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Thermal mismatch in high latitude host-parasite interactions

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Abstract

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Emerging infectious diseases and climate change are urgent wildlife threats responsible for many population reductions and extinctions, but their synergistic effects can be hard to predict, as temperature shifts influence host-parasite dynamics in complex ways. The thermal mismatch hypothesis predicts that cold-adapted hosts will become increasingly susceptible to parasites as temperatures rise. However, temperature effects on host-parasite interactions in high latitudes remain understudied. My thesis investigates the role of temperature and thermal adaptations in shaping host-parasite interactions in high latitudes. For this, I use amphibians and their fungal parasite *Batrachochytrium dendrobatidis* (*Bd*), and perform experimental infections in different temperatures, followed by analysis of the survival, growth and gene expression of each host. The fungus *Bd* causes the disease chytridiomycosis, which has decimated amphibian populations worldwide and is a global conservation concern, with its severity varying between hosts, populations, *Bd* strains and environmental conditions.

In **paper I**, I analyse the transcriptomic responses of two populations of common toads to infection with two *Bd* strains of different origin and uncover tissue differences in gene expression. In **paper II**, I look at the effects of temperature on the survival and growth of four amphibian species from southern Sweden. I discover that the two more cold-adapted species have higher survival and growth when infected with *Bd* at low temperatures compared to one of the warm-adapted ones, that the second warm adapted species is not at all affected by *Bd* infection and that the *Bd* strain I used appears to tolerate high temperature less well than expected. In **paper III**, I make comparisons between the cold-adapted southern species and individuals of the same species from northern Sweden, and find that northern populations may be less susceptible to *Bd* infection despite their overall lower survival. In **paper IV**, I look at the transcriptomic responses of the two cold-adapted species from both populations and find that immune system activity is increased in infected hosts, especially in lower temperatures, but is not always associated with higher survival.

Keywords: host-parasite interactions, high latitude, disease ecology, emerging infectious disease, temperature, thermal adaptation, thermal mismatch, amphibian conservation, chytridiomycosis, climate change, survival, growth, transcriptomics, immune response, differential gene expression, common garden, experimental infection, Batrachochytrium dendrobatidis, Bd, Bufo bufo, Rana arvalis, Bufotes viridis, Pelophylax esculentus

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Στους γονείς μου και στο Γιώργο

«Εὐημεροῦσι δὲ τὰ ζῶα κατὰ τὰς ὥρας οὐ τὰς αὐτάς,
οὐδ' ἐν ταῖς ὑπερβολαῖς ὁμοίως ἁπάσαις·
ἔτι δ' ὑγίειαι καὶ νόσοι κατά τε τὰς ὥρας τοῖς ἑτερογενέσιν ἕτεραι
καὶ τὸ σύνολον οὐχ αἱ αὐταὶ πᾶσιν.»

Αριστοτέλης, Περὶ τῶν ζώων ἱστορίαι

-- --

"Oh, miraculous chameleon, science, who can reverse your doctrine hourly
and never shake our faith!"

Ada Palmer, Seven Surrenders

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Chondrelli, N.**, Kuehn, E., Meurling, S., Cortázar-Chinarro M., Laurila A., Höglund J. *Batrachochytrium dendrobatidis* strain affects transcriptomic response in liver but not skin in latitudinal populations of the common toad (*Bufo bufo*). *Scientific Reports* **14**, 2495 (2024). https://doi.org/10.1038/s41598-024-52975-8

II. **Chondrelli N.**, Eghbal R., Blom S., Fridh F., Waldenström J., Laurila A., Höglund J., Thermal mismatch at low but not high temperatures in high latitude amphibians exposed to *Batrachochytrium dendrobatidis* (Manuscript)

III. **Chondrelli N.**, Waldenström J., Höglund J., Laurila A., Thermal mismatch and latitudinal variation in susceptibility to chytridiomycosis in two high latitude amphibians (Manuscript)

IV. **Chondrelli N.**, Waldenström J., Laurila A., Höglund J., Temperature effects on gene expression of amphibian populations infected with the chytrid fungus along a latitudinal gradient (Manuscript)

Contents

Abbreviations

Bd	*Batrachochytrium dendrobatidis*
Bd-GPL	*Bd*-Global Panzootic Lineage
Bsal	*B. salamandrivorans*
MHC	Major Histocompatibility Complex
qPCR	quantitative Polymerase Chain Reaction
RSW	Reconstituted Soft Water

Introduction

Emerging infectious diseases are viewed as a pressing wildlife threat (Daszak et al. 2000) and their emergence is often linked to human-assisted pathogen transport (Cunningham et al. 2003) and habitat alterations (Patz et al. 2000), which catalyze changes in host and/ or pathogen ecology (Schrag and Wiener 1995). More recently, the role of global warming and climate variability has also been recognised in the context of emerging diseases (Rohr et al. 2011, Raffel et al. 2013), but predicting the effects of climate change on host-parasite interactions remains challenging due to the complexity of ecological interactions involved (Rohr and Cohen 2020). For example, temperature increases may drive the range expansion of pathogens to formerly unhospitable habitats and thus new (sometimes naïve) host populations (Hickling et al. 2006, Dobson 2009), but could also lead to parasite range contractions (Lafferty 2009).

A predictive framework for the outcome of infection in different temperatures was presented by Cohen et al. (2017) through the thermal mismatch hypothesis. The thermal mismatch hypothesis predicts that in a system where the host and parasite are locally adapted to the prevailing temperature, shifts that move the temperature away from the host thermal optima will result in higher host susceptibility. This hypothesis rests on the assumption that pathogens have wider thermal breadths than their hosts due to their smaller size and higher mass-specific metabolic rates (Brown et al. 2004, Kingsolver and Huey 2008, Rohr et al. 2018) and are able for quicker acclimation to track the temperature shift compared to their hosts. According to the thermal mismatch hypothesis, warm-adapted hosts will be more vulnerable to parasites in colder temperatures, while cold-adapted hosts will be susceptible as temperatures rise (Figure 1). In a meta-analysis, Cohen et al. (2020) found support for this hypothesis when examining 2021 host-parasite pairs and predicted that, under various greenhouse gas emission scenarios, disease risk will in general increase in temperate and boreal zones. The risk for high latitude hosts as predicted by the thermal mismatch hypothesis is dire, not least because the rate of climate change will be up to four times faster in and close to the arctic, compared to the tropics (Twardosz et al. 2021, Rantanen et al. 2022). However, research in these areas is lagging behind.

