Received: 4 March 2022 | Accepted: 30 January 2023

DOI: 10.1111/1365-2656.13921

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Effect of predation risk on parasite transmission from first to second intermediate trematode hosts

Annika Cornelius[1] | Christian Buschbaum[1] | Maral Khosravi[2] | Andreas M. Waser[1] | K. Mathias Wegner[1] | David W. Thieltges[3,4]

[1]Alfred Wegener Institute for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstraße 43, D-25992, List, Germany

[2]Department of Marine Ecology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

[3]Department of Coastal Systems, NIOZ Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB, Den Burg, The Netherlands

[4]Groningen Institute for Evolutionary Life-Sciences, GELIFES, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, the Netherlands

Correspondence
Annika Cornelius
Email: annika.cornelius@awi.de

Funding information
Deutsche Bundesstiftung Umwelt, Grant/Award Number: 20018/530

Handling Editor: Isabella Cattadori

Abstract

1. Predators can affect parasite–host interactions when directly preying on hosts or their parasites. However, predators may also have non-consumptive indirect effects on parasite–host interactions when hosts adjust their behaviour or physiology in response to predator presence.
2. In this study, we examined how chemical cues from a predatory marine crab affect the transmission of a parasitic trematode from its first (periwinkle) to its second (mussel) intermediate host.
3. Laboratory experiments revealed that chemical cues from crabs lead to a threefold increase in the release of trematode cercariae from periwinkles as a result of increased periwinkle activity. This positive effect on transmission was contrasted by a 10-fold reduction in cercarial infection rates in the second intermediate host when we experimentally exposed mussels to cercariae and predator cues. The low infection rates were caused by a substantial reduction in mussel filtration activity in the presence of predator cues, preventing cercariae from entering the mussels. To assess the combined net effect of both processes, we conducted a transmission experiment between infected periwinkles and uninfected mussels. Infection levels of mussels in the treatments with crab cues were sevenfold lower than in mussels without crab chemical cues. This suggests that predation risk effects on mussel susceptibility can counteract the elevated parasite release from first intermediate hosts, with negative net effects on parasite transmission.
4. These experiments highlight that predation risk effects on parasite transmission can have opposing directions at different stages of the parasite's life cycle. Such complex non-consumptive predation risk effects on parasite transmission may constitute an important indirect mechanism affecting prevalence and distribution patterns of parasites in different hosts across their life cycle.

KEYWORDS
blue mussel, crab predator, parasite–host-system, partitioning predation risk effects, risk induced, trait-mediated indirect effects, trematode, Wadden Sea

1 | INTRODUCTION

Pathogens and parasites with complex life cycles have to transmit from one host to the next, and the success of the transmission process depends on various factors. For parasites with free-living infective stages, abiotic factors such as temperature are well-known to affect the development and release of infective propagules as well as the susceptibility of hosts (Pietrock & Marcogliese, 2003; Poulin, 2006; Studer et al., 2010). In addition, biotic factors such as predation can have strong effects on parasite transmission, for example when predators preferentially feed on infected hosts (Lopez & Duffy, 2021). This can lead to increased transmission in cases where the predator is a suitable host for trophically transmitted parasites (Lafferty, 1999; Poulin, 2010a). Nonhost predators in turn can lower transmission by reducing contact rates among hosts or reducing the number of hosts releasing infective stages, depending on the mode of transmission (healthy herd hypothesis; Duffy et al., 2005; Johnson et al., 2006; Lopez & Duffy, 2021; Packer et al., 2003). Similar effects can occur when predators reduce the density of hosts below host-density thresholds for epidemics, that is, the minimum host population density below which transmission between hosts ceases (Lafferty, 2004; Ostfeld & Holt, 2004). In some cases, nonhost predation can also lead to increased transmission, for example, when infective stages are released from hosts during consumption (Cáceres et al., 2009). Besides consumption of hosts, predators can also affect transmission from one host to another through the consumption of free-living infective stages of parasites, which reduces parasite exposure of the following hosts due to dilution (Johnson et al., 2010; Johnson & Thieltges, 2010; Thieltges, Bordalo, et al., 2008; Thieltges, Jensen, et al., 2008).

In addition to these direct consumptive impacts, it is becoming increasingly recognised that predators can also affect parasite transmission via non-consumptive effects on prey. These effects result from the ability of prey to respond to predation risk by modifying morphological or behavioural traits in ways that reduce predation risk (Freeman et al., 2009; Leonard et al., 1999; Lima & Dill, 1990; Peacor et al., 2020; Sherker et al., 2017). These risk-induced trait responses in prey organisms can alter the interaction of prey with other species and thus lead to trait-mediated indirect effects of predators on species interactions. Most existing studies focussed on the modification of interactions between prey and its resources or alternative predators (Peacor et al., 2020; Werner & Peacor, 2003). However, there is growing evidence that also parasite–host interactions can be indirectly affected by risk-induced trait responses in prey via altering (1) the exposure to infective stages or infected conspecifics, or (2) the susceptibility to infections when exposed to parasites. For example, tadpoles of *Rana sylvatica* exposed to predator clues reduce their activity to avoid predation, which in turn increases their exposure to free-living infective stages of trematodes, leading to elevated infection levels (Thiemann & Wassersug, 2000). In contrast, decreased infection levels in tadpoles of *Lithobates sylvatica* by the fungal pathogen *Batrachochytrium dendrobatidis* when exposed to predation clues are not related to differences in exposure but a reduced susceptibility due to stress-induced immunoenhancement (Groner & Relyea, 2015). Similar increases in immune responses to predator risk have been observed in the larval dragonfly *Leucorrhinia intacta* (Duong & McCauley, 2016).

These previous studies have revealed changes in parasite–host interactions as a result of trait responses to varying predation risk in down-stream hosts, that is, at the end of the transmission process. In contrast, little is known about predation risk effects in upstream hosts, that is, at the start of the transmission process, as well as about the combined net effect on transmission throughout the whole life cycle. For parasites with free-living infective stages, the production and release of infective propagules in upstream hosts is an important determinant of transmission success as transmission is usually dose dependent, that is, the higher the exposure to infective stages the higher the infection levels and associated disease risks in downstream hosts (Ebert et al., 2000; Liddell et al., 2017; Poulin, 2010b). Hence predation risk-induced trait responses in prey that affect the production and release of infective stages can potentially have strong effects on parasite transmission. In parasites with complex life cycles, hosts releasing infective stages and the ones becoming infected are often different species. Predation risk effects on transmission may thus differ between up- and downstream hosts, leading to potentially complex net effects of predation risk responses. Hence, identifying predation risk effects in hosts at both ends of the transmission process and determining their combined net effect on transmission will be essential to completely understand how predation risk affects parasite transmission.

