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Climate change and parasite transmission: how temperature affects parasite infectivity via predation on infective stages

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Abstract. Climate change is expected to affect disease risk in many parasite-host systems, e.g., via an effect of temperature on infectivity (temperature effects). However, recent studies indicate that ambient communities can lower disease risk for hosts, for instance via predation on free-living stages of parasites (predation effect). Since general physiological theory suggests predation effects to be temperature-dependent, we hypothesized that increases in temperature may lead to reduced parasite infectivity via elevated consumption rates of free-living parasite stages (temperature-predation interaction). We experimentally investigated such interactions in three marine predators of infective parasite stages. Two species (the oyster *Crassostrea gigas*, and the barnacle *Austrominius modestus*) significantly reduced cercarial stages of the trematode *Renicola roscovita* in mussel hosts (*Mytilus edulis*), while the third (the crab *Hemigrapsus takanoi*) did not show a reduction of infective stages at all. In barnacles, cercarial consumption significantly interacted with temperature, with lowest infectivity at highest temperatures. Since these patterns reflected the known thermal responses of the three cercarial predators' feeding rates, parasite consumption rates may be predictable from temperature dependent feeding rates. Our results suggest that integrating temperature-predation interactions into studies on parasite transmission and on climate change effects is essential and that predators of free-living stages of parasites may play an important role in indirectly mediating disease risk under climate change.

Key words: climate change; dilution effect; infectivity; invasive species; parasites as prey; predation on free-living stages of parasites; *Renicola roscovita*; transmission; trematodes; Wadden Sea.

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Introduction

Various studies have suggested that climate change may lead to elevated disease risk in wildlife and humans with wide-ranging ecological and economic effects (Harvell et al. 2002, Patz et al. 2005). However, the actual relationship between temperature and disease is often complex, with varying underlying mechanisms that are better understood for some disease agents than for others (Lafferty 2009, Rohr et al. 2011). A particularly clear and strong effect of temperature on disease dynamics is known from trematodes (flukes) in which it directly affects crucial steps in their transmission between life cycle stages. The production and emergence of their free-living infective stages (cercariae) in the first intermediate hosts (mollusks) is strongly positively correlated with temperature (see review by Poulin 2006). At the same time, the infectivity of cercariae in the down-stream second intermediate hosts (invertebrates or fish, depending on the

species) is also positively correlated with temperature (e.g., Evans 1985, Thieltges and Rick 2006, Studer et al. 2010). Since cercarial transmission is a crucial step in the trematode life cycle, it has been proposed that global warming might dramatically increase future infection levels in hosts (Marcogliese 2001, Poulin 2006, Poulin and Mouritsen 2006).

Parasite-host dynamics are not only influenced by abiotic conditions like temperature but also by interactions with other species in the environment. For example, some organisms of the ecological community from which a parasite-host system is part of can reduce the risk of certain diseases via a process called the *dilution effect*. This effect has mostly been related to varying host compatibility, with the presence of low competency hosts leading to a reduction in disease risk for competent hosts (Keesing et al. 2006). However, the initial concept has recently been broadened to include effects of species which do not serve as hosts at all (non-host; Johnson and Thieltges 2010, Johnson et al. 2010). When such non-hosts prey upon free-living infectious stages of parasites, they can interfere with transmission (equivalent to the 'encounter reduction' mechanism of Keesing et al. 2006) and lead to reduced infection levels in the target host (Johnson and Thieltges 2010). Experimental and observational studies, both from the lab and the field, indicate that such predation effects can occur in many parasites with free-living stages (Thieltges et al. 2008), including trematodes (e.g., Thieltges et al. 2009, Orlofske et al. 2012, Welsh et al. 2014), and they are increasingly proposed as important regulatory mechanisms for many diseases (Keesing et al. 2010, Ostfeld and Keesing 2012).

Given the general positive relationship between metabolic rates of ectothermic organisms and ambient temperature (Q_{10}-rule; Schmidt-Nielsen 1997), non-host predation effects are, like trematode emergence and infectivity, also expected to be mediated by temperature. An increase in metabolism translates into an increase in feeding rates and feeding rates of organisms generally scale positively with temperature up to a maximum, after which they decrease due to temperature stress (e.g., Newell 1970, Englund et al. 2011). This suggests a potential interaction between temperature effects and predation effects: an increase in parasite production and infectivity at elevated temperatures might actually be compensated by increased feeding rates of predators of free-living parasite stages. Although an interaction between temperature and predation effects is very likely, to the best of our knowledge, to date no experimental studies have detected their existence (but see Studer et al. 2013 for a first attempt in this direction).