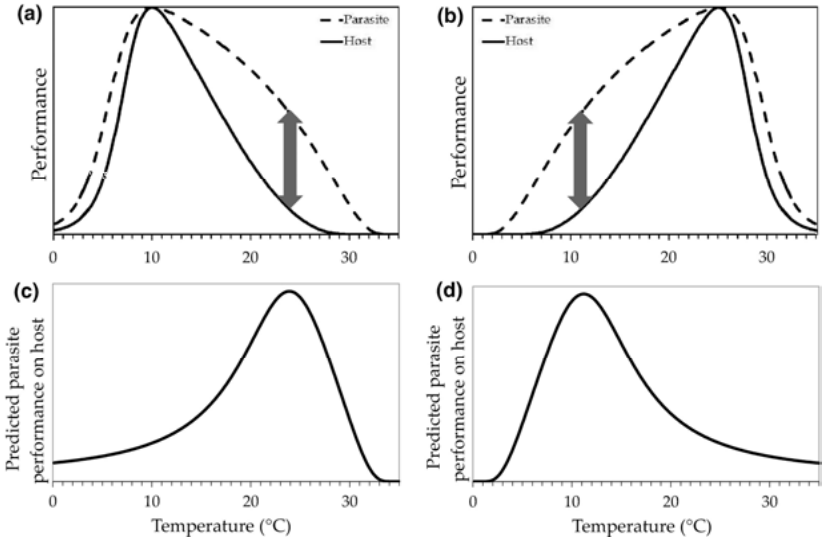


Figure 1. Schematic representation of the thermal mismatch hypothesis. The thermal range of a parasite is often wider than that of its host (top panels) which results in parasite performance (bottom panels) peaking, not at the parasite's thermal optimum, but at the temperature where its performance is highest compared to the host's. Cold-adapted hosts (left-hand panels) are more affected in higher temperatures and warm-adapted host (right-hand panels) in colder temperatures. Figure adapted from Cohen et al. 2017.

One of the parasites that could pose a significant threat to high latitude hosts if temperatures continue to rise is the amphibian skin fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*, Longcore et al. 1999). Zoospores of *Bd* and its sister species *B. salamandrivorans* (*Bsal*, Martel et al. 2013), colonize the skin of amphibians, where they embed themselves and mature, producing and releasing new zoospores on the skin surface, that can reinfect the host or enter the environment (Rosenblum et al. 2010). *Bd* and *Bsal* can cause chytridiomycosis (Berger et al. 1998), a disease characterized by severe dysregulation of the skin osmoregulatory functions, that can lead to electrolyte imbalance and death by cardiac arrest (Voyles et al. 2009, Campbell et al. 2012). Chytridiomycosis has led to the decline of more than 500 species worldwide and is involved in the extinction of 92 of them (Scheele et al. 2019), while *Bd* is able to infect at least 50% of all amphibian species tested thus far (Monzon et al. 2020). In fact, the first definitive record of a species extinction due to disease in the wild was that of the amphibian species *Taudactylus acutirostris* succumbing to chytridiomycosis (Schloegel et al. 2006). Nowadays, many of the enigmatic amphibian declines that have been documented since the 1960s and onward (Houlahan et al. 2000) are also believed to be due to infection with *Bd* (Berger et al. 1998, Fisher and Garner 2020).

The amphibian – chytrid fungi system is very suitable for thermal research. On the one hand, amphibian hosts are ectotherms, so many of their physiological functions, like metabolism, reproduction rate, behavior and immunity are greatly affected by temperature (Kingsolver 2009). On the other hand, the near ubiquity of the fungus *Bd*, which is endemic in South East Asia (O'Hanlon et al. 2018), but has now spread to every continent where amphibians exist through the exotic pet trade (Walker et al. 2008) and human travel (O'Hanlon et al. 2018), presents opportunities for research in different thermal environments, and in amphibians with a multitude of thermal adaptations and from many altitudes and latitudes.

Multiple *Bd* strains are currently recognised, the most widespread being the highly virulent *Bd*-GPL (Global Panzootic Lineage), which has also been contributing to many of the amphibian declines and extinctions (Farrer et al. 2011). Also of interest for thermal research on host-parasite interactions is the fact that *Bd*, originally thought to thrive in temperatures between 17-25ºC with a thermal range between 4 and 28ºC (Piotrowski et al. 2004, Stevenson et al. 2013) has since been found to have varying thermal performance curves across strains and isolates (Sheets et al. 2021), and can survive freezing (Voyles et al. 2017) and heating (Kásler et al. 2022). Temperature affects *Bd* zoospore size and production rate (Woodhams et al. 2008, Voyles et al. 2017), characteristics that seem to affect the virulence of the fungus (Fisher et al. 2009).

Interestingly, the severity of chytridiomycosis differs between populations and species (Gahl et al. 2012, Brannelly et al. 2012) with host responses ranging from complete resistance to total susceptibility (Råberg et al. 2008) to chytridiomycosis. Epidemic breakouts after *Bd* invasion have mostly been reported from the tropics, like in Eastern Australia (Skerratt et al. 2007) and Central America (Berger et al. 1998), which is an important reason why research in this system has focused less on high latitudes. Host characteristics, and especially those related to immune responses, for instance variation in the Major Histocompatibility Complex (MHC, Savage and Zamudio 2011, Cortázar-Chinarro et al. 2022) and antimicrobial skin peptide production (Pasmans et al. 2013), also play a big role in determining disease outcome. Unsurprisingly, the survival of amphibians exposed to *Bd* is also affected by temperature (Andre et al. 2008, Bustamante et al. 2010, Turner et al. 2021), a pattern perhaps partly attributed to the temperature dependence of immune activation (Butler et al. 2013, Rollins-Smith 2017), evident in characteristics such as differential skin sloughing rate (Meyer et al. 2012) and variation in expression of adaptive immune genes (Ellison et al. 2020) depending on ambient temperature.

Research aims

This thesis has been driven by the desire to explore host-parasite interactions in high latitudes, a research priority rendered imperative by the advent of climate change and its capacity to alter disease dynamics in ways that are hard to predict. It has the added benefit of focusing on a system of great conservation concern, namely that of amphibians, the most threatened vertebrate taxon in the world (Luedtke et al. 2023) and their parasitic skin fungus *Bd,* which causes the worst panzootic known to date (Scheele et al. 2019). It was further motivated by the need to follow up with field studies, which showed that *Bd* infection prevalence was higher in southern compared to norther populations of amphibians in Sweden and also differed greatly between species (Meurling et al. 2020).

I aim to address the overarching question:

- Is temperature a significant factor influencing the result of host-parasite interactions in high latitudes?

with focus on the amphibian-chytridiomycosis system, in which I study:

- Do different strains of the fungus *Bd* elicit the same response in individuals from high latitude amphibian populations?

- How does temperature affect the survival and growth of high latitude amphibian species infected with *Batrachochytrium dendrobatidis*?

- Do the thermal adaptations of the host influence infection outcome?

- Do temperature and host thermal adaptations also affect transcriptomic profiles of *Bd*-exposed amphibians?

To investigate those questions, I carry out experimental infections with *Bd* in three temperatures and on four amphibian species from two geographic areas in Sweden and monitor how their survival, growth and transcriptomic responses are affected by these conditions.