In this study, we investigated the predation risk effects of the marine predatory crab *Hemigrapsus takanoi* on the transmission of cercariae of the trematode *Himasthla elongata* from its first intermediate gastropod host *Littorina littorea* to its second intermediate host, the blue mussel *Mytilus edulis* (birds, such as gulls serve as definitive hosts). Along the European Wadden Sea, the brush-clawed shore crab *H. takanoi* is one of the dominant predators on mixed reefs of blue mussels and Pacific oysters *Magallana gigas*, the primary habitat of periwinkles and mussels (Cornelius et al., 2021; Cornelius & Buschbaum, 2020). In general, cercariae released from their first intermediate host infect their second intermediate host within a short time window from a few hours to a couple of days (Morley, 2011). Infection success in the second intermediate host is generally dose dependent (Liddell et al., 2017; Poulin, 2010b) and the effects of infections on hosts are usually density dependent (Fredensborg et al., 2004; Thieltges, 2006) so that any difference in exposure will translate into alterations of the disease risk for the host. Using two separate experiments, we partitioned predation risk effects into exposure (via the release of infective stages from the first intermediate periwinkle host) and susceptibility (via uptake of infective stages in the second intermediate mussel hosts) components. In two additional experiments with both intermediate hosts, we identified the underlying trait changes in periwinkles and mussels. Finally, in a combined transmission experiment, we exposed communities of infected periwinkles and uninfected mussels to crab chemical cues in an effort to identify the net predation risk effect on parasite transmission.

2 | MATERIALS AND METHODS

2.1 | Collection of study organisms

Common periwinkles *Littorina littorea* were collected in October 2018 from an area with known high infection levels of *Himasthla elongata* at of the Danish coast of the Baltic Sea (Jütland, Arosund; 55°15′45.8″N 9°42′39.2″E) (Bommarito et al., 2021). In the laboratory, infected periwinkles were identified by exposing them to intense light at 25°C for several hours and subsequently screening the seawater for the presence of emerged cercariae (periwinkles were screened individually in 6-well plates filled with aerated seawater). After confirming infection status (infected/noninfected) during a second incubation, infected and uninfected periwinkles were individually marked with different colour markings (bee dot). Infected and uninfected periwinkles (shell height of 14–18 mm; corresponding to an age of 2 years (Buschbaum et al., 2007; Fretter & Graham, 1980; Smith & Newell, 1955)) were kept together in aquaria (23.0×47.5×26.0 cm) filled with filtrated seawater and fed every second day with sea lettuce *Ulva lactuca*, until the start of the experiments to guarantee identical conditions for both groups.

Blue mussels, *Mytilus edulis*, were sampled in November 2018 from the west coast of Sylt island (Wenningstedt beach; 54°56′27.4″N 8°18′56.2″E) where trematode infections do not occur naturally (confirmed by screening 50 mussels). Mussels (shell length 25–30 mm) were held in a flow-through tank (60.0×43.0×12.0 cm) and constantly fed with a mix of Instant Algae Iso 1800 TM and Shellfish Diet 1800 TM.

As a predator of both intermediate host species, Asian-brushed shore crabs *Hemigrapsus takanoi* were collected north of Sylt island (55°01′42.0″N 8°26′02.9″E), between November 2018 and March 2019 for each experiment. Crabs (carapace width 20–30 mm) were held separately in flow-through aquaria (23.0×47.5×26.0 cm) and fed every second day with blue mussel flesh. Only male crabs with intact claws and legs were used to obtain consistent crab risk cues and to avoid confounding by potential chemical fertility cues from females. Additionally, *H. takanoi* males prefer mussels in comparison to females (Cornelius et al., 2021).

2.2 | Experimental design

For all experiments, water conditioned with predation risk cues was produced by keeping 15 male *H. takanoi* in a new aquarium containing 1000 mL of filtered aerated seawater (0.45 µm + UV treatment) for 24 h. The water used for control treatments was processed the same way but without adding crabs. For each treatment, we used four aquaria to produce the conditioned water.

2.3 | Effects of predation risk on cercarial release and periwinkle activity

To quantify predation risk effects on trematode cercariae release, we placed two randomly selected infected *L. littorea* in plastic jars (10 cm diameter, 10 cm height). Per treatment, 24 jars were filled with 100 mL of treatment water (predation risk cues present or absent), and the jars were closed with a perforated lid (not touching the water surface and thus preventing escape of cercariae). Control and predator cue jars were randomly positioned in a tank (60.0×43.0×12.0 cm) with a constant temperature of 25°C and artificial daylight (SMD LED Flexbile Stripe, 14.4 W). After 6 h, the content of each jar was sieved (5 µm sieve) and the periwinkles were carefully removed and washed with filtered seawater over the sieve to retain all cercariae. The samples were fixed using 15 mL 96% ethanol and cercariae were counted using a stereomicroscope. All samples were processed within 15 min.

To assess predation risk effects on periwinkle mobility, we determined the capability of periwinkles to return to a crawling position after being turned upside down as an activity proxy. A random selection of infected and uninfected periwinkles was individually placed into glass containers (40 mm diameter) filled with 75 mL of water (25°C) with or without predator chemical cues, resulting in four treatment groups (infected/uninfected periwinkles × treated/untreated water) with 24 replicates each (96 snails in total). All periwinkles were turned upside down, and periwinkles which turned around within 2 h were counted.

2.4 | Effect of predation risk on mussel infection and filtration activity

To identify predation risk effects on infection rates in mussels, we exposed uninfected *M. edulis* to cercariae in both treatments (predator cue or control). To avoid genotype bias and obtain a genetically mixed pool of cercariae for the experiment, 75 infected periwinkles were incubated in a plastic jar (20 cm diameter) in 500 mL of aerated filtered seawater at 25°C and exposed to artificial light (SMD LED Flexbile Stripe, 14.4 W) for 3 h. Fifty cercariae from the mixed pool were added to each of the experimental units. All cercariae were not older than 4 h to avoid confounding effects of declining survival rates which decrease after about 10 h (Thieltges & Rick, 2006).

For the experiment, 20 plastic jars were placed in a tank (60.0×43.0×12.0 cm) filled with water kept at 25°C. Each jar contained two blue mussels (25–30 mm) and 100 mL of water with or without predation risk cues (i.e. 10 jars each). We used two blue mussels per replicate to compensate for possible differences in filtration rate between the individual mussels, which can affect the absorption of parasites and parasitic infection. After an acclimation phase of 30 min, 50 cercariae were added to the jars, which were then closed with a perforated lid (not touching the water surface and thus preventing escape of cercariae). After 6 h, all mussels were removed and put in filtrated seawater for another 48 h to allow full encystment of metacercariae. Afterwards, mussels were dissected, their soft tissue squeezed between two glass plates, and the number of metacercariae counted using a stereomicroscope.

In a separate experiment, we assessed predation risk effects on mussel filtration behaviour, using a fluorometer- and oximeter-equipped flow-through setup (FOFS; see Vajedsamiei et al., 2021 for details) to record the filtration activity of mussels in response to predator risk cues. The 600 L source tank of the original setup was modified to two tanks (each 130 L; 79.0 × 57.0 × 45.0 cm), allowing us to handle the two types of treatment waters (predation risk water, control water) under the same constant conditions. A pump attached to a 0.2 µm filter was added to holding tanks to eliminate possible suspended particles produced by crabs (i.e. faeces or pieces of the moulted exoskeleton). Twenty-four hours before the start of the experiment, both tanks were filled with seawater, and we added 75 male *H. takanoi* to one tank to produce water with predation cues. During the experimental trials, mussels were continuously fed with *Rhodomonas salina*.

