Cold water reduces the severity of parasite-inflicted damage: support for wintertime recuperation in aquatic hosts

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Abstract

The reduction in host fitness caused by parasite infections (virulence) depends on infection intensity and the degree of damage caused per parasite. Environmental conditions can shape both virulence components, but in contrast to infection intensity, environmental impacts on per-parasite damage are poorly understood. Here, we studied the effect of ambient temperature on per-parasite damage, which is jointly determined by the ability of parasites to induce harm (per-parasite pathogenicity) and the ability of hosts to limit damage (tolerance). We experimentally exposed two salmonid species, Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*), to replicated genotypes of the eye fluke *Diplostomum pseudospathaceum*. After development of health damage (eye cataracts) in warm water (16ºC) during the first 12 weeks post exposure, we maintained the fish at either 5ºC (cold water) or 16ºC for another eight weeks and quantified changes in cataracts as a function of parasite load. We found that per-parasite damage was reduced in cold compared to warm water, suggesting that cold temperatures improved host health. Per-parasite damage was also affected by parasite genotype and host species, but these effects did not change with temperature. Our findings suggest that cold-water seasons, which are often neglected in host-parasite studies due to low infection risk, could allow hosts to recuperate and thus, may have important implications for the ecology and epidemiology of parasite infections.

Key words: temperature, tolerance, virulence, salmonid, trematode

Introduction

The virulence of an infection, i.e. the harm caused to the host, is composed of infection intensity and the degree of host damage per parasite, both of which can be influenced by parasite and host factors. As host health generally decreases with infection intensity, virulence studies have traditionally focused on mechanisms shaping parasite load (parasite infectivity, exploitation and host resistance). However, parasites can also show inherent variation in their per capita ability to damage the host, for example, through tissue destruction (De Roode and Altizer 2010; Sternberg et al. 2013; Klemme and Karvonen 2019). Similarly, recent work has emphasized the importance of mechanisms that enable hosts to withstand or repair the damages of a given infection (tolerance) and confirmed a genetic component to this host defence (Best et al. 2008; Medzhitov et al. 2012; Råberg 2014). Thus, processes shaping per-parasite damage may be subject to natural selection and play an important role for overall host health.

In addition to parasite and host background, environmental variation could also influence per-parasite damage. For example, studies exploring the effect of diet on host tolerance have shown both reduced and elevated per-parasite damage under high resource availability (Vale et al. 2011; Cornet et al. 2014; Howick and Lazzaro 2014; Kutzer and Armitage 2016; Knutie et al. 2017), suggesting that higher resources can result in increased host tolerance, but may also alter the ability of parasites to inflict damage. Temperature is another environmental factor that could shape per-parasite damage as it influences many physiological and biochemical processes. For example, evidence from *Daphnia* infected with a bacterial parasite supports temperature-induced variation in host tolerance (Vale et al. 2011). In temperate aquatic systems, low water temperatures during winter inhibit the transmission of most parasites, while many infections that establish during higher summer temperatures can persist across seasons (Poulin 2020). However, cold temperatures slow down parasite

development and activity in ectoparasites and endoparasites within ectothermic hosts (e.g. Macnab and Barber 2012), potentially limiting their ability to inflict further damage. In the absence of new infections, hosts could re-allocate resources from resistance to tolerance and increase their ability to repair damages. This suggests that winter could be an important period for host recuperation, with possible wider implications for the ecology and epidemiology of infections. Although the virulence of most parasite infections is generally known to increase with temperature (Harvell et al. 2002; Marcogliese 2008), evidence for host recuperation in cold temperature is scarce.

Here, we experimentally explored the effects of temperature on per-parasite damage in a trematode parasite – fish host system. *Diplostomum pseudospathaceum* infects many species of freshwater fish, with transmission from the first intermediate snail host occurring primarily during summer, when water temperature exceeds 10ºC (Karvonen 2012). Infections establish in the eye lenses of the fish host, where they cause considerable tissue damage in an intensity-dependent manner, resulting in cataracts that significantly decrease host fitness due to visual impairment (reviewed in Karvonen 2012). Because the eye lens lacks blood circulation, the ability of the host immune system to prevent the parasite after establishment is limited. To reduce pathology in the eye, hosts therefore need to employ tolerance mechanisms that mitigate or repair tissue damages. Recent work has demonstrated different reaction norms between the magnitude of cataracts and parasite load across populations of salmonid fishes, suggesting that such mechanisms exist (Klemme and Karvonen 2017; Klemme et al. 2020). Moreover, genotypes of *D. pseudospathaceum* vary in their per-capita ability to induce cataracts (Klemme and Karvonen 2019).