Methods

Common garden infection experiments

Study species and populations

Bufo bufo

In spring 2021 and 2022 I collected eggs from four amphibian species, the common toad *Bufo bufo*, the moor frog *Rana arvalis*, the edible frog *Pelophylax esculentus* and the green toad *Bufotes viridis*. Of these four species, *B. bufo* and *R. arvalis* have low *Bd* infection prevalence in the field in Sweden, while *P. esculentus* and *B. viridis* have a much higher infection prevalence and higher *Bd* loads (Meurling et al. 2020). Sampling was done in Kalmar, southern Sweden for the first three species, as well as close to Luleå, in northern Sweden, for *B. bufo* and *R. arvalis* (Table 1). In each sampling locality, I collected ~ 50 eggs from 10 – 15 clutches or egg strings. As *B. viridis* is a red-listed (VU) amphibian in Sweden with very few wild populations (SLU Artdatabanken 2020), the *B. viridis* eggs were obtained a captive population in Nordens Ark, a non-profit foundation dedicated to ex-situ conservation.

Rana arvalis

Pelophylax esculentus

Bufotes viridis

Table 1. Name, collection date and coordinates of amphibian egg collection localities. *B. viridis* eggs were acquired from Nordens Ark in 14-May-2021.

Bufo bufo **North**	***Bufo bufo*** **South**	***Rana arvalis*** **North**	***Rana arvalis*** **South**	***Pelophylax esculentus***
Sjulmark1	Stjärnamo	Ersnäs	Mönsterås	Hossmo dammen
26-May-22	16-Apr-21	20-May-22	16-Apr-21	28-May-22
65°33'10.3"N	56°45'25.3"N	65°31.266'N	57°01'00.6"N	56°38'21.6"N
21°26'15.0"E	16°03'42.2"E	21°41.186'E	16°26'51.3"E	16°14'45.0"E
Sjulmark2	Kindbäcksmåla	Arvidsjaur	Anebo1	-
30-May-22	27-Apr-21	20-May-22	16-Apr-21	-
65°34'03.6"N	56°30'25.3"N	65°38'53.0"N	56°37'58.1"N	-
21°26'04.8"E	15°45'15.1"E	19°41'54.8"E	15°55'06.3"E	-
-	-	-	Anebo2	-
-	-	-	16-Apr-21	-
-	-	-	56°38'02.1"N	-
-	-	-	15°54'25.0"E	-

Amphibian rearing

Eggs were brought into the laboratory and placed in 22L water tanks (Picture 1), containing ~18L of Reconstituted Soft Water (RSW, 48mg/L $NaHCO_3$, 61.4mg/L $MgSO_4$ * 7 H_2O, 30mg/L $CaSO_4$ * 2 H_2O and 2mg/L KCl, in de-ionized water, Clescerl et al. 1985). RSW was prepared in 90L buckets at least 24 hours before use and kept under constant aeration. At least one third of the water in each tank was replaced every third day. Eggs and tadpoles were housed familywise, i.e. all individuals from an egg string or egg clump were in the same tank. Tanks contained an air stone that enhanced air and water circulation and artificial plants for enrichment. Tadpoles were fed spinach and fish flakes containing spirulina *ad libitum*. To reflect the different thermal adaptations between the species, I raised *B. bufo* and *R. arvalis* tadpoles in 18°C, while *P. esculentus* and *B. viridis* were kept in 20°C. All experimental animals were kept at an 18/ 6h light/ dark cycle, reflecting the light conditions in southern Sweden in late spring and summer.

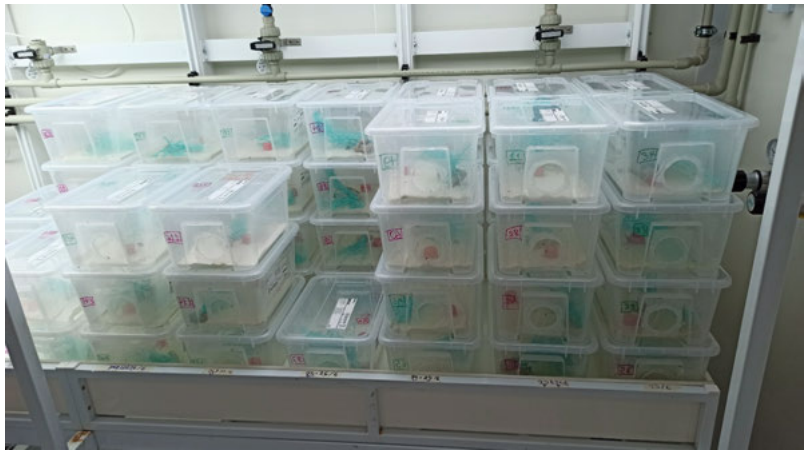
Picture 1. Aquatic tanks for tadpoles

Picture 2. Semi-terrestrial tank

Upon emergence of the first forelimb (Gosner stage 42, Gosner 1960), I moved tadpoles to 5L, semi-terrestrial enclosures (Picture 2), that contained gravel as an artificial shore on one side and 3cm of RSW on the other side, as well as artificial plants and a screw cap as shelter for each individual. Individuals were kept familywise at a density of up to eight per tank and were not fed, as during that stage they utilise nutrients from tail absorption. Water in these tanks was completely replaced daily.

Upon tail absorption (Gosner stage 46), which signifies complete metamorphosis, individuals were moved in completely terrestrial enclosures (Picture 3), which were lined with wettened, unbleached water towels and also contained a screw cap shelter and artificial plant. These enclosures had a mesh-covered hole to enhance air circulation. At this stage individuals were kept individually or in pairs and were given UV light for 10 hours daily, to enhance cutaneous vitamin D3 production (Webb 2006, Chen et al. 2007), an important stage in calcium metabolism (Holick 2003). I also began feeding them again, this time with *Drosophila* fruit flies and occasionally *Callosobruchus maculatus* seed beetles, which was their diet until completion of the experiment.

Picture 3. Terrestrial tanks in infection room



After seven days in the UV light, all metamorphs were placed in individual terrestrial tanks and randomly assigned to one of three climate rooms, at 14, 18 or 22ºC, where they acclimated for a week prior to the experimental inoculations. These temperatures were chosen to represent temperature variation within the activity period of the amphibians ranging from cool to exceptionally warm summer conditions. At that stage, individuals were also randomly assigned to one of two *Bd* treatments, control or *Bd*-exposed.

The total number of experimental individuals that were included in the papers was 1052 (of the 1145 metamorphs raised), corresponding to 176 northern *B. bufo*, 181 southern *B. bufo*, 166 northern *R. arvalis*, 204 southern *R. arvalis*, 183 *P. esculentus* and 142 *B. viridis*.