For each run, three mussels (25–30 mm) were individually placed in one of the four experimental chambers ($n = 16$). The filtration activity was recorded in response to a 150 min no-stimulus phase (control water) followed by a 150 min stimulus phase (water with water predation risk cues). In each run, mussel-induced changes in the chlorophyll concentrations were determined as the difference between measurements taken from the mussel-filled and the mussel-free flow-through path (see Vajedsamiei et al., 2021 for details).

In order to ensure that the observed filtration performance of the mussels was due to predator cues and not a decline in filtration rates over time, measurements were also done with control water over 300 min with three mussels. In addition, we investigated whether the observed filtration reduction also occurred over longer time periods by conducting an experiment with another three mussels exposed to predation risk cues over a period of 800 min.

2.5 | Combined transmission experiment with periwinkle and mussel hosts

To investigate the combined effects of predation risk effects on cercarial production in periwinkles and susceptibility of mussel hosts, we conducted an experiment using 36 cylindrical plastic jars (10 cm diameter, 10 cm height) filled with 100 mL of treatment water (25°C; $n = 18$ each for predation risk cues present and absent). The jars were randomly placed into a tank (60.0 × 43.0 × 12.0 cm) filled with water (9 cm high) kept at 25°C and exposed to artificial daylight (SMD LED Flexbile Stripe, 14.4 W). Two infected periwinkles (shell height 14–18 mm) and two uninfected mussels (25–30 mm) were added to each jar and the jars were closed with a perforated lid (not touching the water surface and thus preventing escape of cercariae). After 6 h, the mussels were removed and left in seawater of the same temperature for another 48 h to allow full encystment of metacercariae. Following this, the tissues of each mussel were squeezed between two large glass slides and encysted metacercariae counted under a dissection microscope.

2.6 | Statistical analysis

Statistical analyses for cercarial production, behavioural assessment and susceptibility of blue mussels were conducted in the statistical software R (version 4.2.0, R Core Team, 2017). The predefined functions 'MASS' (Venables & Ripley, 2002), 'CAR' (Fox & Weisberg, 2019) and 'GGPLOT2' (Wickham, 2009) packages were used to perform the analyses and visualise the results. Data are given as arithmetic means with standard error (SE). Effects were considered to be statistically significant if the p-value was <0.05.

To analyse different numbers of emerged cercariae of *H. elongata* present in both treatments (control water, water with predation risk cues), data were analysed by fitting a generalised linear model (GLM; Poisson distribution) with 'cercariae number' as response variable and 'predation treatment' as predictor variable. We estimated model fit by examining residual versus predicted and QQ plots. The effect of infection status and predation risk on the activity (turned vs. not turned) of periwinkles was tested using a generalised linear model (GLM; binomial distribution) by fitting infection status and predation risk and their interaction. To analyse the predation risk effects on blue mussels, the number of successful infections versus the number of unsuccessful infections with cercariae of blue mussels for each treatment (control water, water with predation risk cues), was analysed by fitting a GLM(binomial distribution). In the transmission experiment, differences in metacercarial loads of mussels between the two treatments (control water, water with predation risk cues) were analysed by fitting a GLM(Poisson distribution).

The data from the fluorometer- and oximeter-equipped flow-through experiment were processed using Python (Python Software Foundation) based on the scripts and the protocol described in Vajedsamiei et al. (2021). To analyse differences for filtration rates between the experimental treatments (control water, water with predation risk cues) the R packages 'MGCV', 'VISREG' and 'ITSADUG' (Wood, 2017) were used to analyse the data with a GLM(Gaussian distribution).

3 | RESULTS

3.1 | Effect of predation risk on cercarial release and periwinkle activity

The mean number of cercariae released from periwinkles over 6 h was higher in treatments with predation risk cues than in control treatments (GLM analysis of deviance: $\chi^2 = 388.96$, df = 1, $p < 0.001$; Figure 1a). Periwinkles exposed to predation risk water released almost three times higher cercariae numbers of *H. elongata* (58.00 ± 5.90 total no. of cercariae emerged from two periwinkles over 6 h) than periwinkles in the control water treatment (22.50 ± 3.00).

Periwinkles exposed to water with predation risk cues showed a higher turning rate than periwinkles exposed to control water, both in infected and noninfected periwinkles (GLM analysis of deviance: $\chi^2 = 9.95$, df = 1 $p = 0.001$; Figure 1b). In general, turning rates of infected periwinkles were higher than the ones of noninfected

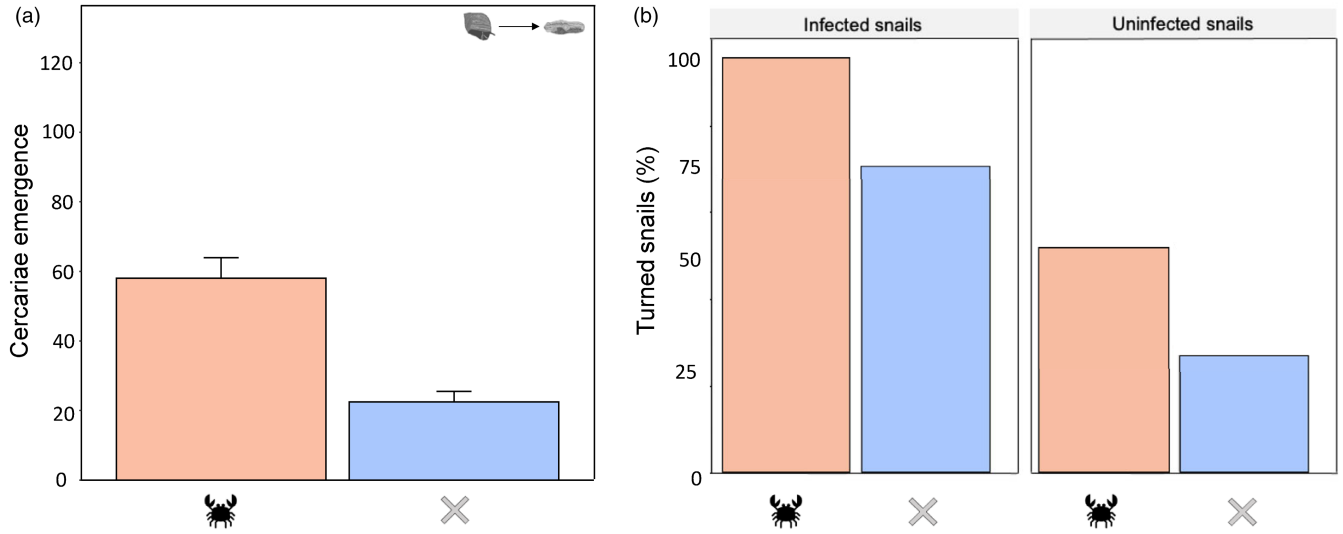

FIGURE 1 (a) Mean emergence (± SE) of cercariae of *Himasthla elongata* released from the first intermediate periwinkle hosts (two snails per replicate) in treatments with water including predation risk cues from crabs or control water; $n = 12$ replicates per treatment. (b) Percentage of periwinkles that turned back into upright position after being placed upside down in four different treatments: (i) infected snails exposed to water with crab predation risk cues, (ii) infected snails exposed to control water, (iii) uninfected snails exposed to water with crab predation risk cues and (iv) uninfected snails exposed to control water ($n = 24$ replicates per treatment). Infected snails were infected with the trematode *Himasthla elongata*.

