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Under the cover of ice: trematode infections affect survival and growth of wintering mussels

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Abstract

Parasites play many regulating roles in ecosystems that are increasingly recognized. In coastal ecosystems, the trematode *Himasthla elongata* infects blue mussels *Mytilus edulis*, a foundation species that shapes the functioning of intertidal communities. Although the largest impacts of infections are during the summer months, the parasite forms long-lived cysts in the mussels that can be harmful to their hosts under winter conditions. Here, we experimentally show that even moderate infection levels by *H. elongata* have a detrimental effect on the survival and growth rate of wintering blue mussels. These parasite-induced costs during winter may potentially affect the populations of blue mussels in coastal habitats, with ramifications for the whole ecosystem.

Keywords: Parasite, Bivalvia, *Mytilus*, *Himasthla*, Mortality, Growth, Field Experiment, Winter Ecology

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1. Introduction

Along the North Atlantic coastlines, blue mussels *Mytilus edulis* are a central ecosystem engineering and foundation species that shape inter- and subtidal communities (Melzner et al. 2020). In these habitats, *M. edulis* often form extensive mussel beds and biogenic reefs which filter out large amounts of nutrients and organic matter and serve as shelter and substrate for other organisms (Ragnarsson and Raffaelli 1999, Norling and Kautsky 2007). In return, the dynamics of these crucial ecosystem engineers are regulated and controlled by other taxa, such as competitors, predators, or parasites.

One of the most common parasites of *M. edulis* is the digenean trematode *Himasthla elongata*. Blue mussels and other bivalves, such as cockles, serve as intermediate hosts for the parasite and become infected by free-swimming larval transmission stages, the cercariae, that are released from the periwinkle snail *Littorina littorea* (Werding 1969). Inside the mussel, the cercariae encyst as metacercariae in the mantle and foot tissues, which can directly influence the mussels' growth rate, or affect their ability to produce byssal threads and attach to the substrate, rendering them more vulnerable to predation by shorebirds, the parasites final host (Lauckner 1983, Bech 2008, Bakhmet et al. 2017).

The parasites' transmission to their mollusc intermediate host predominantly occurs during the warmer months of the year, when the productivity of short-lived cercarial stages peaks (Nikolaev et al. 2020). However, once encysted inside the mussel, the metacercarial resting stages are robust and can survive for several years (Nikolaev et al. 2006). The main impact of *Himasthla* metacercariae on their intermediate hosts is observed during the summer months, with the most severe damage to host individuals reported shortly after the penetration of the cercariae (Jensen et al. 1999, Bakhmet et al. 2019). While low to moderate infection intensities (<100 metacercariae per host for infections in cockles *Cerastoderma edule*, de Montaudouin et

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al. 2009) appear to be relatively harmless and cause little damage to their cockle and mussel hosts (Wegeberg & Jensen 2003, Bakhmet et al. 2019), high infection levels (>100 metacercariae) with *Himasthla* metacercariae have been reported to cause high mortality in mussels and cockles during the winter months (Lauckner 1983, Nikolaev et al. 2006, 2020). However, it remains to be tested if and to what extent moderate infection intensities that are common in blue mussel populations along Atlantic shorelines can impact the population dynamics of these central ecosystem engineers during the harsh winter season, particularly in field experiments that take natural fluctuations in environmental conditions and food availability into account (Wegeberg & Jensen 2003).

Here, we experimentally investigate the impact of *Himasthla elongata* infections in *Mytilus edulis* on the bivalves' survival and growth rates during the wintering period. We expected that even low and moderate infection levels (<100 metacercariae) could have detrimental effects on the mussels' development and survival under stressful winter conditions. Such negative impacts of parasites on wintering mussels could play an important but often overlooked regulatory role in the functioning of blue mussel populations in the intertidal.