Experimental inoculation and disease monitoring

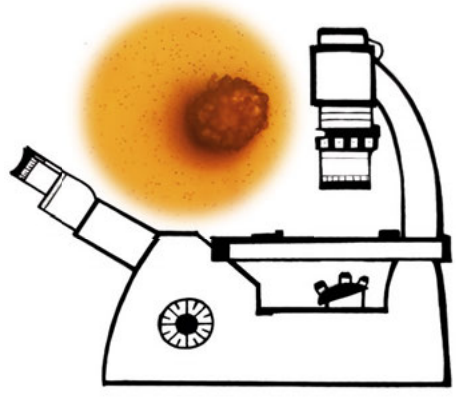
After the one-week acclimation period in the climate rooms, individuals were weighed and placed in individual petri dishes containing 30ml RSW, where they were sprayed (Picture 4) with either 200ul of liquid *Bd* culture containing ~ 30 000 motile zoospores ("*Bd*-exposed" treatment), or 200ul sterile *Bd* culture media ("control" treatment). Individuals were left in the petri dishes for 5h before being returned to their individual terrestrial tanks. After inoculation, each individual was monitored daily until the experimental endpoint, defined as either the point when an individual showed severe signs of chytridiomycosis (i.e. loss of righting reflex) and was euthanised or, at maximum, as 40 days past *Bd*-exposure, when all remaining individuals were euthanised. At the end point, individuals were weighed, to determine their growth during the experiment, swabbed, to quantify *Bd* infection load on their skin, and then humanely euthanised using submersion in 2% tricaine mesylate (MS222) solution followed by pithing. After euthanasia, skin and liver from each individual was excised and stored in RNAlater in -70ºC, until transcriptomic analysis.

Picture 4. "Spraying" with *Bd*

Fungal cultures maintenance and quantification

For the experimental inoculations, I used the *Bd*-GPL strain SWED-40-5, provided by the Zoological Society of London. This strain has been isolated from a wild *B. viridis* caught in Malmö (Norra Hamnen), southern Sweden, in 2015, and passaged 17–20 times prior to the experiment. *Bd* cultures were grown in 20ºC in T25 culture flasks with sterilised culture media, consisting of 0.8% tryptone, 0.2% gelatin hydrolysate and 0.4% lactose in dH_2O, and were kept in the fridge when not in use. I used a haemocytometer for zoospore quantification (Picture 5) in the *Bd* cultures and culture media if dilutions were necessary to achieve the desired concentration.

Picture 5. *Bd* sporangium and zoospores under the microscope



Molecular methods

RNA extraction, library preparation and sequencing

To explore the effects of temperature on the expression profiles of individuals in the experiment, I extracted RNA from a subset of the *B. bufo* and *R. arvalis* skin and liver samples, from both populations, using the Qiagen RNeasy Mini kit. I did the mRNA library preparation myself for the southern individuals using the Illumina TruSeq Stranded mRNA kit, and Novogene processed the samples from northern individuals with proprietary kits. Sequencing was performed on a NovaSeq 6000 system for southern individuals and on a NovaSeq X Plus for northern individuals, each time yielding 150bp paired-end reads.

DNA extraction and qPCRs

In order to quantify the *Bd* infection load on the skin of each individual, or to verify the absence of *Bd*, I extracted DNA from the skin swabs taken at the experimental endpoint, using the PrepMan Ultra reagent. I used the same method to extract *Bd* DNA from filtered *Bd* cultures with a known amount of zoospores and create *Bd* standards of known zoospore concentrations for use in the qPCRs (quantitative Polymerase Chain Reactions).

Afterwards, I ran qPCRs, adjusted from Boyle et al. (2004), targeting the internal transcribed spacer (ITS)–5.8S rRNA region on the *Bd* genome. Each

20ul reaction contained 10ul QuantiTect Probe Mix (2X), 0.2ul of each 50uM primer (ITS-3_Chytr and 5.8S_Chytr), 0.2ul of 20uM MGB probe (5.8S_MGB2), 5ul DNA sample, an internal positive control (i.e. 1ul 10X Exo IPC master mix and 0.45-0.5ul 50X Exo IPC DNA, Hyatt et al. 2007) and water. The reactions were run for 15 min at 95ºC, 45 cycles of 15 sec at 95ºC and 1 min at 60ºC, and 30 sec at 40ºC. I ran each qPCR plate with a negative control and *Bd* standards corresponding to 1000, 100, 10, 1 and 0.1 zoospore equivalents. Samples were run once, and repeated if the positive control failed to amplify.

Data analysis

I used RStudio (Posit team 2023) for all statistical analysis, with R versions spanning 4.2.2 to 4.4.1 (R Core Team 2024). For all plots, I used a combination of package-specific plotting tools and ggplot2 (Wickham 2016).

Survival and growth analysis

To analyse the survival of each species, I used mixed effects Cox proportional hazards models, fit by maximum likelihood using the packages *survival* (Therneau 2024) and *coxme* (Therneau 2022). I also used log-rank tests with Benjamini-Hochberg adjusted p-values, to calculate pairwise comparisons of survival between treatment regimes. For daily growth, defined as the total mass change of an individual divided by the number of days it survived, I used linear mixed-effects models with package *nlme* (Pinheiro and Bates 2000, Pinheiro et al. 2024). As explanatory variables in the models, I used treatment (control or exposed to *Bd*) and temperature (14, 18 or 22ºC) as factorial variables and initial body mass as a continuous covariate. I also included family, i.e. the egg string or clump from which each individual originated, as an additive random effect. When comparing the *B. bufo* and *R. arvalis* populations, I also used population (north or south) as a factorial explanatory variable. In the models, I initially included the interaction terms between all explanatory variables. Then, for model selection, I removed the least significant interactions stepwise, starting with the highest order ones, and evaluated each reduced model against the more complex one with F-tests using the *anova* function. If the more complex model did not offer a significantly ($p < 0.1$) better fit compared to the simpler one, the more complex model was discarded and the process continued.

The survival and growth of *B. bufo* and *R. arvalis* individuals were also analysed separately only for controls, to verify the hypothesis that the populations harbour different thermal adaptations.

Bd infection loads

To evaluate the effects of temperature, initial body mass and population on the log10 transformed infection load of individuals, I fit linear models with normal error distribution with the function *lm*, for the individuals of each species that tested positive for *Bd*. The initial model for each species included interaction terms, which were evaluated and potentially dropped as described above.

To assess the differences in percentage of individuals that tested positive for *Bd* in each temperature, I used Pearson's Chi-squared tests on the *Bd*-exposed individuals of each species.

Bioinformatics

I used Trimmomatic (Bolger et al. 2014) to remove sequencing reads of low quality as well as to trim adapters and evaluated the resulting reads using FastQC (Andrews 2010) and MultiQC (Ewels et al. 2016). Then, I mapped the *B. bufo* samples to the *B. bufo* genome (Streicher 2021a) and the *R. arvalis* samples to the *R. temporaria* genome (Streicher 2021b) using STAR (Dobin et al. 2013). To quantify transcripts in each sample, I used RSEM (Li and Dewey 2011).