To determine the impact of water temperature on per-parasite damage, we experimentally exposed two salmonid fish species, Atlantic salmon (*Salmo salar*) and sea trout (sea-migrating brown trout, *Salmo trutta*), to replicated genotypes of *D.*

pseudospathaceum. We then allowed cataracts to develop and subsequently followed changes in their per-parasite magnitude either in unaltered warm water temperature (16ºC) or in decreased water temperature (5 ºC) for eight weeks. We predicted lower per-parasite damage and improved fish health in cold compared to warm water. We also explored temperature by parasite/host genotype interactions to study whether these responses were genotype-specific.

Material and Methods

Host and parasite sources

Three-month-old Atlantic salmon and sea trout from ten replicate captive populations (five per species) were obtained from a breeding programme of the Natural Resource Institute Finland two weeks before the experiment commenced. The fish had been maintained in lake water at natural temperatures (May-July) and following transfer, were kept in replicated 180 L flow-through tanks with lake water at constant 17ºC. After one week (see Table 1 for the timeline of the experiment), 80 individuals of each population were individually marked with PIT tags (8 mm, Oregon RFID, Portland, OR, U.S.A.) under light anaesthesia (MS-222, 100 mg/L). The fish were fed daily with fish pellets *ad libitum*.

Parasites originated from six naturally infected *Lymnaea stagnalis* snails collected from Lake Vuojärvi (62° N, 25° E) five weeks before the exposures. Genotyping of 20 parasite larvae (cercariae) per snail with four microsatellite markers (Louhi et al. 2010) confirmed single genotype infections. The snails were maintained in individual containers with 1 l of lake water (4ºC) and lettuce *ad libitum*. To induce cercarial release, each snail was transferred

to 250 ml of lake water (22ºC) 3-4 hours prior the exposure. Cercarial density of each genotype was estimated from five 1 ml samples.

Experimental procedure

For experimental exposures, fish were placed individually in containers with 500 ml of lake water (17ºC) and 500 cercariae for 30 minutes. Altogether 680 fish were exposed to one of six parasite genotypes. For the exposures, 120 fish (12 from each of the 10 populations) were randomly chosen for each parasite genotype, except for one genotype with low parasite production to which 80 randomly chosen fish (8 from each population) were exposed. Following the exposure, fish were distributed among four 1400 L flow-through tanks (16ºC), with each host and parasite genotype present evenly in each tank. Note that the parasite does not transmit directly between fish. In the hatchery from which the fish were obtained, eye infections are very rare and the transfer to the experimental unit occurred at the end of the transmission period of *D. pseudospathaceum*. Thus, possible uncontrolled infections were considered unlikely. However, two weeks after the exposures, an unexpected outbreak with the ectoparasitic flagellate *Ichthyobodo necator* in all four tanks caused mortality in 113/680 fish (26 salmon and 87 trout). The infection was cleared with formalin baths.

Twelve weeks after the experimental exposures, parasites (metacercariae) were fully grown and had begun to induce eye cataracts (Karvonen 2012). Both eye lenses of each fish were examined for cataracts under light anaesthesia (MS-222, 100 mg/L) using a slit lamp microscope (Kowa SL-15). The area as well as thickness of cataract coverage (i.e. volume) were considered and scored as 0-100% in increments of 10% (Karvonen et al. 2004b; Klemme and Karvonen 2017). The length of all fish was also measured (mean ± SE = 99.4 ± 0.9 mm). At this stage, all uninfected fish (due to low infectivity of some parasite genotypes,

see below) were discarded and individuals with confirmed infection in one or both of the lenses (303/567 exposed fish that survived; 162 salmon and 141 trout) were again randomly distributed among four large 1400 L flow-through tanks (16ºC). Each tank contained 72-77 fish, including individuals from both species (supplementary Table S1).

Temperature manipulations were initiated on the following day. In two randomly selected tanks, the water temperature was gradually lowered (2ºC/day to allow for acclimation) to 5ºC ± 0.5ºC, while remaining at 16ºC ± 0.5 ºC in the other two tanks. These temperatures are well within the thermal limits of Atlantic salmon (0-28°C) and sea trout (0-25°C, Jonsson and Jonsson 2011) and correspond roughly to winter and summer temperatures naturally experienced by these species. Target temperatures were reached after six days. After four and eight weeks, the fish were again surveyed for eye cataracts and length as described above. Cataracts were scored every time by the same person who was blind to temperature treatment. Before the final scoring at eight weeks, fish were euthanized with an overdose of MS-222. After scoring, all eyes were dissected and lens size measured as well as parasite load counted under a microscope.