2. Material & Methods

2.1 Sample collection

Blue mussels *Mytilus edulis* were supplied by the Danish Shellfish Centre, Mors, Denmark. These mussels grow in sublittoral waters of the Danish Limfjord (56°53'29.2"N 9°09'58.0"E) where no *Littorina littorea* occur, ensuring that no mussels were infected with trematodes. To obtain the parasites for infection experiments, periwinkle snails *L. littorea* were collected at an intertidal zone near Knebel, Eastern Jutland, Denmark (56°12'32.2"N 10°28'47.2"E). Snails

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were screened for infections with *Himasthla elongata* by inducing cercarial shedding in warm sea water (25°C) under a light source, and infected snails were isolated and kept dry in a climate chamber at 16°C for the infection of treatment groups. In total, 350 infected snails were used in the experimental infection. All treatment preparations and experiments were carried out at the Marine Biological Station, Rønbjerg harbour, Limfjorden, Denmark (56°53'26.0"N 9°10'03.4"E).

2.2 Experimental infection of treatment group

Blue mussels were experimentally infected with freshly emitted *H. elongata* cercariae to obtain parasitized mussels for the treatment group (infected treatment). For this, 340 mussels (shell length 30-38mm) were established in a 80 l tank with running filtered sea water and air supply. Infected snails were placed in glass jars with filtered sea water at approximatively 25°C and placed under a light source to induce cercarial shedding. After 2h, the glass jars were examined for released cercariae, and free-swimming *H. elongata* were added to the tank containing mussels. At the same time, algae concentration (TETRASELMIS 3600, Instant Algae, Reed Mariculture, final concentration of 20 million cells per litre) was added to the aquarium to induce filtration activity in mussels and thus increase infection success of the parasites. To allow a gradual build-up of trematode infections, mussels were exposed five times to cercariae over the course of three days. After the last infection treatment, mussels were kept for 24h before deployment to allow for cercarial encystment. In parallel, another group of 340 mussels were prepared in the same way but without the addition of cercariae (control group). Pre-experimental dissections of mussels from the infected treatment revealed that individuals had acquired parasites (ranging from 17 to 44 metacercariae, mean ± SD = 28 ± 11, n = 8), while mussels in the control group were parasite-free (n = 6). The infection intensities reached here represent low to moderate parasite loads encountered in young mussels in Danish coastal

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93 regions (personal observation, Bech 2008), and are within the range of infection intensities
94 found in more northern *H. elongata* populations (Nikolaev et al. 2006).

95 **2.3 Experimental design: winter study**

96 For the overwinter experiment, mussels from each treatment were divided into mussel socks
97 (interwoven mesh tubes used in mussel mariculture, 1.2 m long, 10cm diameter), resulting in
98 four socks per treatment with 85 individual mussels in each sock. The socks were tagged and
99 deployed in Rønbjerg harbour from 18 October 2019 to 29 April 2020, i.e. during the cold half
100 of the year from late autumn to late spring. The socks were horizontally attached to a floating
101 jetty in the harbour next to naturally occurring blue mussel populations (ca. 30cm below the
102 surface) where no first intermediate snail hosts occurred. A temperature logger (HOBO Pendant
103 Data Logger) was attached to the socks to record water temperature. During the experimental
104 period, mussel socks were regularly inspected but not interfered with. In spring, the mussel
105 socks were recovered, and all mussels frozen until processing. In the laboratory, all intact (i.e.
106 surviving) mussels were counted, measured, and screened for parasites; empty shells or missing
107 mussels were recorded as dead individuals. To quantify the infection intensity with *H. elongata*,
108 the soft tissue of each mussel was firmly squeezed between two glass plates and present
109 metacercariae were counted under a stereomicroscope (ZEISS Stemi 2000c, Germany).
110 Maximum and minimum water temperatures recorded in the mussel socks throughout the
111 experiment ranged from 15.95°C in October to 1.98°C in January; by the end of the experiment
112 in April, temperatures reached up to 13.94°C.