This study aimed to investigate whether predation effects caused by non-hosts can interact with temperature effects so that increasing infection levels due to elevated temperatures may be counterbalanced by predation on free-living stages of parasites. We used controlled lab experiments and a marine parasite-host system widespread in the eastern North Atlantic (cercariae of the trematode *Renicola roscovita*, its second intermediate host, the blue mussel M*ytilus edulis*) and three common cercarial predators (Pacific oysters *Crassostrea gigas*, Asian shore crabs *Hemigrapsus takanoi* and Australasian barnacles *Austrominius modestus*), to investigate the following specific questions: (1) Does the strength of the predation effect depend on temperature? (2) If so, could this predation effect compensate for increased infection levels with rising temperatures? (3) Do predation effects vary between cercarial predators and can this be explained by their known temperature responses in feeding rates? Identifying potential interactions of temperature and predation effects has important implications for our understanding of parasite transmission in general and the role of non-hosts in mediating effects of climate change in particular.

Methods

Organisms

To obtain a source of *Renicola roscovita* cercariae, its first intermediate host, the periwinkle *Littorina littorea*, was collected from different mussel beds and dykes on the east coast of the island of Texel (Wadden Sea, The Netherlands). After an acclimation period of at least 24 h at 15°C, the periwinkles were divided over 6-well plates, filled with seawater (16 mL per well) and placed in an incubator at 25–29°C. After 3 h, the wells were screened for cercariae of *R. roscovita* under a dissection microscope. Infected snails

were kept in groups of 20 in aerated aquaria (3.6 L) and fed ad libitum with *Ulva* spp. Uninfected blue mussels (*Mytulis edulis*; 30–35 mm) were used as target hosts and collected from groins along the west coast of Texel. Here the first intermediate host of the parasite does not occur and the mussels are therefore uninfected (verified by dissecting 30 mussels—no infections found). The three cercarial predators were collected as follows: Pacific oysters (*Crassostrea gigas*; average volume of 38.3 ± 8.5 mL) were collected from a small oyster bank in the Mokbaai inlet at the southeast end of the island of Texel (53°00′21″ N; 4°46′10″ E). At the same location, Asian shore crabs (*Hemigrapsus takanoi*; average carapace size of 1.0–1.5 cm) and empty mussel shells covered with the Australasian barnacle (*Austrominius modestus*) were collected between the oysters. All organisms were kept in aerated sand filtered seawater at 15°C and fed ad libitum with blue mussel flesh (crabs) or with the unicellar algae *Isochrysis galbana* (mussels, barnacles, and oysters).

Experimental design

The experiment was carried out as a partly nested two-factorial split-plot design (Quinn and Keough 2002), with temperature (between plots: 12.5°, 18° and 25°C) and cercarial predator presence (within plots: yes or no) as fixed factors and water bath (plots) as random factor, nested in temperature (see details below). The temperatures used were within the normal physiological temperature range of the three cercarial predators (Pacific oyster: Bougrier et al. 1995, Ren et al. 2000; Asian shore crab: Dehnel 1960; Australasian barnacle: Southward 1955) and reflect the average spring temperature (12.5°C) in the study area (Western Wadden Sea) over the last 140 years (van Aken 2008) and temperatures that are commonly (18°C) or occasionally observed during extreme warm days during summer (25°C) in tidal pools and shallow waters on the tidal flats (the habitat of the parasite-host system and cercarial predators; Kühl 1952, van Aken 2008, Onken et al. 2010). Moreover, the highest temperature level (25°C) is predicted to occur more frequently and for prolonged periods under future climate change scenarios (Philippart and Epping 2009). The standardized amount of cercarial predators was within the range observed in the field (see *Discussion* for details) and was as follows: one oyster, two crabs and empty mussel shells with a total of approximately 7 cm^2 of barnacle cover (≈ 140 individuals). To all experimental units three individuals of uninfected blue mussels were added as the target hosts for cercariae.

