explained by the GLMM including the interactive effects of salinity and infection status was 46%.

3.2. Filtration rate

The statistical analysis revealed a significant positive correlation between FR and salinity, especially in the end measurement (GLM, start measurement: 2nd degree polynomial: $p = 0.021$; last measurement: 1st degree polynomial: $p = 0.006$; Tables S8 & S9, Fig. 3). In the start measurement, FR reached a maximum at 18 psu, while at the end measurement, maximum FRs were achieved at a range of salinities from 18–24 psu. Infection status did not affect the FR in either measurement. Variances explained by the GLM were 72 and 97% for the start and end measurements, respectively.

4. DISCUSSION

As shown by previous studies, the impacts of abiotic factors on aquatic organisms may be more pronounced when combined with biotic factors, such as parasitism. For instance, Shameena et al. (2021) observed increased mortality of the fish host *Carassius auratus* exposed to parasitic co-infection and elevated temperature. Reisser & Forward (1991) found no significant effect of parasite and salinity on the osmoregulation and mortality of the crab host. At the community level, Friesen et al. (2021) detected a decline in amphipod population abundance when exposed to parasitic infection and elevated water temperature, whereas the isopod population was not affected. Few studies have focused on the effects of trematode infections in non-optimal environmental conditions on bivalve hosts and their function. Among them, Nikolaev et al. (2006) found that mussels infected by *Himasthla elongata* and *Cercaria parvicaudata* (possibly a synonym of *Renicola roscovita*; see Stunkard & Shaw 1931, Werding 1969) were more sensitive to cold winter conditions compared to non-infected individuals. Several other studies have found negative effects of trematode infection on bivalve growth and CI as well as filtration and heart rates (de Montaudouin et al. 2012, Stier et al. 2015, Bakhmet et al. 2017). To our knowledge, the present study is the first to investigate the combined effects of