113 Growth of mussels after the experiment was determined by measuring the distance (± 0.1mm)
114 along the longitudinal axis between (1) the growth interruption line formed as a consequence
115 of laboratory storage prior to deployment in October and (2) the full shell-length at the point of

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mussel recollection in April. In addition, the shell-length at the point of deployment was measured as the distance from umbo to the laboratory-induced interruption line.

2.4 Data analysis

Data was analysed using IBM SPSS Statistics (28.0). Difference in mortality between treatments (infected and uninfected) was tested using Fisher's exact test on mussels from all four socks per treatment combined, as n=4 provided insufficient statistical power for alternative approaches. Gompertz growth model (see Kaufman 1981) was applied to the growth data, and hence, Specific Growth Rate (SGR; ln(final length)-ln(initial length)) was plotted against ln(initial length) for each treatment separately, resulting in significant linear negative relationships ($p \leq 0.002$). A full-model ANCOVA was then applied to the growth data, entering SGR as dependent variable, treatment (infected/uninfected) as fixed factor and initial shell length (ln(initial length)) as covariate. The full-model analysis showed no interaction ($F_{1,261} = 0.716$, $p = 0.398$), and hence, only summary statistics from a reduced model incorporating the interaction-term in the error variance is presented.

3. Results

After the six-month experimental period, 101 of the infected and 164 of the uninfected mussels were recovered alive, meaning that infected mussels had experienced a significantly higher mortality rate (70.0%, n = 340) than uninfected conspecifics (51.8%, n = 340) (Fisher's Exact test, $p < 0.001$, Fig. 1). The SGR of surviving mussels showed an expected significant negative relationship with initial shell length regardless of infection status (Table 1, Fig. 2). However, the growth rates of infected mussels were on average significantly lower (18% based on grand means) than that of uninfected mussels (Table 1, Fig. 2). Prevalence (i.e., proportion of infected individuals) in the surviving mussels from the infection treatment was 100%. Mean infection intensity in the recovered mussels was 36 (± SD 21, n = 101) and ranged from 4 to 123

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metacercariae. No trematodes were found in the control treatment, showing that no natural infections occurred during the experimental time.

4. Discussion

Trematodes and other parasites and pathogens have been shown to cause or contribute to mass mortalities in bivalves, but the exact interplay between parasitism and host population dynamics often remains insufficiently understood (see Burdon et al. 2014 and references therein). The results of our in-situ experiment show that even low to moderate infection levels (<100 metacercariae per mussel) by *Himasthla elongata* can have detrimental effects on growth and survival of blue mussels *Mytilus edulis* during the wintering period. This contrasts with previous findings that suggest low infection intensities with *Himasthla* metacercaria to cause little damage to their bivalve intermediate hosts with regard to survival and growth during the summer (Wegeberg & Jensen 2003), or condition and heart rate (Bakhmet et al. 2019). Severe winters have been shown to lead to high mortality rates in cockles (*Cerastoderma edule*, see Thieltges 2006), and it is possible that the multiple stressors of mild parasite infections and harsh winter conditions can have synergistic harmful effects on blue mussel populations.

The detrimental impacts of parasites on wintering *M. edulis* could play an important but largely overlooked regulatory role for blue mussel populations and their crucial functioning in the intertidal zone. The reduction of growth and the increased mortality during the wintering period can be expected to have cascading impacts in the following active spring and summer seasons, when competition and predation pressure increases again. Smaller mussels are easier prey items for a range of predators, and the reduced growth rate will mean that infected mussels will be slower at reaching a sufficient size to protect them from predation (see Hamilton et al. 1999, Mascaró & Seed 2001). Thereby, the reduced growth rates likely enhance predation rates and the flow of energy in coastal systems. Moreover, the increased mortality rate in infected blue

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mussels will result in the removal of a significant number of bivalves and infective metacercariae from the intertidal zone each winter, highlighting the importance of trematode infections for the regulation of population dynamics of both parasites and their hosts. Interestingly, no apparent reduction in infection intensity was observed in the infected mussels before and after the experiment, suggesting that the impacts observed under the study conditions might not be intensity-related, as it usually is the case in second intermediate hosts (e.g., Desclaux et al. 2004, Fredensborg et al. 2004). Future studies should further explore this possibility, especially due to the low sample size of pre-experimental mussels in the present study. Furthermore, it remains to be tested if *Mytilus* populations at higher latitudes that are better adapted to the cold (e.g., Nikolaev et al. 2006, Galaktionov et al. 2015, Bakhmet et al. 2019) are more robust under these conditions or show similar reactions to our temperate model system.