For each of the three temperature levels (12.5°, 18° and 25°C), we used four individually heated and temperature controlled (to avoid the common pseudoreplication present in temperature experiments; Rohr et al. 2011) water baths (functioning as plots; 40 × 30 × 25 cm) that were distributed randomly within a single climate chamber (10°C). Within each water bath we placed two sets of two plastic containers (12 × 10 × 22 cm, filled with 1.5 L of seawater) that served as the experimental units to apply the within-plot factor cercarial predator presence. Two of the four containers included cercarial predators, while the other two served as controls, containing hosts only. During the experiments and the acclimation periods, the organisms experienced full light exposure. Cercariae only emerge at daylight and their infectivity ceases after 10–15 hours (Greve 1997, Thieltges and Rick 2006), thus for our experiment (cercariae already about 3 hours old when added) a day-night cycle was considered irrelevant. No food was supplied during the experiment as cercarial predators are known to maintain cercarial consumption under starvation (Orlofske et al. 2012, Liddell 2014).

Experimental procedure

Due to logistical constraints, three separate experiments (one for each cercarial predator) were conducted. All organisms (cercarial predators and hosts) were acclimated for 24 hours in the experimental set-up as described above. After 24 h, 50 mL of cercariae stock (seawater with cercariae) was added to all containers. The stock was obtained by incubating 112 infected snails in 3.1 L seawater in an incubator at 28–29°C. After 3 h, the snails were removed and the stock was transferred into a glass beaker and processed immediately (thus the cercariae had a maximum age of ~3 h at the start of the experiment). The stock was carefully stirred (3 times clockwise and 3 times counter-clockwise) each time before 50 mL was removed, to avoid aggregations of cercariae on the bottom of the beaker. A pre-

study showed that this method was appropriate to provide consistently similar numbers of cercariae. To estimate the added dose, two subsamples of 50 mL were taken before, four in between, and two after adding of cercariae stock (i.e., 8 subsamples in total). They were fixed with 10 mL of 96% ethanol, stained with 1 mL Rose Bengal and immediately counted under a dissection microscope (mean number of cercariae (±SE) added to the containers: oysters: 299 ± 10; crabs: 51 ± 7 and 246 ± 8; barnacles: 45 ± 2 and 55 ± 7). In the oyster experiment only a single dose was administered as the first dose was already above our target of at least 100 cercariae (considered to be a realistic dose) while in the other two experiments a second round of infection was necessary due to low numbers of cercariae shed by the snails in the first infection round. The second infection round was done on the next day in the same way as the first. The resulting different total doses were considered unproblematic as cercarial dose does not affect infectivity and does not lead to satiation of cercarial predators at the levels used in our experiment (Liddell 2014). After 24 h in the oyster and 48 hours in the other two experiments, all mussels were placed into seawater and incubated for an additional 48 h to ensure full encystment of metacercariae. Finally, the mussels were dissected, the complete tissue squeezed between two large glass slides, and the numbers of metacercariae determined under a stereomicroscope.

Data analysis

Infectivity of cercariae was calculated as the ratio of the mean number of metacercariae encysted per mussel host divided by the number of cercariae added to the experimental unit. The analysis of the partly nested two-factorial split-plot design followed Quinn and Keough (2002), using R (R Development Core Team 2014). Temperature (A) and cercarial predator presence (C) were used as fixed factors. The water baths (B) functioned as plots and were nested within temperature (B(A)) and analyzed as a random factor. Between plots, we tested for the effects of temperature (A). Within plots, we tested for the effects of cercarial predator presence (C) as well as the interaction between temperature and cercarial predator presence (A × C). For all analyses we averaged the two replicates of cercarial predator presence within each bath as an independent replicate (Quinn and Keough 2002). To check for potential differences in mussel size within or among the three experiments, Welch two sample t-tests were used for within trials (diluter vs control) and one-way ANOVA for among trials. For all analyses, the assumptions of normality and homogeneity of variances were checked by inspecting residual plots. To meet the assumptions we applied an arcsine square-root transformation to the infectivity data of all experiments.