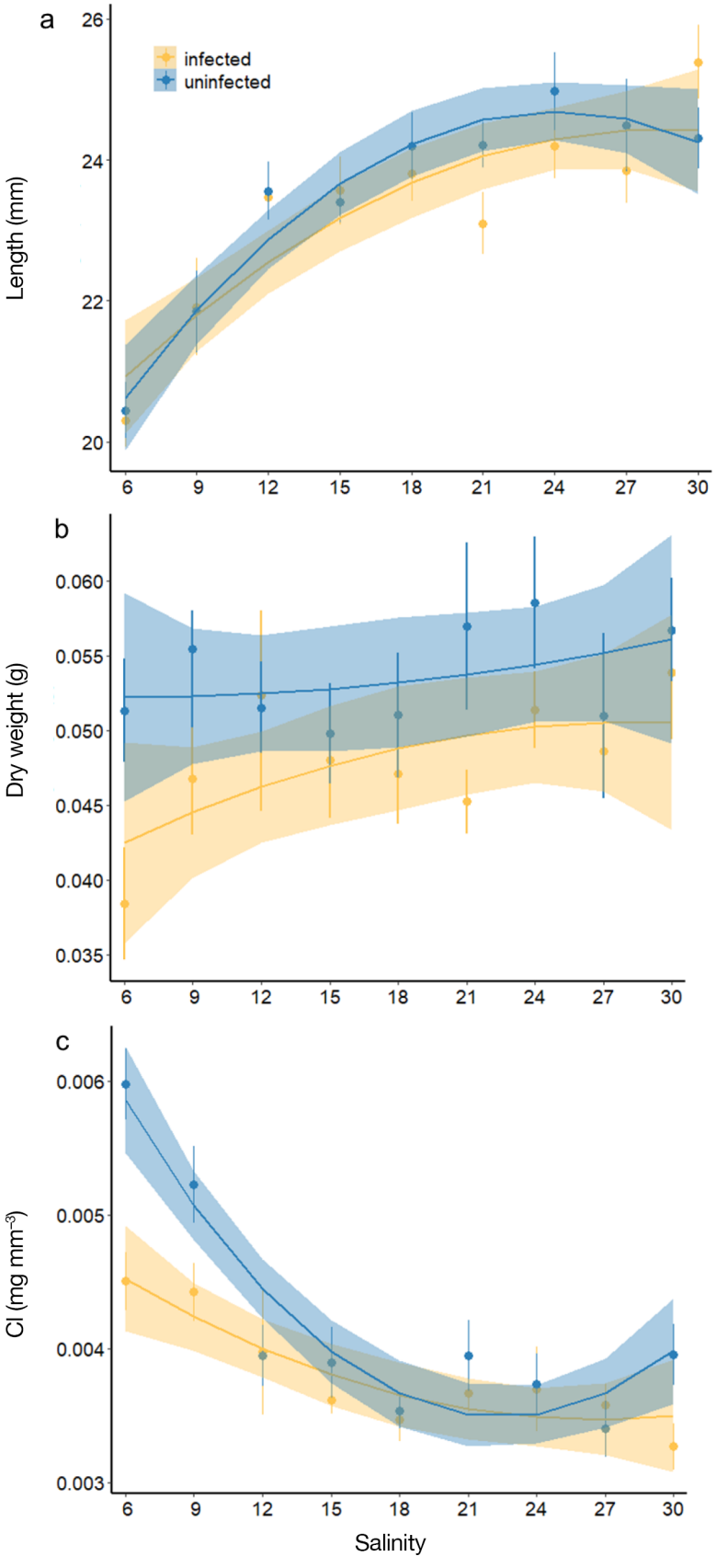

Fig. 2. Modelled (a) final length, (b) final dry weight and (c) condition index (CI) of infected and uninfected mussels at the different salinity levels applied (from 6–30 psu, with an interval of 3 psu between each salinity level). Error bars: SE; dots: mean of the measures of 4 mussel individuals in each experimental unit (cylinder)

salinity and trematode infection on a benthic filter feeder species. Our most novel finding is that *H. elongata* reduced the CI of blue mussels, i.e. soft-tissue mass with respect to shell length, especially at low salinities (<10 psu) representative of the central part of the Baltic Sea (Baltic Proper). Furthermore, we found that salinity strongly affected mussel filtration and shell length growth rates. Besides these main findings, our results revealed species-dependent infection success when exposing mussels to known doses of cercariae: while *H. elongata* successfully infected all experimental individuals, this was not the case for *R. roscovita*.

4.1. Mussel growth and condition in response to salinity and *H. elongata* infection

Regarding effects on mussel condition, our results showed an interaction of trematode infection and salinity, suggesting that the effect of parasites on soft tissues becomes more pronounced at low, potentially stressful, salinities. These findings are corroborated by those on the dry tissue weight, showing a negative effect of *H. elongata* infection. Trematode infections are known to cause loss of host exudates and increased hemocyte production by their penetrations into the second intermediate host tissue (Lauckner 1983, Wegeberg & Jensen 2003, Stier et al. 2015). This tissue damage could be even more profound in the case of *H. elongata*, which is characterised by large-sized cercariae compared to other species such as *H. interrupta*, which does not negatively affect the growth of juvenile cockles (Lauckner 1983, Wegeberg & Jensen 2003). These adverse effects in-