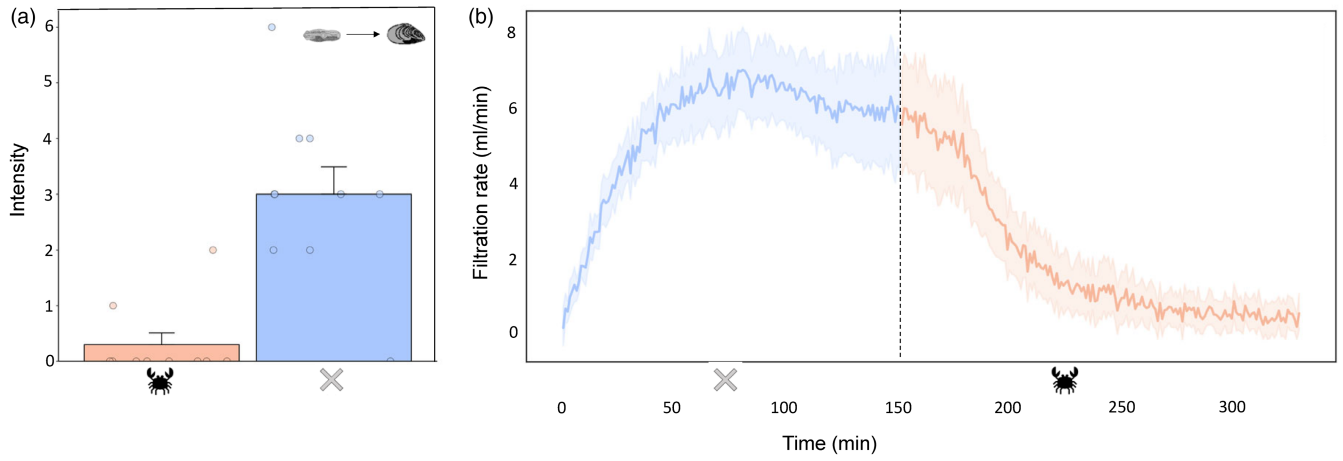

FIGURE 2 (a) Mean intensity (± SE) of metacercariae (in two mussels) after exposure of uninfected mussels to 50 cercariae of *Himasthla elongata* in treatments with water including predation risk cues from crabs or in control water; $n = 18$ replicates per treatment). (b) Mean filtration rate (ml/min) of mussels ($n = 16$) over time when kept in control water (blue) and after adding of water including predation risk cues from crabs (red).

periwinkles (GLM analysis of deviance: $\chi^2 = 23.08$, df = 1, $p < 0.001$; Figure 1b), with a slight interaction with predation risk cues ($GLM_{treatment \times infection\ status}$ analysis of deviance: $\chi^2 = 4.08$, df = 1, $p = 0.043$).

3.2 | Effect of predation risk on mussel infection and filtration activity

The mean number of metacercariae infecting the two mussels in each experimental unit in experimental infections was about 10 times lower in mussels exposed to predation cues (0.30 ± 0.21) compared to mussels kept in control water (3.00 ± 0.49; GLM analysis of deviance: $\chi^2 = 26.396$, df = 1, $p < 0.001$; Figure 2a).

Mussels exposed to water with predation risk cues reduced their filtration activity by 49% under predation risk cue period compared to the control cues (GLM analysis of deviance: $\chi^2 = 2317.2$, df = 1, $p < 0.001$; Figure 2b).

The control experiments supported that the observed reduction in filtration performance of the mussels was due to predator clues and not a decline in filtration rates over time as mussels exposed to control water only showed constantly high filtration activity over

the 300 min experimental period (Figure S1b). In addition, mussels exposed to predation risk cues continued to show a reduced filtration activity also over a longer period of 800 min (Figure S1c).

3.3 | Combined transmission experiment with periwinkle and mussel hosts

Realised transmission from periwinkle to mussel host measured as metacercarial infections was lower in the treatment group with predator risk cues compared to the ones without (GLM analysis of deviance: $\chi^2 = 605.02$, df = 1, $p < 0.001$; Figure 3). Mussels exposed to predation risk cues had a seven times lower infection intensity (8.55 ± 2.39 total number of metacercariae in two mussels) than mussels kept in experimental units with control water (63.11 ± 13.75).

4 | DISCUSSION

Predation risk cues can influence parasite transmission in different ways. Our experiments suggest that the effects of predation risk on parasite transmission must be considered at each step of the transmission chain to understand the full impact on parasite transmission. While the presence of predator risk cues lead to an almost threefold increase in cercarial release in the first intermediate periwinkle hosts, and thus a higher exposure of downstream mussel hosts to infective stages, susceptibility of mussels to cercarial infections was 10 times lower when predator cues were present. The latter effect was dominated in the combined transmission experiment (where infected periwinkles were housed together with uninfected mussels)

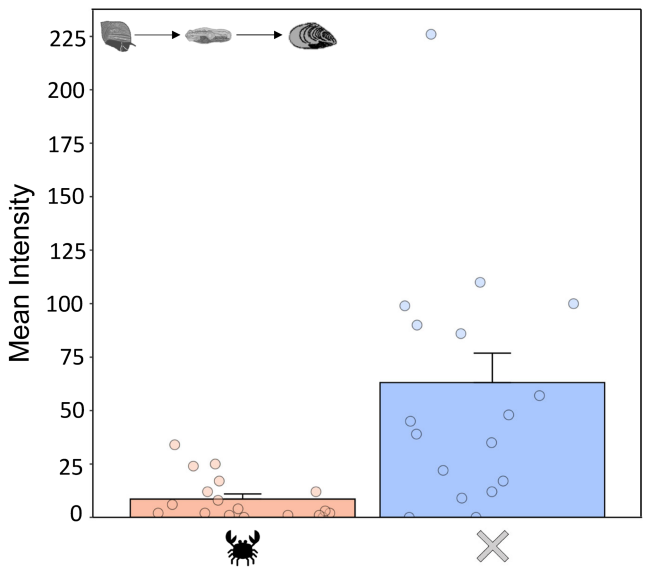

FIGURE 3 Mean intensity (± SE) of metacercariae of *Himasthla elongata* in two mussels kept together with infected snails in mesocosms exposed to treatments with water including predation risk cues from crabs or control water; $n = 10$ per treatment.

as suggested by the sevenfold decrease in infection levels of mussels exposed to predation risk cues.