Results

The three cercarial predators showed different responses resulting in different cercarial infectivity with increasing temperature (Fig. 1). While temperature had a significant effect on cercarial infectivity in the experiments using crabs as cercarial predators, the presence of crabs had no effect (Table 1): cercarial infectivity followed a similar pattern as in the absence of crabs (Fig. 1). In contrast, in the other two experiments (oysters and barnacles) the presence of cercarial predators had a significant effect on infectivity with a decrease in infectivity compared to the controls (Fig. 1, Table 1). In the presence of oysters, all cercarial predator treatments showed lower infection levels in mussels compared to the controls but no significant temperature-predation interaction (Fig. 1, Table 1). In contrast, in the barnacle experiment, the observed predation effect showed a significant interaction with a temperature effect: infectivity in the presence of the predators decreased with an increase in temperature, suggesting an increase in the strength of the predation effect with rising temperatures. The mean infectivity of cercariae per individual mussel differed among the three experiments: it was lowest in the oyster experiment (0.7–2.3%), intermediate in the crab (2.6–4.4%) and highest in the barnacle experiment (3.6–15.9%). Sizes of mussels did not differ among treatments within and among the three trials (all $p > 0.3$).

Discussion

Our experiments demonstrated a strong interaction between temperature and predation ef-

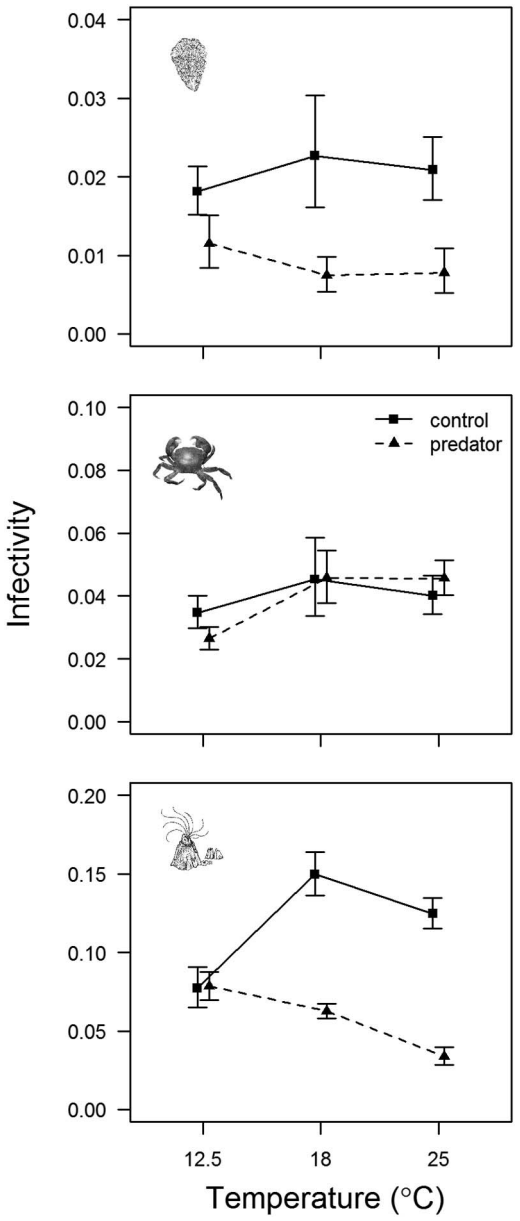

Fig. 1. Infectivity of *Renicola roscovita* cercariae in the mussel *Mytilus edulis*, depending on temperature and the presence of cercarial predators: Pacific oysters *Crassostrea gigas* (top panel), Asian shore crabs *Hemigrapsus takanoi* (middle panel) and Australasian barnacles *Austrominius modestus* (lower panel). The infectivity is the average number of cercariae found per mussel divided by the amount of cercariae added to each container. Infectivity values are back transformed asin(sqrt()) averages. The bars denote ±1 SE.

Table 1. Results of the partly nested two-factorial split-plot ANOVA for the three separate experiments (oysters, crabs, barnacles). We tested for the effects of temperature (T) and water bath (nested in temperature) between plots and the effects of cercarial predator presence and an interaction between temperature and predator presence within plots. Temperature and predator presence were fixed factors, while water bath (nested in temperature) was a random factor. The analyses and presentation of results follow Quinn and Keough (2002).