Data considerations

Three of six parasite genotypes showed unexpectedly low infectivity, which resulted in low replicate host numbers (supplementary Table S1) with a small range of parasite loads (46/55 fish harboured only 1 parasite, range 1-4). Thus, fish infected with these genotypes were excluded from the statistical analysis. Further, six individuals died during the eight weeks of temperature manipulation. This resulted in final sample sizes of 128 fish at 5ºC (77 salmon and 51 trout) and 110 fish at 16ºC (52 salmon and 58 trout).

All statistical analyses were conducted in SAS v. 9.4. To account for differences in fish (and eye lens) size, percent cataract coverages were transformed to volume (mm^3) of lens tissue affected by cataracts, using eye lens size. Lens sizes at the first and second cataract screening were estimated as a function of fish length, using a linear regression of lens size with fish length at the final screening ($r^2 = 0.770$, $P < 0.001$, $N = 238$).

Per-parasite damage was analysed using a linear mixed model that included cataract coverage (mm^3) at the final screening as response variable, temperature, fish species and parasite genotype as categorical factors, parasite load as covariate, and fish population as well as tank as random effects. Per-parasite damage was quantified as slope of cataract coverage against parasite load (Råberg et al. 2009). Both variables were log +1 transformed to linearize the relationships. The initial model also included the quadratic term of parasite load to account for possible non-linear relationships between this variable and cataract coverage (Råberg et al. 2009). However, the term was not significant and thus omitted to reduce model complexity. The final model included all two-, three- and four-way interactions. As cataracts were never observed in uninfected individuals, and all infected individuals had at least some cataracts, the intercept of the slopes was set to zero (Klemme and Karvonen 2017).

Development of cataract coverage at different temperatures over time was explored with a linear mixed model, including log +1 transformed cataract volume at all three measurements as response variable. Time and temperature were entered as categorical factors and log +1 transformed parasite load as covariate. To account for repeated measures within the same individual, fish ID was entered as random effect. *P*-values in pairwise comparisons were *Bonferroni* corrected.

Results

Per-parasite damage was significantly affected by temperature, host species and parasite genotype, indicated by their significant interactions with parasite load (Table 2). Eight weeks after temperature manipulation, fish maintained at 5ºC showed significantly lower per-parasite damage than at 16ºC (Fig. 1). Salmon had also lower per-parasite damage than trout (Fig. 1). All other two-, three- and four-way interactions were not significant, suggesting absence of genotype by temperature interactions.

Cataract volume, measured repeatedly during the experiment, was affected by an interaction between temperature and time ($F_{2,699} = 30.20$, $P < 0.001$). At 5ºC, cataract volume did not change during the first four weeks of temperature manipulation ($t_{699} = -1.99$, $P = 0.701$), but decreased significantly during the following four weeks ($t_{699} = -4.15$, $P = 0.001$, Fig. 2). At 16ºC cataract volume increased significantly throughout the study (first 4 weeks: $t_{699} = -3.35$, $P = 0.001$; 4 to 8 weeks: $t_{699} = -4.43$, $P < 0.001$, Fig. 2).

Discussion

Environmental conditions can shape the dynamics of host-parasite interactions and consequently host health. Here, we provide evidence for temperature effects on per-parasite damage in salmonid fishes with established eye fluke infections. We found smaller parasite-induced eye damages at a given parasite load at 5ºC compared to 16ºC, with cataract volume decreasing in cold and increasing in warm water. Per-parasite damage was also affected by host and parasite genotypes, but these effects were stable across temperatures. Overall, our results suggest opportunities for aquatic hosts to recuperate from parasite-inflicted damage during cold-water seasons.

Parasite-induced eye cataracts in this system are caused primarily by parasite movement and metabolic excretions in the eye lens, leading to structural damage (Shariff et al. 1980). As cataract size increases, fish hosts become less efficient at feeding (Karvonen and Seppälä 2008) and more susceptible to predation (Seppälä et al. 2005). Here, the reduced cataract volume in cold water (Fig. 2) is suggestive of tissue repair by the host and thus, the smaller per-parasite damage could indicate increased tolerance at these temperatures. This could be explained by a drastically reduced infection risk from eye flukes (Karvonen et al. 2004a) and many other parasites at cold temperatures, potentially allowing hosts to re-allocate resources from resistance mechanisms towards tolerance. Fish immune functions vary seasonally and are typically down-regulated at low temperatures (Bly and Clem 1992). While this is mainly related to substantial costs involved in maintaining the immune system (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000) and physiological constraints in the cold (Le Morvan et al. 1998), downregulating redundant immune functions may also release some additional resources for tolerance.