The observed differences in direction of predation risk effects between the first and second intermediate hosts likely resulted from different predator-induced modifications of host behavioural traits, that then indirectly affected parasite transmission. In the case of the first intermediate periwinkle hosts, the observed increase in cercarial production at exposure to predation risk cues may have been related to behavioural anti-predator responses of the periwinkles. In general, gastropods are known to show multiple behavioural responses after detection of chemical cues that indicate a predation threat (Behrens Yamada et al., 1998; Ojima & Wada, 2013). The species used in our experiments (*L. littorea*) shows active escape behaviour and increased activity when exposed to predator cues (Jacobsen & Stabell, 1999). Although turning rates of periwinkles after flipping over are only a very limited proxy for overall periwinkle activity, the significantly higher turning rates under predation risk cues observed in our experiments represent a conservative estimator of a stimulus response that is compatible with increased activity. In any case, higher turning rates were observed in infected as well as in uninfected snails, suggesting that higher turning activity is generally induced by a perceived risk of predation. Should the periwinkles in our experiment indeed have been more active when exposed to predation risk cues, this could have led to an elevated cercarial release as a higher activity of gastropods is known to lead to increased cercarial shedding, likely due to an increase in metabolic rates of the hosts (Anderson et al., 1976; Mouritsen, 2002). While the exact mechanism remains to be confirmed for our system, it is clear from our experiments that exposure to predation risk cues can lead to a higher release of infective stages from the periwinkle hosts. This in turn can be expected to result in a higher level of exposure for the mussel second intermediate hosts due to the general dose dependency of metacercarial infections in second intermediate hosts (Liddell et al., 2017; Poulin, 2010b). Therefore, any predator-induced changes in the production and release of infective stages from first intermediate hosts would be expected to affect the exposure and associated disease risk for second intermediate hosts.

Like gastropods, bivalves such as mussels can also detect and react to predation risk cues released by predators, including short-term changes in their behaviour (Eschweiler & Christensen, 2011; Freeman & Byers, 2006; Irlandi & Peterson, 1991; Smee & Weissburg, 2006b). A common response is a closure of the valves and a subsequent reduction in filtration activity in the presence of predators. For example, hard clams *Mercenaria mercenaria* reduce filtration activity and feeding as a reaction to chemical cues from blue crabs, *Callinectes sapidus* (Smee & Weissburg, 2006a) and blue mussels decrease their filtration rate in the presence of starfish, *Asterias rubens* (Kulakovskii & Lezin, 2002). Also, the clearance rate of zebra mussels *Dreissena polymorpha* is reduced by predation risk cues (Naddafi et al., 2007). Blue mussels in our experiments also showed anti-predator behaviour in response to crab predation cues by closing their valves and strongly reducing filtration activity. This affected the uptake of cercarial infective stages from the water and

lowered the infection rates of mussels. As mussels also reduced filtration activity for periods of more than 10 h, the effects of predation risk on host susceptibility seem to be relatively long-lasting, and could thus present a significant limiting factor for parasite transmission dynamics as cercarial stages are usually relatively short-lived (infective period <12 h, Evans, 1985; Lowenberger & Rau, 1994; McCarthy, 1999). As a consequence, the temporal and spatial presence of predator cues may influence the distribution and infection patterns of trematodes in blue mussel beds. In addition, potentially confounding chemical fertility cues from females (not investigated in our study as we focussed on male crabs) might further contribute to spatiotemporal variation in crab effects.

In general, any predator-induced reductions in infection levels should be of benefit for second intermediate hosts of trematodes as the fitness effects of metacercarial infections are usually density dependent (Fredensborg et al., 2004; Stier et al., 2015; Thieltges, 2006). The presence of predators may thus indirectly lower the known detrimental effects of trematode infections on invertebrate intermediate hosts such as reducing survival, growth and condition (Fredensborg et al., 2004; Jensen et al., 1998; Thieltges, 2006). However, in the case of mussels, the reduced filtration activity may reduce the negative effects of infections but it will at the same time also impair the mussels' fitness because reduced filtration activity will also result in a lower energy uptake (Gosling, 2003). How this trade-off will affect overall mussel fitness is likely to depend on local infection and predation pressures as well as growth conditions, but this remains to be studied. Likewise, it remains to be studied whether the predation risk-induced elevated cercarial shedding from the first intermediate snail hosts has fitness consequences for the snails. For example, increased shedding is probably energetically demanding and may lead to reduced fitness of infected snails that are exposed to predator cues. This in turn may lead to lower cercarial production by infected snails in the long run which could affect local transmission dynamics.

The four separate experiments indicate that predation risk effects on parasite transmission can have opposite signs for the first and the second intermediate host of the transmission chain. The combined transmission experiment with both host species showed that predation risk effects on the second intermediate host counteracted the effects of increased parasite exposure through increased cercarial release on the first intermediate host, leading to an overall reduced parasite transmission. However, the predation risk-induced reduction in mussel infection levels was only sevenfold and not 10-fold, suggesting that elevated cercarial production in the presence of predators partly compensated the effect of the reduced filtration activity. The net effect of the two diverging predation risk effects on the first and second intermediate host side is likely to be context dependent. Higher numbers of infected snails or weaker predator cues in the water may have changed the net outcome and lead to higher infection levels in mussels. In addition, in natural settings, periwinkles and mussels will encounter many different types, sizes and sexes of predators as well as different predator densities and this is likely to result in complex effects on host behavioural traits which in turn can indirectly affect parasite transmission. In addition, state-dependent factors such as mussel feeding status and general food availability could also play a mediating role in these interactions. In our experiment, mussels were constantly fed with optimal levels of algae and other levels of food supply may alter anti-predator responses, something which should be explored in future studies.

In other aquatic systems, similar predation risk effects on parasite transmission may exist but the responses of other types of intermediate hosts to predator cues and their effects on parasite transmission may be very different from the ones observed in our system. For example, tadpoles of *Rana sylvatica* reduce their activity in response to predator cues which in turn increases their susceptibility to cercarial stages possibly through extended proximity of tadpoles to cercaria (Thiemann & Wassersug, 2000). In contrast, an elevated activity when exposed to predator cues seems to be a general response in gastropods (Behrens Yamada et al., 1998; Behrens Yamada & Boulding, 1998; Jacobsen & Stabell, 1999; Ojima & Wada, 2013). Given the fact that snail activity and cercarial release are positively correlated (Anderson et al., 1976; Mouritsen, 2002), it seems likely that predator presence may often lead to elevated cercarial shedding and thus an increase in exposure for the following hosts in the transmission process. Such predator-induced effects on the production and release of infective stages in hosts responding to predator clues are likely not limited to trematodes. A large diversity of behavioural and other trait changes in prey have been identified in many different taxonomic groups (Lima & Dill, 1990; Trussell et al., 2003; Werner & Peacor, 2001, 2003) and it seems likely that many of these trait changes could also affect the production and release of infective stages. More research on a range of parasites and hosts is needed to identify the presence and magnitude of predation risk effects on parasite propagule production. Likewise, more research is warranted on predation risk effects on the susceptibility of hosts. For example, immune responses to predator risk clues leading to reduced susceptibility have been observed in a few cases (Duong & McCauley, 2016; Groner & Relyea, 2015) but it is likely that those play a role in other systems as well.