Source of variation	df	MS	F	p
Oyster				
Between plots				
Temperature	2	0.0000	0.048	0.954
Water bath (T)	9	0.0009		
Within plots				
Predator	1	0.0148	12.068	0.007
Temperature × Predator	2	0.0008	0.622	0.558
Residual	9	0.0012		
Crab				
Between plots				
Temperature	2	0.0036	4.337	0.048
Water bath (T)	9	0.0008		
Within plots				
Predator	1	0.0001	0.032	0.863
Temperature × Predator	2	0.0007	0.381	0.694
Residual	9	0.0019		
Barnacle				
Between plots				
Temperature	2	0.0066	3.449	0.077
Water bath (T)	9	0.0019		
Within plots				
Predator	1	0.0721	110.363	<0.001
Temperature × Predator	2	0.0192	29.462	<0.001
Residual	9	0.0007		

fects on cercarial infectivity in one of the cercarial predators tested (barnacles). While infectivity of cercariae generally increased with elevated temperatures in the absence of cercarial predators, it decreased when barnacles where present. This suggests that temperature-mediated increases in infectivity and host infection levels can be counteracted by predation on free-living parasite stages. This has several important implications.

First, an increase in the strength of predation effects with rising temperatures suggests that predicted effects of climate change (Poulin 2006, Poulin and Mouritsen 2006) may not be so severe or even non-existing because temperature induced increases in predation on free-living parasite stages could counterbalance increases in parasite infectivity. Our experiments focused on identifying potential temperature-predation

interactions on cercarial infectivity by keeping cercarial doses constant, i.e., they did not integrate elevated doses due to increases in cercarial production at higher temperatures. However, given the known temperature response of cercarial production of *Renicola roscovita*, a potential compensation of increased cercarial production by cercarial consumption of barnacles at elevated temperatures under climate change seems likely. While snails infected with *R. roscovita* shed 3.4 times more cercariae at 20°C compared to 15°C, production is 2.3 times lower at 25°C compared to 20°C (Thieltges and Rick 2006). This suggests that the observed increased cercarial consumption by barnacles will not keep up with the increase in cercarial production from low to intermediate temperatures (3.4× higher production but only a 2.4× decrease in infectivity when barnacles are present; see Fig. 1) but could easily override the production difference between 20° and 25°C (2.3 times lower cercarial production but also a 4.2× decrease in infectivity when barnacles are present; see Fig. 1). Since the last step is the one relevant for climate change scenarios in our region (Philippart and Epping 2009), the net effect is likely to be a lowered transmission due to increased cercarial consumption. Field experiments and observations in our (Thieltges et al. 2009) and other ecosystems (e.g., Upatham and Sturrock 1973, Mouritsen and Poulin 2003, Kaplan et al. 2009, Venesky et al. 2014) suggest that laboratory derived indications for predation effects of a variety of predators of free-living parasite stages hold true under field conditions. Hence, the observed temperature-predation interaction is likely to translate into a compensation of elevated parasite production under climate change.

However, these calculations are based on the specific densities used in our experimental set-up. The experimental densities of all three cercarial predators fall within the range observed in the field, but this range actually spans over several orders of magnitude, depending on the habitat where mussel hosts occur (mussel dominated beds, mixed mussel-oysters beds, oyster dominated beds, and hard substrates like the extensive Dutch dykes and harbors) and on local factors (e.g., tidal height, exposure). For example, the barnacle used in our experiments occurs at densities ranging from a few individuals to mean densities of about 70,000 individuals m^{-2} on mussel/oyster beds in the Wadden Sea ecosystem (Witte et al. 2010). Hence, the actual strength of predation effects will probably be strongly mediated by the density of cercarial predators and may be locally much stronger than observed in our experiments (about 12,000 barnacles m^{-2}). In addition, predation effects of different cercarial predators may also be additive when they co-occur, resulting in further reductions of infection levels (Thieltges et al. 2009). With all three cercarial predators co-occurring locally in the Wadden Sea (Troost 2010, Witte et al. 2010, Landschoff et al. 2013), the actual total cercarial consumption will thus depend on the species composition around target mussels. Interestingly, the mussels themselves will also act as sentinels, i.e., increasing mussel densities will lead to reduced infection levels per individual host (Thieltges et al. 2009). However, experiments with trematode-mussel (e.g., Thieltges et al. 2009) and other parasite-host systems (see e.g., review by Thieltges et al. 2008) have shown that predation effects by cercarial predators exist in the presence of target hosts, suggesting that they actually cause an additional dilution of infective stages. Finally, there are also other factors besides cercarial predators and sentinel hosts that mediate infectivity. For example, host condition and also abiotic factors are likely to affect transmission of cercariae to mussels (Pietrock and Marcogliese 2003) and the observed differences in infectivity between the three experiments may have resulted from differences of some of these factors among trials. In conclusion, the exact net effect of temperature increases on infectivity will depend on the interplay of cercarial production, cercarial predator and host densities as well as other infectivity mediating factors. More detailed studies focusing on entangling the relative contributions of these factors will be a promising avenue for future research. Moreover, studies on the effects of climate change on parasitism would generally benefit from integrating effects of predation on parasite free-living stages given the potential for temperature-predation interactions.