Differential gene expression

For the differential gene expression of *B. bufo* and *R. arvalis* individuals, I used a subset of genes present in the genomes of species to be able to make comparisons between them. I used the package *DESeq2* (Love et al. 2014) to build a DESeqDataSet object, filter low gene counts (I kept genes with at least ten counts in 5 or more samples), and perform the differential expression analysis using function *DESeq*. The factorial explanatory variables I used in the analysis were species (*B. bufo* or *R. arvalis*), population (north or south), tissue (skin or liver), treatment (control or *Bd*-exposed) or infection status (control, *Bd*-positive or cleared), temperature (14, 18 or 22°C), survival (surviving or dead at the experimental endpoint), *Bd* test results (positive or negative), and I also included initial body mass as a continuous covariate. I repeated these steps, with appropriate subsets of the variables, for different subsets of the data, from the full dataset containing all individuals of both species and populations, down to very small parts of the dataset, like samples from one tissue from a single population and species in one of the temperatures. For lists of genes that were differentially expressed between samples for the comparisons outlined above, I used the function *results*, with different contrasts. Differentially expressed genes were considered those with a log2 fold change significantly different than zero (bigger or smaller), using false discovery rate corrected p-values < 0.05.

To functionally profile the sets of differentially expressed genes I used the online tool g:Profiler (Kolberg et al. 2023) with the g:SCS multiple testing correction method, and a 0.05 significance threshold. I tried to use custom annotation data for *B. bufo* and *R. arvalis* from UniProt, but due to the very small number of annotated genes in the species, the enrichment analysis yielded no results. I thus resorted to the *Homo sapiens* dataset as a reference, and searched the gene ontology database for biological processes.

Paper I individuals

The data of paper I originated from a previous infection experiment, aimed at investigating the effects of different *Bd* strains on the survival, growth and transcription of exposed *B. bufo* individuals. That infection experiment was similar to the ones I carried out, with a few key differences:

a) There was only one temperature regime, 19ºC, kept constant throughout the experiment.
b) Apart from the Swedish *Bd* isolate in my infection experiments, a second isolate from the UK was used.
c) Metamorphs were followed for 30 instead of 40 days past infection.
d) The southern populations from which eggs were sampled were in Skåne, 0.3-1 degree of latitude south of my sampling localities in Småland.

I used skin and liver tissue harvested from this experiment, to investigate potential differences in gene expression between the populations and *Bd* treatments. The bioinformatic and data analysis approach was as described above.

Results and discussion

Effects of *Bd* strain on *B. bufo* transcription (Paper I)

In **paper I**, I analysed the skin and liver transcriptomic profiles of *B. bufo* metamorphs from two populations, one from northern and one from southern Sweden, exposed to the fungus *Bd*. The tissue samples were collected during an earlier infection experiment (Meurling et al. 2024) using two strains of *Bd*, one from Sweden and one from the UK, and one temperature regime, 19°C. Mortality of metamorphs in that infection experiment was mediated by treatment, with *Bd*-exposed individuals having lower survival, and body size, with heavier individuals having higher survival (Meurling et al. 2024). In the subset of individuals used in this gene expression study, all liver and skin samples from control individuals were from individuals that survived the 30-day infection experiment, while mortality in *Bd*-exposed individuals was higher in northern compared to southern individuals (Table 2).

Table 2. Number of samples from skin and liver of *B. bufo* metamorphs from two latitudinal populations in Sweden, exposed to either of two *Bd* strains (from Sweden or the UK) or sham-exposed to *Bd* growth medium (controls). In parenthesis is the number of samples from individuals surviving the 30-day infection experiment.

Treatment	**Population**	**Skin**	**Liver**
Control	North	11 (11)	13 (13)
	South	16 (16)	16 (16)
***Bd*-SWE**	North	18 (7)	14 (6)
	South	14 (10)	13 (11)
***Bd*-UK**	North	22 (3)	18 (0)
	South	13 (12)	11 (11)

Gene expression differences in the full dataset, containing 22 178 genes, were driven primarily by tissue type with ~ 75% of all genes being significantly up- or down-regulated in skin compared to liver samples. The skin is an important organ in amphibians, responsible for oxygen intake and osmoregulation (Campbell et al. 2012) and the site of *Bd* infection (Berger et al. 1998, 2005b), with many active immune pathways, such as the MHC and antimicrobial peptides (Grogan et al. 2018b). The liver of amphibians is involved in energy and

protein metabolism and glycogen storage (Crawshaw and Weinkle 2000) and also has some immune related functions, like the development of B-cells (Hadji-Azimi et al. 1982). As these organs have largely different functions, the differences in gene expression are not unexpected (Engwerda and Kaye 2000), and I proceeded to analyse the expression profiles of skin and liver separately.

A second axis along which both skin and liver samples were differentiated was that of survival status, i.e. whether each individual died prior to the end of the 30-day experiment or survived and was euthanised at the endpoint. 46% of genes in skin and 25% in liver samples were differentiated based on this variable. There were genes related to regulation of cell death, the expression of glucocorticoids, stress and some immune functions upregulated in skin samples of dead individuals, and developmental terms in liver samples, but the responses were not strong. The lack of big expression differences between samples from surviving and dead individuals may be related to sampling constraints, namely the fact that some metamorphs were euthanised and others found dead and sampled many hours after death. It may also be a result of the misregulation of liver and skin metabolites caused by *Bd* infection (Grogan et al. 2018c). For further analysis, I focused only on surviving individuals trying to elucidate what gene expression patterns are associated with survival in the species, and whether they differ by *Bd* strain.

In the skin, gene expression differed mostly between control individuals and those exposed to either of the *Bd* strains (39% of genes in SWE vs controls and 32% of genes between UK and controls), while there were only very few differences between surviving individuals exposed to the two *Bd* strains (1.2% of genes), despite the differences in mortality between the *Bd* treatments. A noteworthy difference between the strains was the activation of a complement component only in skin of individuals exposed to the Swedish strain of *Bd*, which could be in part responsible for the higher survival rates of those individuals, as the complement system is known to enhance other immune factors (Rodriguez and Voyles 2020). Savage et al. (2020) also found that skin gene expression did not differ as much in amphibians with different levels of susceptibility to *Bd*, while they observed major differences in spleen samples. Further zooming in within each population, revealed that more genes in the northern compared to the southern samples were differentially expressed between control individuals and those exposed to *Bd*. In *Bd*-exposed individuals, these genes were functionally enriched for terms related to both the innate (e.g. cytokines, macrophages) and the adaptive arm (e.g. B and T – cells) of the immune system. The immune-related enriched terms were more by number for northern samples, but similar by percentage over all enriched terms between the two populations (Figure 2). Interestingly, 40% of the upregulated genes in *Bd*-exposed compared to control individuals were shared across populations and *Bd* strains.