Winter and under-ice conditions are increasingly recognized as ecologically important, productive and active periods that shape population dynamics and food web structures in aquatic environments (Vasseur et al. 2014, Sutton et al. 2021). The role of parasite infections under these conditions remains critically understudied, even though individual studies have highlighted the importance of cold seasons on fish-parasite interactions in aquatic environments. For instance, eye flukes *Diplostomum* spp. were shown to depress the metabolic rate and reduce the growth rate of their second intermediate host, Arctic charr *Salvelinus alpinus*, at low winter temperatures (Seppänen et al. 2009, Voutilainen et al., 2010). Contrastingly, cold water has also been shown to act as a recuperation period during which infected fish hosts can recover from trematode infections (Klemme et al. 2021). Moreover, in fish communities in subarctic lakes, the ice-covered winter period is an important transmission window for trophically transmitted parasites. Here, the seasonal dietary change of fish lead to a shift in the prevalence, intensity, and community composition of intestinal parasites (Prati et

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189 al. 2020). Our results indicate a similarly complex host-parasite interaction in the *Mytilus*-
190 trematode system under winter conditions that warrants further testing and investigation.

191 Climate change and global warming will affect the productivity, transmission, and infectivity
192 of trematodes in aquatic systems in a multitude of ways (e.g., Selbach & Poulin 2020, Díaz-
193 Morales et al. 2022). The resulting changes in parasite infection prevalence and intensity can
194 therefore be expected to have cascading effects during the wintertime when infections lead to
195 increased mortality and reduced growth. However, while extreme heat events are expected to
196 increase in frequency under current climate change predictions, cold events and ice-covered
197 winters will become rarer (Vasseur et al. 2014). It is therefore possible that milder winters could
198 offset the negative effects of increased parasite pressure in a warming world to some extent. To
199 better predict the complex outcomes of climatic shifts, we need to understand the regulating
200 effects of parasitism that take place under the cover of ice and shape bivalve populations.

201

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297 **Table 1**. Summary statistics of reduced model ANCOVA including the surviving mussels
298 (*Mytilus edulis*) specific growth rate (SGR) as dependent variable, treatment
299 (infected/uninfected) as fixed factor and shell-length (ln(initial length)) as covariate. Partial eta
300 squared gives the proportion of variance explained.

Source	df	F	P	Partial η^2
Treatment	1	34.273	<0.001	0.106
Shell-length	1	31.166	<0.001	0.102
Error	262			