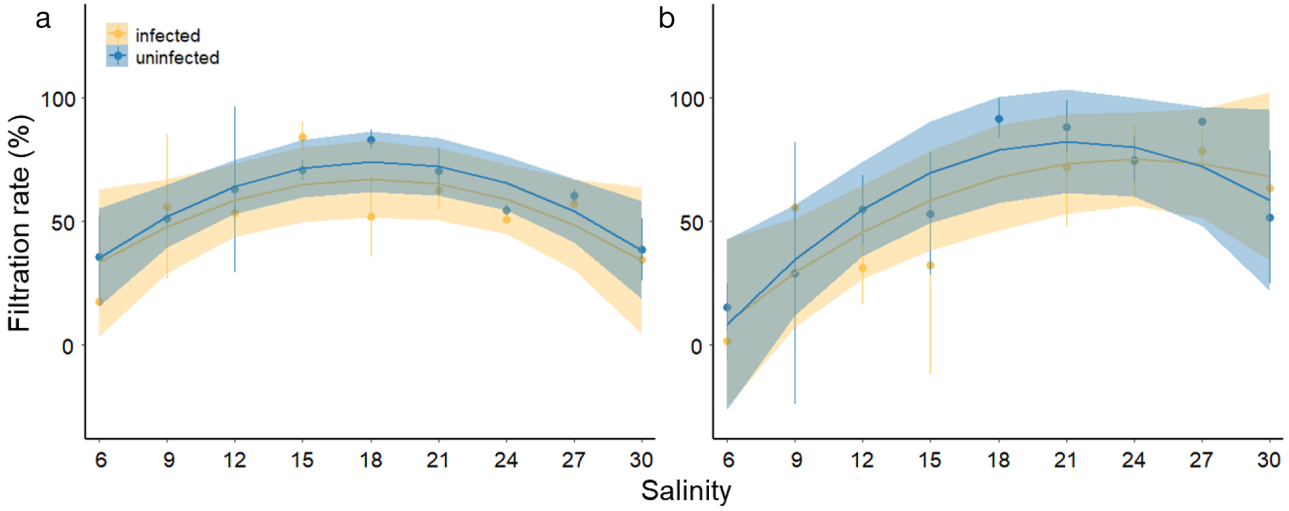

Fig. 3. Modelled filtration rate of infected and uninfected mussels at different salinities applied (from 6–30 psu, with an interval of 3 psu between each salinity level) at the (a) start and (b) end of the experiment, based on the equation from Pleissner et al. (2013). Error bars: ±SE; dots: mean of the measures taken in 4 mussel individuals in each experimental unit (cylinder)

duced by tissue damage may reduce the tolerance of host organisms exposed to stressful environmental conditions such as salinity fluctuations (Pascoe & Woodworth 1980, working on sticklebacks), which possibly explains the more substantial effect of *H. elongata* infections on mussel condition at low salinities.

As opposed to soft tissue biomass, our results did not show significant infection effects on mussel shell length growth. This finding is in line with Wegeberg & Jensen (2003), who did not find any significant impact of *H. interrupta* infections on cockle shell growth (in contrast to strong effects of tidal level). A significant negative correlation between *R. roscovita* infection and mussel shell growth was reported by Thieltges (2006). However, also in this case, the effect of tidal level was much stronger, with mussels from lower tidal levels growing more, presumably due to increased food uptake during longer immersion periods (Thieltges 2006). These results indicate a possible major role of abiotic drivers such as tidal exposure or other drivers such as food supply on bivalve shell growth, which might be further altered by parasitism (Wegeberg & Jensen 1999, Thieltges 2006). The magnitude of the effect of parasitism in second intermediate hosts is determined by the metacercariae intensity inside the host tissue (density-dependent effect; see Fredensborg et al. 2004, Thieltges 2006) The intensity of metacercariae in our experiment was approximately 25 per mussel individual, which is in the same order of magnitude compared to other studies investigating the effects of *Himasthla* species on bivalve functioning (Wegeberg & Jensen 2003, Bakhmet et al. 2017) and reflects natural infection levels in mussels (Galaktionov et al. 2015, Goedknegt et al. 2019, Bommarito et al. 2021) but might be not sufficient to provoke detectable effects on shell length growth.