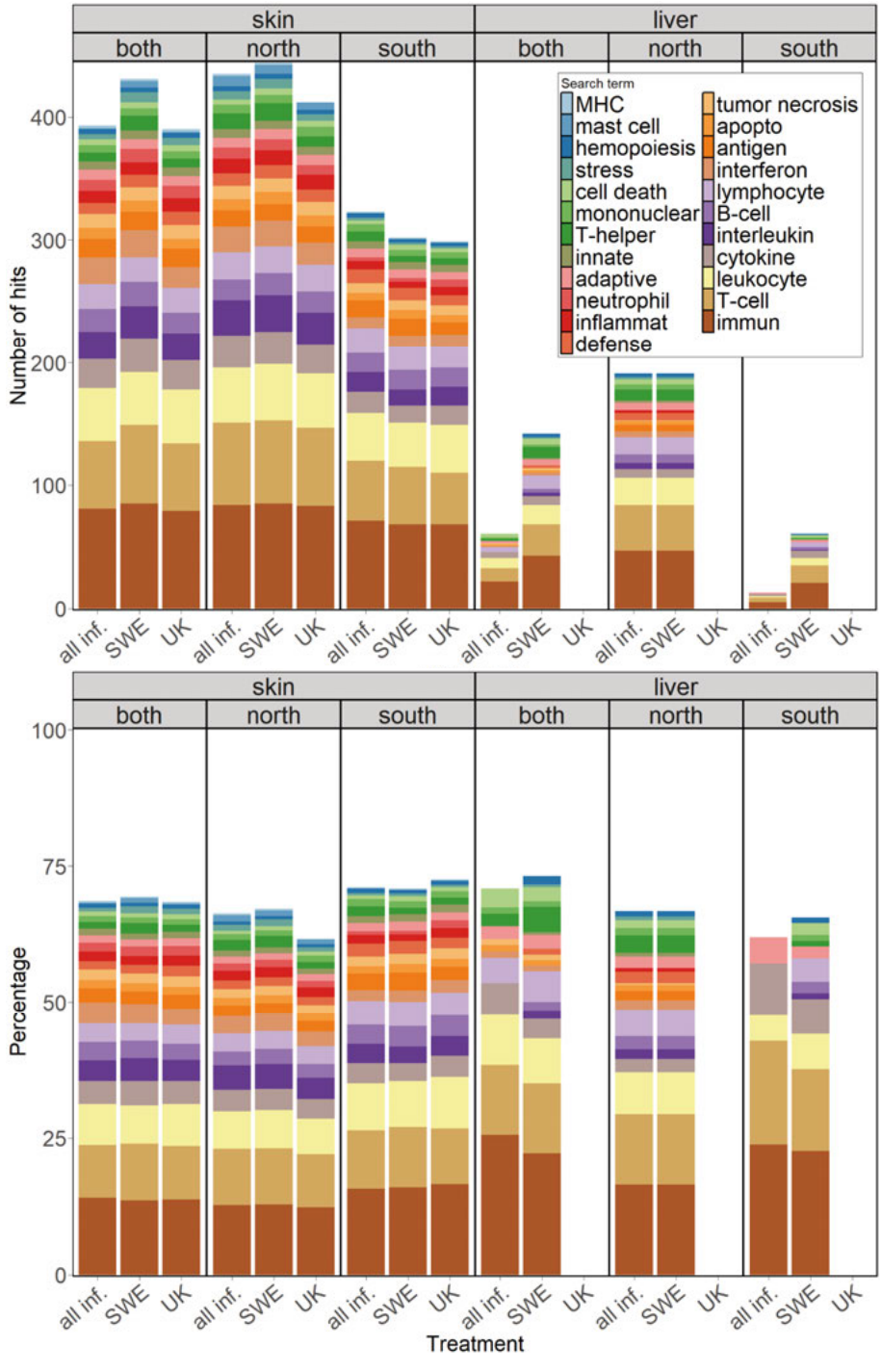

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Figure 2. Number of immune related gene ontology terms (top) and percentage over all terms (bottom), which are functionally enriched in sets of genes upregulated in surviving *Bd*-infected compared to control *B. bufo* metamorphs using the *Homo sapiens* database for biological processes in g:Profiler. The data is presented separately for each tissue sampled (skin–liver), and for population of origin for the *B. bufo* metamorphs (both, north only or south only). The metamorphs were exposed to one of two *Bd* strains, a Swedish one and one from the UK, and results are presented for comparisons of all *Bd*-exposed individuals to controls (all inf.: all infected individuals), as well as for individuals infected with each *Bd* strain separately (SWE: individuals infected with the Swedish *Bd* strain, UK: individuals infected with the UK *Bd* strain).

Liver gene expression presented a stark difference to skin gene expression, as in this tissue the two *Bd* strains produced different responses. There were still large expression differences between samples from control individuals and those exposed to the Swedish *Bd* strain (39% of genes), but the UK *Bd* strain elicited almost no response in liver (only 4.4% of genes differentially expressed between controls and UK *Bd*-exposed liver samples), a pattern that persisted when analysing only samples from southern individuals (analysis with the northern liver samples was not possible as none of them are from surviving individuals). *Bd* strain effects on other variables, like survival time (Berger et al. 2005a), infection load (Meurling et al. 2024), growth rate (Retallick and Miera 2007) and expression of corticosteroids (Gabor et al. 2015) have been reported in the literature, but this is the first study to examine the full range of amphibian transcriptomic responses to different strains of *Bd*. It is possible that the UK isolate used in this study, which has undergone more passages in the laboratory than the Swedish one (both isolates are listed in O'Hanlon et al. 2018), has accumulated mutations that allow it to escape detection from the liver response mechanisms. Only genes overexpressed in liver samples of *Bd*-SWE-exposed individuals were enriched for immune-related terms, and, as in skin samples, more so when originating from northern compared to southern individuals. The population differences likely represent genetic differences between the populations, which have been shaped by their different colonization routes to Scandinavia (Thörn et al. 2021) and could affect the process of immune system activation. Both a non-helpful, prolonged immune reaction (Savage et al. 2020) and suppression of the immune system (Rosenblum et al. 2009, Fites et al. 2013, Savage et al. 2020) have been documented in different amphibians following exposure to *Bd*, the former scenario being more likely in this system, as northern individuals suffer higher *Bd*-mediated mortality.

Survival and growth analysis (papers II and III)

In **papers II** and **III**, I analysed the effects of temperature on the survival and growth of four amphibian species, from a total of six populations, exposed to *Bd*. Through the same experimental set-up, I studied how host thermal adaptations influence the outcome of *Bd* exposure, both among species from the same latitude and in latitudinal populations of the same species.