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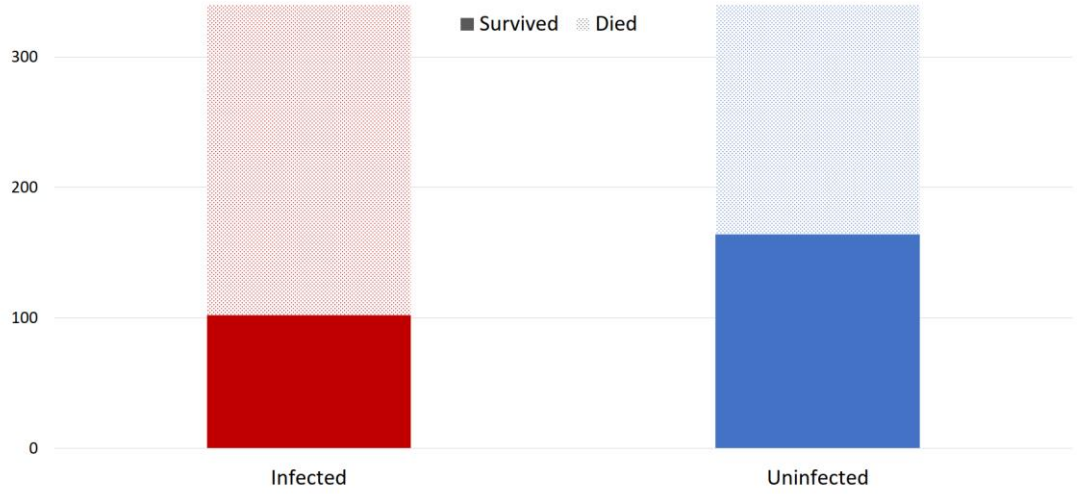
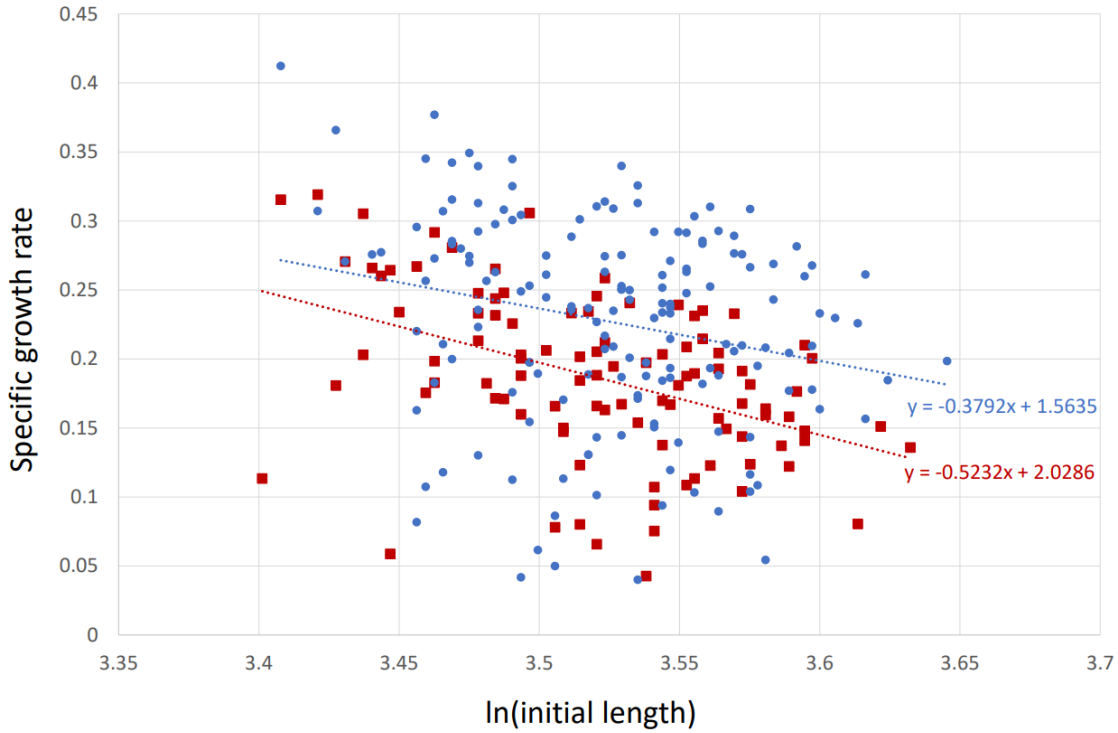


302

303 **Fig.1.** Number of surviving mussels at the end of the experiment in the infection (101 out of

304 340 mussels) and control (uninfected) treatments (164 out of 340 mussels).

305



306

307 **Fig.2.** Specific growth rates (SGR; ln(final length)-ln(initial length)) of surviving uninfected
308 (●, n=164) and infected (■, n=101) blue mussels (*Mytilus edulis*) as a function of ln(initial
309 length).

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