A reduced per-parasite damage at lower temperatures could also be attributed to parasite activity. As in many other trematode taxa, the activity and development of larval *D. pseudospathaceum* stages are reduced with decreasing temperature (Karvonen 2012; Karvonen and Marcogliese 2020). This could reduce parasite movement and production of metabolites in the eye lens and thus, slow down or halt cataract growth. In consequence, a decrease in temperature may simultaneously increases host tolerance and reduce the degree of parasite-inflicted damage. Alternatively, tolerance may be insensitive to temperature, but reduced per-parasite pathogenicity in lower temperatures could increase the impact of tissue repair.

As our experimental design included a temperature change in one treatment group (5ºC), while remaining constant in the other (16ºC), it could also be argued that the observed

reduction in per-parasite damage is related to the temperature shift and subsequent acclimation. Due to their smaller size and faster metabolism, parasites are expected to thermally acclimate faster than their hosts (Raffel et al. 2013). This could result in an apparent decrease in per-parasite damage after a sudden drop in temperature, if parasite activity was reduced, but costly host tolerance mechanisms were not yet down-regulated. However, we consider this unlikely as per-parasite damage was measured 8 weeks after a gradual temperature change, which is considerably longer than the two weeks generally sufficient for thermal acclimation in fish (Talo and Tirri 1991). Thus, while an acclimation effect cannot be fully excluded, we suggest that temperature impacts per-parasite damage.

Independent of the underlying mechanism, our results are suggestive of seasonal variation in per-parasite damage. We therefore propose that winter could be an important, but so far neglected period of host recuperation in temperate aquatic systems. A recent multi-annual study on a natural population of Galápagos mockingbirds (*Mimus parvulus*) demonstrated temporal changes in tolerance to experimental infections with a parasitic fly (*Philornis downsi)*, which was related to annual variation in rainfall and, consequently, resource availability (McNew et al. 2019). This emphasizes the importance of studying the effect of temporal environmental variation on per-parasite damage, in addition to infection intensity (Nelson and Demas 1996; Wolinska and King 2009), for a more realistic view of virulence in the wild.

Besides the observed temperature effects, we also found host and parasite genotype-specific variation in per-parasite damage. The first finding is in accordance with our previous results in this system, demonstrating a genetic trade-off between resistance and tolerance, with sea trout showing higher resistance and lower tolerance than Atlantic salmon (Klemme and Karvonen 2017; Klemme et al. 2020). Although both species are closely related and share many life-history traits, sea trout are known to be more active and aggressive than

Atlantic salmon (Jonsson and Jonsson 2011). Thus, it is possible that sea trout experience a higher risk of parasite exposure and, consequently, employ effective resistance mechanisms at the cost of lower tolerance (Sears et al. 2013; Klemme and Karvonen 2017; Klemme et al. 2020). Interestingly, however, this species-specific pattern was consistent across the experimental temperatures, suggesting that sea trout also invest less into tolerance during periods of low infection risk.

The parasite-genotype effect suggests that genotypes differ in per-parasite pathogenicity, which also confirms earlier findings (Klemme and Karvonen 2019). As described above, an increase in cataract size is associated with increased predation risk by birds (Seppälä et al. 2005). This is beneficial for the parasite, which needs an avian final host to complete its life cycle (Karvonen 2012) and should therefore lead to selection favouring increased ability to induce cataracts. Our present results indicate that temperature does not alter genotype-specific differences in this ability, but it is known that other types of environmental factors, such as the competitive environment experienced within a host, can change genotype rank order for per-parasite pathogenicity (Klemme and Karvonen 2019).

Taken together, we show that the per-parasite damage of an established infection can be shaped by combined effects of hosts, parasites and the environment. Variation in temperature could either modulate the host's ability to repair damage, the per-parasite pathogenicity, or both, leading to host recuperation in cold temperatures. Such positive health effects, however, could be at risk due to the ongoing climate change. Not only are water temperatures predicted to rise generally, but cold-water periods will likely shorten and be subject to more pronounced temperature fluctuations (Heino et al. 2009). This emphasizes the importance of studying condition-dependency of per-parasite damage to understand the future consequences of parasitism on host health.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

All applicable institutional and/or national guidelines for the care and use of animals were followed.