Species comparisons

In **Paper II**, I explored the effects of temperature on the survival and growth of four amphibian species from southern Sweden, *B. bufo*, *R. arvalis*, *B. viridis* and *P. esculentus*, exposed to a high dose of *Bd*. The former two species are early breeders (March-April), with distribution ranges reaching to the northernmost parts of Sweden (Sillero et al. 2014) and are considered more cold-adapted. The latter have a limited distribution Sweden where they are restricted in the southernmost part of the country, reproduce later during the year (May-June) and are in this context considered more warm-adapted. According to the thermal mismatch hypothesis, we expected to see more dire effects of *Bd* infection for each species in temperatures departing from their thermal optimum.

The original assessment of the study species as cold- or warm-adapted was confirmed by the survival and growth patterns of control individuals in the three temperature regimes (Fig. 3c-f, 4c-f). *B. bufo* and *R. arvalis* had lower mortality in the lowest temperature, 14ºC, and maximum growth in 18ºC, while *B. viridis* was more warm-adapted, with highest survival and growth in 22ºC. *P. esculentus* also had highest growth at 22ºC, which justifies its classification as warm-adapted, but exhibited an unexpected survival pattern, discussed below.

With the exception of *P. esculentus*, exposure to *Bd* was detrimental for all species, which suffered from excess mortality and reduced growth. However, the warmest temperature regime, 22ºC, appears to be at the edge of the thermal optimum of the *Bd* isolate used, as is evident from multiple experimental results. The three species managed to clear infection at a higher rate in 22ºC compared to the lower temperature regimes and in *Bd*-positive individuals, infection load was lower in 22ºC. In that temperature, survival of *Bd*-exposed individuals was also significantly improved in comparison to the lower temperatures (Fig. 3c-e).

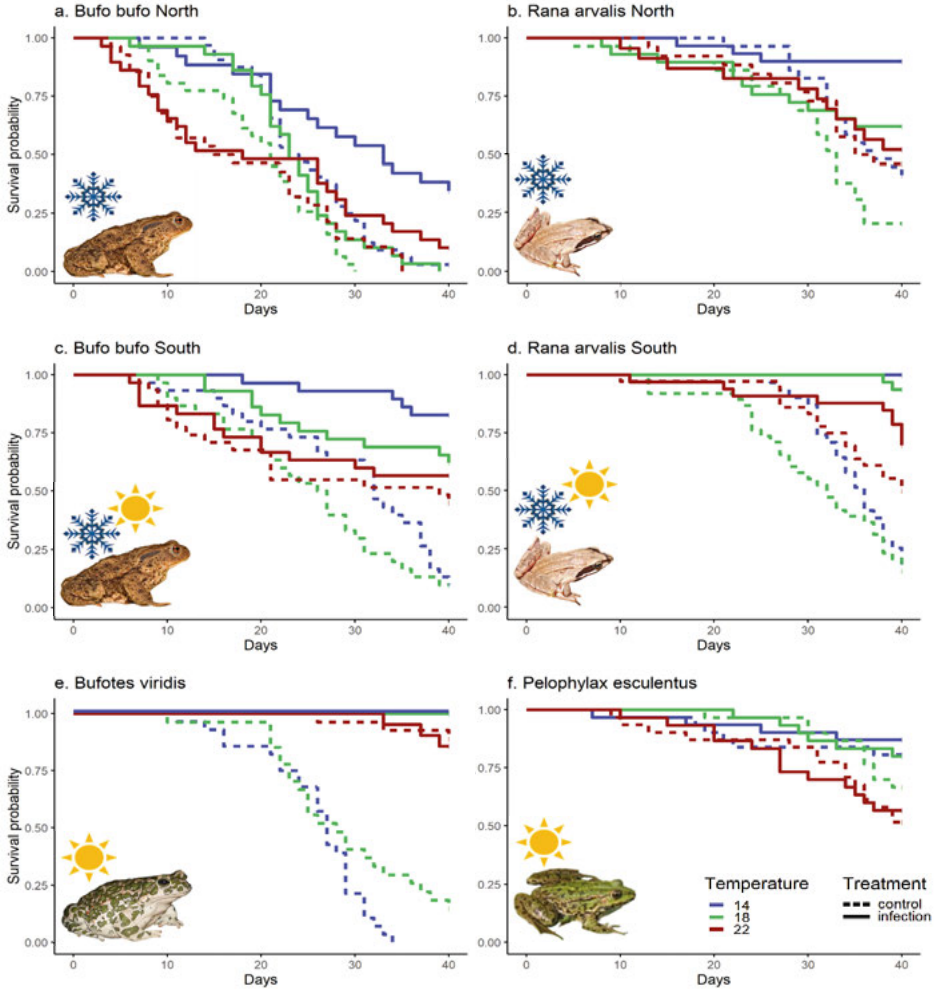

Figure 3. Survival curves for individuals from six high latitude populations exposed to the fungus *Bd*. Survival is shown by temperature (blue: 14ºC, green: 18ºC, red: 22ºC) and by treatment (solid line: control, dashed: exposed to *Bd*). Each individual was followed until death for a maximum of 40 days past exposure. Snowflakes and suns represent the thermal adaptations of each population, with the northern *B. bufo* and *R. arvalis* being the most cold-adapted, their southern counterparts being intermediate and *B. viridis* and *P. esculentus* being the most warm-adapted amphibians in the experiment.

This result was unexpected, as the thermal optima of the early breeders, *B. bufo* and *R. arvalis*, is below 22ºC, and based on the thermal mismatch hypothesis, they are expected to be more vulnerable to *Bd* in temperatures warmer than their optima. It is also a surprise, as parasites generally have wider thermal ranges compared to their hosts (Kingsolver and Huey 2008). The *Bd* isolate used in this experiment may have evolved a lower thermal tolerance than the generally reported for *Bd*-GPL strains of 17-25ºC (Longcore et al. 1999, Piotrowski et al. 2004), either as an adaptation to the cooler climate in Sweden (Fisher et al. 2009) or as a result of prolonged maintenance under laboratory conditions (Kásler et al. 2022). As a remedy for this uncertainty, simultaneous culturing of *Bd* cultures *in vitro* in the same temperature regime as hosts experience would be recommended (as in Raffel et al. 2013, Stevenson et al. 2020). Alternatively, the observed departure from the thermal mismatch hypothesis may be hinting at the wider thermal breadth of high latitude hosts (Rohr et al. 2018), which may allow them withstand infection at a broader range of temperatures. This has already been noted by Cohen et al. (2019), who showed that in higher latitudes, temperature was not as strongly associated with *Bd* prevalence. In the lower temperatures, the thermal mismatch hypothesis is better at describing the survival and growth differences between the species, namely that the early breeders had a higher survival compared to *B. viridis* when exposed to *Bd* in lower temperatures (Fig. 3c-e).