Another possible reason why trematode infections had an impact on mussel condition but not on shell length growth could be attributed to the allocation of energy resources. The allocation of energy to building tissue or non-living structures (like shell or byssus threads) in mussels can differ depending on physical conditions that, in turn, are habitat-dependent (Elliott et al. 2008). Adverse factors like trematode infection might have negatively affected allocation to reproductive tissue rather than to shell growth. However, we can only speculate on these mechanisms, and further experiments investigating the different allocation of the energy budget in *Mytilus* sp. infected by trematodes will be required. Finally, it must be underlined that our experiment lasted 1 mo (plus 8 d for infection and acclimation); a longer-term experiment may have led to even more pronounced effects of *H. elongata* infections on condition and size. We are not aware of previous studies investigating the time-dependent effects of metacercariae on the host, and further experimentation could lead to informative results.

Not surprisingly, our results revealed a strong correlation between salinity and mussel shell length, indicating an increase in shell length with increasing salinity until an optimum of 18–24 psu followed by a decline at salinities above 24 psu, as also detected by several other authors (Bøhle 1972, Almada-Villela

1984, Kossak 2006). In the Baltic Sea, growth rates of the blue mussel decrease below a salinity of 10 psu, where maximum size is 35 mm (Sanders et al. 2018). Previous studies investigating *M. edulis* shell growth recorded lower calcification at low salinities (around 10 psu) (Kossak 2006, Riisgård et al. 2014), and the reason might be represented by a salinity-dependent change in the metabolism of amino acids and the excretion of nitrogen, which results in a less favourable energy balance at low salinities (Tedengren et al. 1990). However, the growth of soft body tissue displayed more stability (Kautsky et al. 1990, Riisgård et al. 2014). These observations suggest that low salinity impairs calcification rather than tissue growth. Another mechanism underlying reduced shell length at low salinity might be related to osmosis. Mussels are osmoconformers and are able to adjust organic osmolyte concentrations to iso-osmotic water rather than changing inorganic ions (Silva & Wright 1994). However, at salinities <8 psu, the ion concentration is reduced, suggesting a critical threshold below which ions like K^+ and Na^+ are being depleted to prevent an increase in cell volume (Podbielski et al. 2016). These potential intracellular changes in ions at low salinities might also cause reduced efficiency of biochemical processes involved in growth, as suggested by Sanders et al. (2018).

4.2. Mussel filtration in response to salinity and *H. elongata* infection

Our findings showed an optimal salinity for mussel filtration (hereafter, S_{Fopt}). At the start of the experiment, filtration at different salinities followed a pronounced bell-shaped curve, with S_{Fopt} at approximately 18 psu. The filtration–salinity curve changed at the end of the experiment, with a S_{Fopt} range of 18–24 psu, suggesting compensational acclimation to higher salinities (Tedengren et al. 1990). These findings are partially consistent with those of Riisgård et al. (2013). In their experiment, mussels exposed to salinity of 5 psu displayed lower filtration than those exposed to salinities of 10–30. When acute shifts in salinity (i.e. sudden freshening) occur, mussels rapidly close their valves and the filtration is then reduced to prevent osmotic stress (Riisgård et al. 2012). Unlike our experiment, in Riisgård et al. (2013), mussels at salinities of 10–30 psu displayed almost identical FRs. Both sampling sites, the Great Belt (Riisgård et al. 2014) and Kiel fjord (this study), experience a similar range of salinity fluctuations; however, the average salinity in the Great Belt is 20 psu (Riisgård et al. 2014), while average summer salinity in Kiel Fjord is 16 psu (Nour et al. 2021). This difference in average salinity might explain the humped-shaped curves of the FRs in our experimental mussels in the early phase of the experiment.

In contrast to salinity, we did not detect any effect of trematode metacercarial infections on mussel FRs. According to Stier et al. (2015), the only previous study examining this issue, infections with *R. roscovita* negatively impacted mussel FR. This discrepancy with our study is because *R. roscovita* metacercariae mainly infect palp and gill tissue (Lauckner 1983, Svärdh & Thulin 1985), which results in a direct impact on the FR, whereas *H. elongata* metacercariae encyst in the foot and mantle tissues of mussels, which are not involved in filtration. As reported above, in our experiment *R. roscovita* infection rates were very low and thus the observed effects were mainly caused by *H. elongata*.