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Table 1 Overview of the experimental timeline and procedures

Week number	Experimental procedure
0	Fish transferred
1	PIT tagging
2	Experimental parasite exposures
14	First determination of eye cataracts and fish length
	Temperature manipulation commenced
15	Target temperatures reached
19	Second determination of eye cataracts and fish length
23	Third determination of eye cataracts and fish length
	Eyes dissected for lens size and parasite load

Table 2 Linear mixed model analyses of variation in per-parasite damage. Parasite induced cataract volume (mm^3) was entered as response variable, temperature (5ºC and 16ºC), host species (Atlantic salmon and sea trout) and parasite genotype (N=3) as fixed factors, parasite load as continuous predictor, and population as well as tank as random effects. Non-significant interactions are not shown

factor	*df* numerator	*df* denominator	*F*	*P*
temperature	1	2	0.99	0.425
host species	1	8	2.88	0.128
parasite genotype	2	202	3.46	0.033
parasite load	1	202	700.68	<0.001
temperature × load	1	202	11.56	0.001
species × load	1	202	14.63	<0.001
genotype × load	2	202	10.70	<0.001

Figure 1

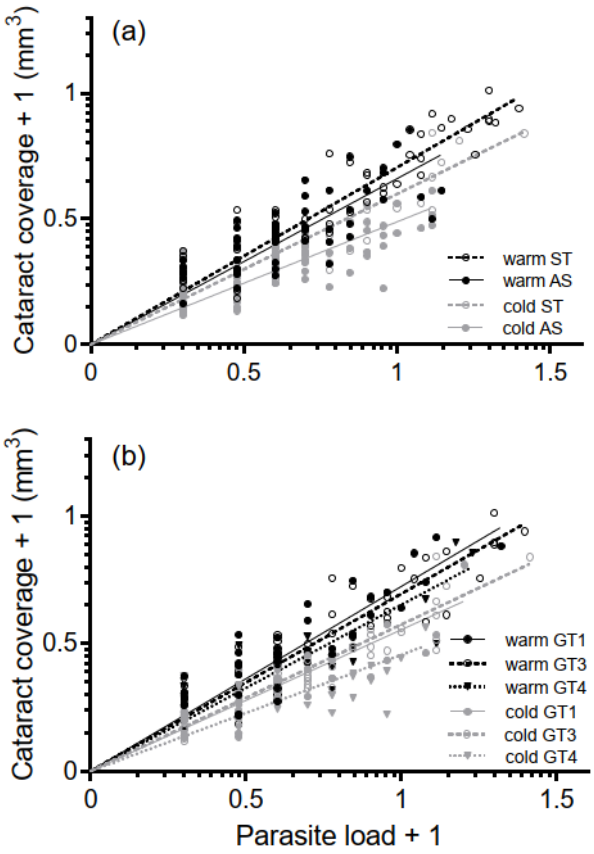

Fig. 1 Per-parasite damage of fish hosts infected with *D. pseudospathaceum*, expressed as slope of parasite-induced cataract volume (tissue damage) against parasite load across (a) two host species, Atlantic salmon (AS, N=129) and sea trout (ST, N=109), and (b) three parasite genotypes (GT1 N=72, GT3 N=84, GT4 N=82). Infected hosts were maintained at either 5ºC (cold, N=128) or 16ºC (warm, N=110) for eight weeks. Note log-scale used on both axes

Figure 2

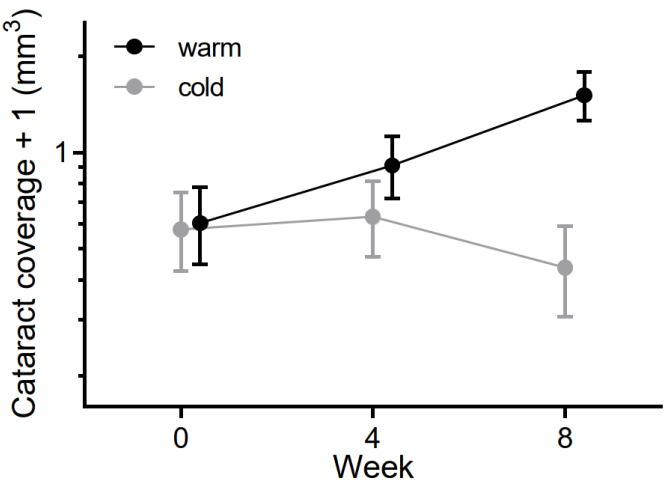

Fig. 2 Predicted parasite-induced cataract volume (mean ± SE) combined for Atlantic salmon and sea trout hosts and three parasite genotypes (N=238). Cataract volume was measured before temperature manipulation (week 0), and 4 and 8 weeks after maintenance at 5ºC (cold) or 16ºC (warm). Note log-scale used on y-axis