In addition to predation risk effects on parasite transmission via behavioural or physiological trait changes in their hosts, predators may also indirectly affect transmission via the parasites themselves. Various free-living infective stages of parasites are well known to perceive chemical cues, in particular related to host finding and seeking (Chaisson & Hallem, 2012; Haas, 2003). It could be possible that certain chemical clues released by predators affect the host seeking behaviour and ultimately the infection success of infective stages by masking or decoy effects. However, to the best of our knowledge, we are not aware of any study that has investigated such potential transmission interference effects of predation risk cues. Much better understood, in contrast, are the indirect effects that parasites can induce on their hosts via infection risk-induced trait changes of their hosts (Buck et al., 2018; Daversa et al., 2021; Koprivnikar et al., 2021). Similar to prey that respond to predation risk cues with trait changes that reduce predation risk, hosts can change traits in the presence of infection risk cues by infective parasite stages that reduce infection risk. This also applies to mussels (Mouritsen et al., 2022) and can in turn can lead to various non-consumptive effects of parasites, similar

to the ones known from predator–prey interactions (Koprivnikar et al., 2021). In some cases, predation and infection risk-induced trait responses may lead to opposing effects on the realised predation and infection risks. For example, as already mentioned above, tadpoles that reduce their activity in response to predation risk cues lead to increases in infection risk by cercariae (Thiemann & Wassersug, 2000), which leads to complex outcomes in the presence of both predators and parasites (Rohr et al., 2015). Hence, disentangling the relative importance of parasite- and predator-induced non-consumptive effects on parasite transmission and prey/host fitness will be an interesting direction for future studies.

In conclusion, the experiments presented in this study highlight opposing predation risk effects on parasite transmission at different steps of the transmission process and they also point to complex, context-dependent net effects on parasite transmission. Such predation risk effect on parasite transmission constitutes a potentially important indirect mechanism of how predators can affect parasite transmission via non-consumptive effects next to their well-known direct consumptive effects on infective stages of parasites (Thieltges, Bordalo, et al., 2008; Thieltges, Jensen, et al., 2008; Johnson et al., 2010; Johnson & Thieltges, 2010). Future research should evaluate the relative importance of these direct and indirect effects of predators on parasite transmission to disentangle and understand the complex effects of predatory organisms on parasite transmission.

AUTHOR CONTRIBUTIONS

Annika Cornelius, Christian Buschbaum and David W. Thieltges conceived the ideas and designed the experiments. Annika Cornelius and Maral Khosravi carried out all experiments. Annika Cornelius, Maral Khosravi, Andreas M. Waser, K. Mathias Wegner and David W. Thieltges designed the analysis and carried out all analyses. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Eike Petersen and Katerina Wagner for her help in the field and during the experimental procedure. The project was made possible by financial support of the German Environmental Foundation(DBU), grant no. 20018/530. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the PANGE Repository https://doi.org/10.1594/PANGAEA.956828 (Cornelius et al., 2023).

ORCID

Annika Cornelius https://orcid.org/0000-0001-9764-4715
Christian Buschbaum https://orcid.org/0000-0002-0223-1916
Maral Khosravi https://orcid.org/0000-0001-5734-6502
Andreas M. Waser https://orcid.org/0000-0002-9455-4447
K. Mathias Wegner https://orcid.org/0000-0002-2410-8898
David W. Thieltges https://orcid.org/0000-0003-0602-0101

REFERENCES

Anderson, P. A., Nowosielski, J., & Croll, N. A. (1976). The emergence of cercariae of *Trichobilharzia ocellata* and its relationship to the activity of its snail host *Lymnaea stagnalis*. *Canadian Journal of Zoology*, *54*(9), 1481–1487. https://doi.org/10.1139/z76-171

Behrens Yamada, S., & Boulding, E. G. (1998). Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. *Journal of Experimental Marine Biology and Ecology*, *220*(2), 191–211. https://doi.org/10.1016/S0022-0981(97)00122-6

Behrens Yamada, S., Navarette, S. A., & Needham, C. (1998). Predation induced changes in behavior and growth rate in three populations of the intertidal snail, *Littorina sitkana* (Philippi). *Journal of Experimental Marine Biology and Ecology*, *220*(2), 213–226. https://doi.org/10.1016/S0022-0981(97)00106-8

Bommarito, C., Thieltges, D. W., Pansch, C., Barboza, F. R., Pranovi, F., & Wahl, M. (2021). Effects of first intermediate host density, host size and salinity on trematode infections in mussels of the South-Western Baltic Sea. *Parasitology*, *148*(4), 486–494. https://doi.org/10.1017/S0031182020002188

Buck, J. C., Weinstein, S. B., & Young, H. S. (2018). Ecological and evolutionary consequences of parasite avoidance. *Trends in Ecology & Evolution*, *33*(8), 619–632. https://doi.org/10.1016/j.tree.2018.05.001

Buschbaum, C., Buschbaum, G., Schrey, I., & Thieltges, D. W. (2007). Shell-boring polychaetes affect gastropod shell strength and crab predation. *Marine Ecology Progress Series*, *329*, 123–130. https://doi.org/10.3354/meps329123

Cáceres, C. E., Knight, C. J., & Hall, S. R. (2009). Predator-spreaders: Predation can enhance parasite success in a planktonic host–parasite system. *Ecology*, *90*(10), 2850–2858. https://doi.org/10.1890/08-2154.1

Chaisson, K. E., & Hallem, E. A. (2012). Chemosensory behaviors of parasites. *Trends in Parasitology*, *28*(10), 427–436. https://doi.org/10.1016/j.pt.2012.07.004

Cornelius, A., & Buschbaum, C. (2020). Introduced marine ecosystem engineers change native biotic habitats but not necessarily associated species interactions. *Estuarine, Coastal and Shelf Science*, *245*, 106936. https://doi.org/10.1016/j.ecss.2020.106936

Cornelius, A., Buschbaum, C., Khosravi, M., Waser, A. M., Wegner, K. M., & Thieltges, D. W. (2023). Data from: Parasite transmission from first to second intermediate trematode marine mollusc hosts under predation pressure. *PANGEA Repository*, https://doi.org/10.1594/PANGAEA.956828

Cornelius, A., Wagner, K., & Buschbaum, C. (2021). Prey preferences, consumption rates and predation effects of Asian shore crabs (*Hemigrapsus takanoi*) in comparison to native shore crabs (*Carcinus maenas*) in northwestern Europe. *Marine Biodiversity*, *51*, 75. https://doi.org/10.1007/s12526-021-01207-7

Daversa, D. R., Hechinger, R. F., Madin, E., Fenton, A., Dell, A. I., Ritchie, E. G., Rohr, J., Rudolf, V. H. W., & Lafferty, K. D. (2021). Broadening the ecology of fear: Non-lethal effects arise from diverse responses to predation and parasitism. *Proceedings of the Royal Society B: Biological Sciences*, *288*, 20202966. https://doi.org/10.1098/rspb.2020.2966

Duffy, M. A., Hall, S. R., Tessier, A. J., & Huebner, M. (2005). Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? *Limnology and Oceanography*, *50*(2), 412–420. https://doi.org/10.4319/lo.2005.50.2.0412