Second, a significant interaction between temperature and predation effects has also important implications for our understanding of the role of non-hosts in mediating parasite transmission in

general (dilution effects sensu lato). Many organisms have been identified to act as predators for free-living infectious stages of parasites, not just for trematodes but also for many other macroparasites as well as microparasites (Thieltges et al. 2008, Johnson and Thieltges 2010, Ostfeld and Keesing 2012). The activity levels and feeding rates of most predators of parasite free-living stages will be temperature-dependent, following basic physiological rules (Newell 1970, Englund et al. 2011). Hence, the strength of their respective predation effects can also be expected to be strongly temperature-dependent. This suggests that parasite consumption rates of predators may vary greatly under different temperature regimes, strongly calling for an integration of temperature as an additional factor in experimental studies on the role of non-host diversity in mediating parasite transmission.

Finally, our results point to a potentially important role of invasive species in mediating the effects of climate change on host infection levels. Many invasive species are generally expected to extend their ranges poleward (Hellmann et al. 2008) and increase in population size (Dukes and Mooney 1999, Walther et al. 2009). This is also true for the three cercarial predators used in our study which are all invasive in the study area and expected to benefit from climate change by increasing in abundance (Diederich et al. 2005, Witte et al. 2010, van den Brink et al. 2012). Such an increase in abundance together with temperature-mediated increases in predation rates on parasite stages may have profound effects on future disease risks. Negative effects on the transmission of parasite free-living stages have also been reported from other invasive species (e.g., Bartoli and Boudouresque 1997, Kopp and Jokela 2007) and may be more common than currently known. Hence, invasive species may play an important role in mediating disease risk due to climate change thus integrating the indirect effects of invaders on disease risk would be highly relevant for impact assessments of invasive species.

Although two of the cercarial predators we tested showed a significant predation effect, the type of their response differed: while oysters showed similar predation effects at intermediate and high temperatures, the predation effect by barnacles increased with rising temperatures showing highest predation effects at 25°C. This pattern exactly mirrors the known temperature response of both species regarding their feeding rates. In the Pacific oyster, the thermal response curve is hump-shaped, with clearance rates increasing from 10° to about 20°C and then levelling off at higher temperatures (Bougrier et al. 1995, Ren et al. 2000). In contrast, the feeding activity of Australasian barnacles increases from 4° to 25°C (Southward 1955). This close match of the temperature response of predation effects with the general feeding activity of the respective predators suggests that predation rates can be predicted from temperature response curves of feeding rates. However, this does not mean that there will always be predation effects. Asian shore crabs have a similarly shaped activity rate response curve as the barnacles, with increasing activity rates from 5° to 20°C (Dehnel 1960), but they did not show any predation effect. This was probably due to a size-selective mismatch between predator and prey, with the cercariae of *R. roscovita* (129–330 μm; Werding 1969) being too small to serve as prey for the crabs. In contrast, the size of *R. roscovita* cercariae are within the prey size range of oysters (Möhlenberg and Riisgård 1979) and barnacles (Crisp and Southward 1961), allowing predation effects to occur. Since size-dependent match-mismatches between predator and cercarial sizes have also been observed in juvenile fish preying on cercariae (Kaplan et al. 2009), a match of cercarial size and prey size range of a predator is crucial in determining which species has the potential to act as a cercarial predator. This in turn suggests that the capacity to prey on free-living stages of parasites is, to a certain extent, predictable from the general prey size spectrum of predators.

In conclusion, our experiments revealed that there can be strong interactions between temperature and predation effects. Such interactions can potentially offset predicted increases in disease risk under climate change and have important implications for our general understanding of the effects of non-hosts on parasite transmission, because consumption rates of most predators will be temperature-dependent. In addition, our results suggest that invasive species may play an important role in mediating disease risk in the course of climate change. All this calls for the integration of temperature in future studies on

the role of non-hosts in parasite transmission as well as in studies on the effects of climate change on disease risk and on the impact of invasive species.

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