4.3. Parasite-specific infection success

Our primary objective was to test for the main and interactive effects of salinity and trematode infection (in general) and salinity. Because co-infection with 2 species of trematode in mussels is a common occurrence in their natural habitats, our initial objective was to assess the responses of co-infected mussels. It would have been ideal, but logistically impossible due to the low number of first intermediate hosts, to test the effects of sole infection and co-infection for both parasite species simultaneously. Notwithstanding, we conducted a co-infection that nearly failed for *R. roscovita* and resulted in a sole infection by *H. elongata*, which was an advantage because it allowed us to explain the effect of *H. elongata*.

The species-dependent infection success during our controlled infections is notable. The number of *R. roscovita* cercariae administered to mussels during our infection events was lower than for *H. elongata;* however, infection success of almost zero was surprising. Similar patterns were observed in cockles, in which infection success of *H. elongata* was significantly higher than for *R. roscovita* (Magalhães et al. 2020). The mechanism underlying a higher infection success of *H. elongata* might be linked to the larger size of this species compared to other trematodes, with larger cercariae being more active swimmers as previously hypothesised by Daly & Johnson (2011). Another mechanism underlying different infection success might be the different swimming behaviours, as shown by Nikolaev et al. (2017). After emergence,

cercariae of *H. elongata* actively swim at the bottom, displaying a positive geotactic response which increases with their age. In *C. parvicaudata*, the cercariae are more concentrated in the upper layer. Only several hours after their emergence, the cercariae sink to the bottom. The different strategies of the 2 species might be influenced by their daily cercarial outputs: in *H. elongata* this output is around 3 times lower than in *Renicola* sp. (Prokofiev et al. 2016). Therefore, increasing the chance of meeting the second intermediate host immediately after emergence is advantageous. In contrast, in Renicolidae, the high number of cercariae released promotes the risk of hyper-infection. As an adaptation to avoid this risk, the cercariae swim in the upper layer, less inhabited by mussels. We located our experimental mussels at the bottom of each beaker; in turn, the swimming behaviour and infection strategy of *H. elongata* might have been more successful than that of *R. roscovita*. Further experiments, including different levels of infection (i.e. single and double infections with both species), would be needed to clarify the different infectivity success and the different impacts on blue mussel functionality.

4.4. Conclusions and perspectives

Our experiment showed that reduced salinity and *H. elongata* infections have negative impacts on mussels. Both impacted different mussel traits, with salinity directly affecting mussel filtration and shell length growth and trematode *H. elongata* infections negatively affecting mussel dry weight. The combination of reduced salinity and trematode infections led to interactive negative effects on mussel condition, with particularly strong effects of infections at low salinities. As multiple studies on global change have predicted a freshening of low salinity regions, including the Baltic Sea (Durack et al. 2012, Gröger et al. 2019), such freshening could be detrimental to mussel functioning. In addition to freshening, levels of parasitism may also increase as a consequence of climate change (Marcogliese 2001, Lafferty et al. 2004). In particular, cercarial emergence and infection success of trematodes in the Baltic Sea appear to be enhanced by an increase in water temperature (Díaz-Morales et al. 2022). However, this might not be the only possible scenario, since warming was found to be detrimental to the first intermediate gastropod host survival as well as the survival of cercariae and also because freshening reduces the infectivity of cercariae (Bommarito et al. 2020, Díaz-Morales et al. 2022). Longer-term laboratory experiments including multiple abiotic factors followed by field experiments in more natural conditions and including all intermediate hosts involved in the trematode life cycle would be promising approaches to better understand the consequences for both the mussel host and the parasites.

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