Duong, T. M., & McCauley, S. J. (2016). Predation risk increases immune response in a larval dragonfly (*Leucorrhinia intacta*). *Ecology*, *97*(6), 1605–1610. https://doi.org/10.1890/15-1964.1

Ebert, D., Zschokke-Rohringer, C. D., & Carius, H. J. (2000). Dose effects and density-dependent regulation of two microparasites of *Daphnia magna*. *Oecologia*, *122*, 200–209. https://doi.org/10.1007/PL00008847

Eschweiler, N., & Christensen, H. T. (2011). Trade-off between increased survival and reduced growth for blue mussels living on Pacific oyster reefs. *Journal of Experimental Marine Biology and Ecology*, *403*(1–2), 90–95. https://doi.org/10.1016/j.jembe.2011.04.010

Evans, N. A. (1985). The influence of environmental temperature upon transmission of the cercariae of *Echinostoma liei* (Digenea: Echinostomatidae). *Parasitology*, *90*(2), 269–275. https://doi.org/10.1017/S0031182000050976

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). SAGE Publications.

Fredensborg, B. L., Mouritsen, K. N., & Poulin, R. (2004). Intensity dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: Experimental infections and field observations. *Journal of Experimental Marine Biology and Ecology*, *311*(2), 253–265. doi:10.1016/j.jembe.2004.05.011

Freeman, A. S., & Byers, J. E. (2006). Divergent induced responses to an invasive predator in marine mussel populations. *Science*, *313*, 831–833. https://doi.org/10.1126/science.1125485

Freeman, A. S., Mészáros, J., & Byers, J. E. (2009). Poor phenotypic integration of blue mussel inducible defenses in environments with multiple predators. *Oikos*, *118*(5), 758–766. https://doi.org/10.1111/j.1600-0706.2008.17176.x

Fretter, V., & Graham, A. (1980). The prosobranch molluscs of Britain and Denmark; part 5 marine Littorinaceae. *Journal of Molluscan Studies (Suppl)*, *7*, 241–284.

Gosling, E. (2003). *Bivalve molluscs: Biology, ecology and culture*. Wiley-Blackwell.

Groner, M. L., & Relyea, R. (2015). Predators reduce *Batrachochytrium dendrobatidis* infection loads in their prey. *Freshwater Biology*, *60*(8), 1699–1704. https://doi.org/10.1111/fwb.12602

Haas, W. (2003). Parasitic worms: Strategies of host finding, recognition and invasion. *Zoology*, *106*(4), 349–364. https://doi.org/10.1078/0944-2006-00125

Irlandi, E. A., & Peterson, C. H. (1991). Modification of animal habitat by large plants: Mechanisms by which seagrasses influence clam growth. *Oecologia*, *87*, 307–318. https://doi.org/10.1007/BF00634584

Jacobsen, H. P., & Stabell, O. B. (1999). Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: Dependence on season, light conditions, and chemical labelling of predators. *Marine Biology*, *134*, 551–557. https://doi.org/10.1007/s002270050570

Jensen, T., Jensen, K. T., & Mouritsen, K. N. (1998). The influence of the trematode *Microphallus claviformis* on two congeneric intermediate host species (*Corophium*): Infection characteristics and host survival. *Journal of Experimental Marine Biology and Ecology*, *227*(1), 35–48. https://doi.org/10.1016/S0022-0981(97)00260-8

Johnson, P. T. J., Dobson, A., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S. A., Poulin, R., & Thieltges, D. W. (2010). When parasites become prey: Ecological and epidemiological significance of eating parasites. *Trends in Ecology & Evolution*, *25*(6), 362–371. https://doi.org/10.1016/j.tree.2010.01.005

Johnson, P. T. J., Stanton, D. E., Preu, E. R., Forshay, K. J., & Carpenter, S. R. (2006). Dining on disease: How interactions between infection and environment affect predation risk. *Ecology*, *87*(8), 1973–1980. https://doi.org/10.1890/0012-9658(2006)87[1973:DODHIB]2.0.CO;2

Johnson, P. T. J., & Thieltges, D. W. (2010). Diversity, decoys and the dilution effect: How ecological communities affect disease risk. *Journal of Experimental Biology*, *213*, 961–970. https://doi.org/10.1242/jeb.037721

Koprivnikar, J., Rochette, A., & Forbes, M. R. (2021). Risk-induced trait responses and non-consumptive effects in plants and animals in response to their invertebrate herbivore and parasite natural enemies. *Frontiers in Ecology and Evolution*, *9*, 667030. https://doi.org/10.3389/fevo.2021.667030

Kulakovskii, E. E., & Lezin, P. A. (2002). Filtration activity of the blue mussel, *Mytilus edulis*, under influence of the starfish, *Asteries rubens* (Forcipulata, Asteriidae). *Zoologicheskiy Zhurnal*, *81*(3), 292–297.

Lafferty, K. D. (1999). The evolution of trophic transmission. *Parasitology Today*, *15*(3), 111–115. https://doi.org/10.1016/s0169-4758(99)01397-6

Lafferty, K. D. (2004). Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications*, *14*(5), 1566–1573. https://doi.org/10.1890/03-5088

Leonard, G. H., Bertness, M. D., & Yund, P. O. (1999). Crab predation, waterborne cues and inducible defence in the blue mussel, *Mytilus edulis*. *Ecology*, *80*(1), 1–14. https://doi.org/10.1890/0012-9658(1999)080[0001:CPWCAI]2.0.CO;2

Liddell, C., Welsh, J. E., van der Meer, J., & Thieltges, D. W. (2017). Effect of dose and frequency of exposure to infectious stages on trematode infection intensity and success in mussels. *Diseases of Aquatic Organisms*, *125*, 85–92. https://doi.org/10.3354/dao03133

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619–640. https://doi.org/10.1139/z90-092

Lopez, L. K., & Duffy, M. A. (2021). Mechanisms by which predators mediate host–parasite interactions in aquatic systems. *Trends in Parasitoloy*, *37*(10), 890–906. https://doi.org/10.1016/j.pt.2021.06.006

Lowenberger, C. A., & Rau, M. E. (1994). *Plagiorchis elegans*: Emergence, longevity and infectivity of cercariae, and host behavioural modifications during cercarial emergence. *Parasitology*, *109*(1), 65–72. https://doi.org/10.1017/S0031182000077775

McCarthy, A. (1999). The influence of temperature on the survival and infectivity of the cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology*, *118*(4), 383–388. https://doi.org/10.1017/S003118209900400X

Morley, N. J. (2011). Thermodynamics of cercarial survival and metabolism in a changing climate. *Parasitology*, *138*(11), 1442–1452. doi:10.1017/S0031182011001272

Mouritsen, K. N. (2002). The *Hydrobia ulvae–Maritrema subdolum* association: Cercarial emergence controlled by host activity. *Journal of Helminthology*, *76*(4), 349–353. https://doi.org/10.1079/JOH2002137

Mouritsen, K. N., Dalsgaard, N. P., Flensburg, S. B., Madsen, J. C., & Selbach, C. (2022). Fear of parasitism affects the functional role of ecosystem engineers. *Oikos*, *2022*(5), e08965. https://doi.org/10.1111/oik.08965

Naddafi, R., Pettersson, K., & Eklöv, P. (2007). The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. *Freshwater Biology*, *52*(5), 823–842. https://doi.org/10.1111/j.1365-2427.2007.01732.x

Ojima, H., & Wada, S. (2013). Contrasting anti-predation responses in the intertidal periwinkle *Littorina sitkana*: Effects of chemical cue, body size and time of day. *Plankton & Benthos Research*, *8*(1), 38–45. https://doi.org/10.3800/pbr.8.38

Ostfeld, R. S., & Holt, R. D. (2004). Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. *Frontiers in Ecology and the Environment*, *2*(1), 13–20. https://doi.org/10.1890/1540-9295(2004)002[0013:APGFYH]2.0.CO;2

Packer, C., Holt, R. D., Hudson, P. J., Lafferty, K. D., & Dobson, A. P. (2003). Keeping the herds healthy and alert: Implications of

predator control for infectious disease. *Ecology Letters*, *6*(9), 797–802. https://doi.org/10.1046/j.1461-0248.2003.00500.x
Peacor, S. D., Barton, B. T., Kimbro, D. L., Sih, A., & Sheriff, M. J. (2020). A framework and standardized terminology to facilitate the study of predation-risk effects. *Ecology*, *10*(12), e03152. https://doi.org/10.1002/ecy.3152
Pietrock, M., & Marcogliese, D. J. (2003). Free-living endohelminth stages: At the mercy of environmental conditions. *Trends in Parasitology*, *19*, 293–299. https://doi.org/10.1016/S1471-4922(03)00117-X
Poulin, R. (2006). Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology*, *132*(1), 143–115. https://doi.org/10.1017/S0031182005008693
Poulin, R. (2010a). Parasite manipulation of host behavior: An update and frequently asked questions. *Advances in the Study of Behavior*, *41*, 151–186. https://doi.org/10.1016/S0065-3454(10)41005-0
Poulin, R. (2010b). The scaling of dose with host body mass and the determinants of success in experimental cercarial infections. *International Journal for Parasitology*, *40*(3), 371–377. https://doi.org/10.1016/j.ijpara.2009.09.001
R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/
Rohr, J. R., Civitello, D. J., Crumrine, P. W., Halstead, N. T., Miller, A. D., Schotthoefer, A. M., Stenoien, C., Johnson, L. B., & Beasley, V. R. (2015). Predator diversity, intraguild predation, and indirect effects drive parasite transmission. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 3008–3013. https://doi.org/10.1073/pnas.1415971112
Sherker, Z. T., Ellrich, J. A., & Scrosati, R. A. (2017). Predator-induced shell plasticity in mussels hinders predation by drilling snails. *Marine Ecology Progress Series*, *573*, 167–175. https://doi.org/10.3354/meps12194
Smee, D. L., & Weissburg, M. J. (2006a). Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. *Journal of Chemical Ecology*, *32*, 605–619. https://doi.org/10.1007/s10886-005-9021-8
Smee, D. L., & Weissburg, M. J. (2006b). Clamming up: Environmental forces diminish the perceptive ability of bivalve prey. *Ecology*, *87*(6), 1587–1598. https://doi.org/10.1890/0012-9658(2006)87[1587:CUEFDT]2.0.CO;2
Smith, J. E., & Newell, G. E. (1955). The dynamics of the zonation of the common periwinkle (*Littorina littorea* (L.)) on a stony beach. *Journal of Animal Ecology*, *24*, 35–56. https://doi.org/10.2307/1878
Stier, T., Drent, J., & Thieltges, D. W. (2015). Trematode infections reduce clearance rates and condition in blue mussels *Mytilus edulis*. *Marine Ecology Progress Series*, *529*, 137–144. https://doi.org/10.3354/meps11250
Studer, A., Thieltges, D. W., & Poulin, R. (2010). Parasites and global warming: Net effects of temperature on an intertidal host–parasite system. *Marine Ecology Progress Series*, *415*, 11–22. https://doi.org/10.3354/meps08742
Thieltges, D. W. (2006). Parasite induced summer mortality in the cockle Cerastoderma edule by the trematode Gymnophallus choledochus. *Hydrobiologia*, *559*, 455–461. https://doi.org/10.1007/s10750-005-1345-4
Thieltges, D. W., Bordalo, M. D., Hernández, A. C., Prinz, K., & Jensen, K. T. (2008). Ambient fauna impairs parasite transmission in a marine parasite-host system. *Parasitology*, *135*(9), 1111–1116. https://doi.org/10.1017/S0031182008004526
Thieltges, D. W., Jensen, K. T., & Poulin, R. (2008). The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology*, *135*(4), 407–426. https://doi.org/10.1017/S0031182007000248
Thieltges, D. W., & Rick, J. (2006). Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea: Renicolidae). *Diseases of Aquatic Organisms*, *73*, 63–68. https://doi.org/10.3354/dao073063
Thiemann, G. W., & Wassersug, R. J. (2000). Patterns and consequences of behavioural responses to predators and parasites in *Rana tadpoles*. *Biological Journal of the Linnean Society*, *71*(3), 513–528. https://doi.org/10.1111/j.1095-8312.2000.tb01272.x
Trussell, G. C., Ewanchuck, P. J., & Bertness, M. D. (2003). Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. *Ecology Letters*, *5*(2), 241–245. https://doi.org/10.1046/j.1461-0248.2002.00304.x
Vajedsamiei, J., Melzber, F., Raatz, M., Kiko, R., Khosravi, M., & Pansch, C. (2021). Simultaneous recording of filtration and respiration in marine organisms in response to short-term environmental variability. *Limnology and Oceanography: Methods*, *19*(3), 196–209. https://doi.org/10.1002/lom3.10414
Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). Springer.
Werner, E. E., & Peacor, S. D. (2001). The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(7), 3904–3908. https://doi.org/10.1073/pnas.071061998
Werner, E. E., & Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology*, *84*(5), 1083–1100. https://doi.org/10.1890/0012-9658(2003)084[1083:AROTII]2.0.CO;2
Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. https://doi.org/10.1007/978-0-387-98141-3
Wood, S. N. (2017). *Generalized addative models: An introduction with R* (2nd ed.). Chapmann & Hall/CRC.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: (a) Mean filtration rate (mL/min) of mussels ($n = 16$) over time when kept in control water (blue) and after adding of water including predation risk cues from crabs (red). (b and c) Mean filtration rate (ml/min) of mussels over a longer time period (300 or 800 min) when kept in control water (a, $n = 3$) and after addition of water including predation risk cues from crabs (*b*, n = 3).

How to cite this article: Cornelius, A., Buschbaum, C., Khosravi, M., Waser, A. M., Wegner, K. M., & Thieltges, D. W. (2023). Effect of predation risk on parasite transmission from first to second intermediate trematode hosts. *Journal of Animal Ecology*, *00*, 1–10. https://doi.org/10.1111/1365-2656.13921