In this experiment, I also observed that *Bd* reduced the growth of *B. bufo*, *R. arvalis* and *B. viridis*, especially in the two lower temperature regimes (Fig. 4c-e). This is a known sublethal effect of *Bd* exposure and may be attributed to the inappetence of infected individuals (Berger et al. 1999) or trade-offs between development and mounting an immune response against the fungus (Lochmiller and Deerenberg 2000). A lower growth rate is likely to reduce the fitness of amphibians, as it increases predation risk (Wilbur 1984, Werner 1986) and decreases overwintering survival (Møller et al. 1998).

Conversely to the other three species, only two *P. esculentus* individuals, both of which were euthanised before day 40, tested positive for *Bd* at the experimental endpoint. Their survival was only nearly significantly affected by *Bd* exposure, perhaps attributed to the increased cost of pathogen clearance (Lochmiller and Deerenberg 2000). *P. esculentus* is reported as a highly *Bd* tolerant species (Woodhams et al. 2012), and *P. lessonae*, one of *P. esculentus'* parental species, has been reported to clear infection in the wild in Sweden (Kärvemo et al. 2020). More intriguingly, the survival of *P. esculentus* was negatively affected by the highest temperature (Fig. 3f) despite their growth rate being higher as temperature increased (Fig. 4f). This observation warrants further studies on the species, ideally experiments where the food source of amphibians is richer in nutrients, as the fruit flies may have been nutritionally inadequate for the fast growing *P. esculentus*.

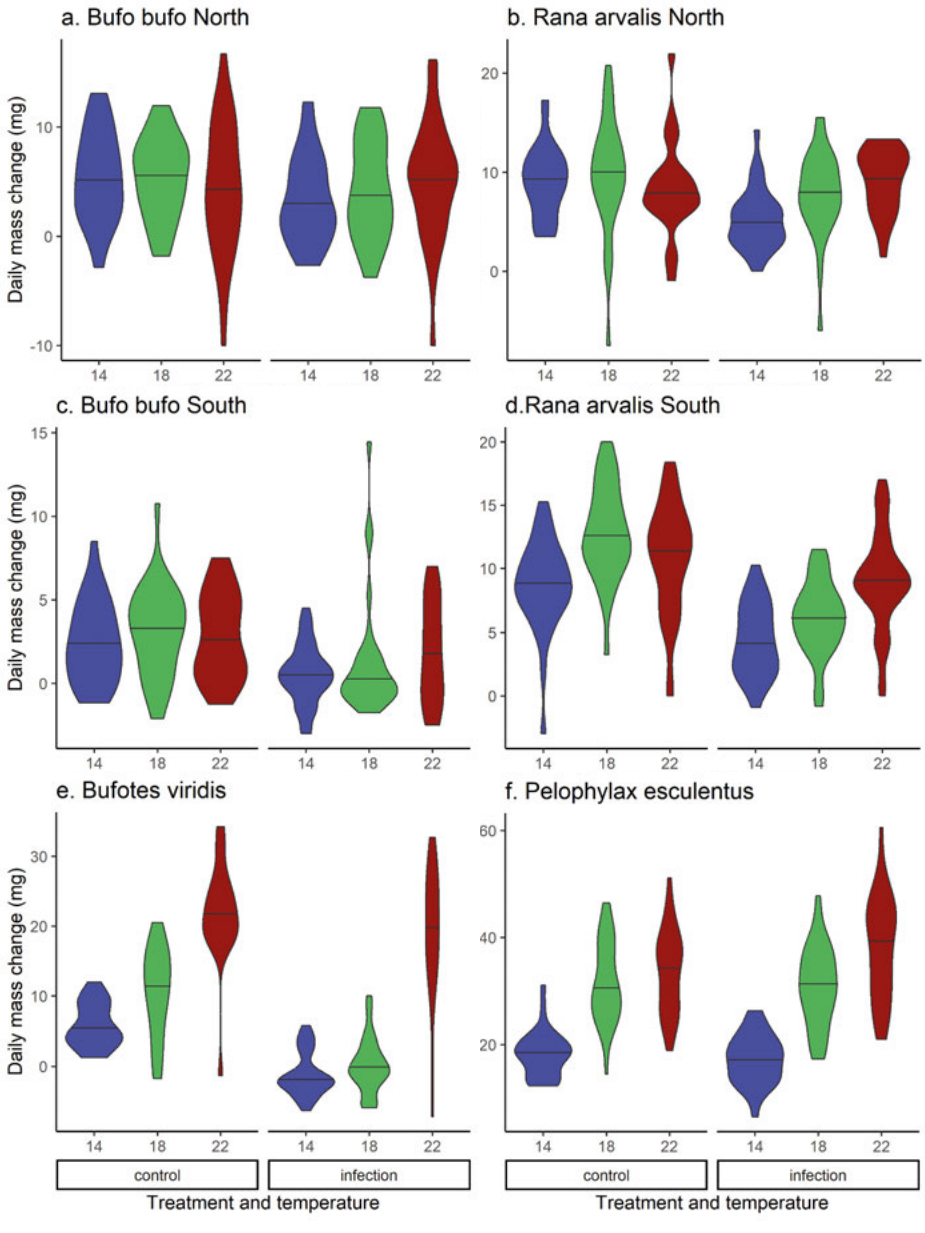

Figure 4. Daily change in mass (i.e. total mass change divided by survival days) in mg, for the six high latitude amphibian populations exposed to the fungus *Bd*. Note the difference in scale in the y axis!

Adding to the mix: population comparisons

In **Paper III**, I take a closer look at the two species with a wider distribution, *B. bufo* and *R. arvalis*, by adding individuals from northern populations, which have a unique genetic background, having colonized the Scandinavian peninsula following a different route (Cortázar-Chinarro et al. 2017, Thörn et al. 2021), and are presumably more cold-adapted than their southern counterparts. Focusing on the survival and growth patterns of control individuals verified the assumption of divergent thermal adaptations in each population (Angilletta 2009). Northern individuals were more cold-adapted than their southern counterparts, evident in their growth rate which was decreasing as temperature increased (Fig. 4a-d). Survival also decreased more drastically with increasing temperature for the northern amphibians (Fig. 3a-d).

Corroborating the limitations of the thermal mismatch hypothesis to explain survival and growth patterns in high latitude amphibians following *Bd* exposure in high temperatures, which I discovered in paper II, the northern populations of *R. arvalis* also exhibited lower survival differences (Fig. 3b) and comparable growth rate (Fig. 4b) between control and infected individuals in 22ºC. In both species and populations, infection load and the percentage of *Bd*-positive individuals was also lowest in 22ºC. Additionally to the hypothesis of a lower thermal optimum for *Bd*, this finding could be attributed to the temperature-dependence of immune system functions in amphibians (Rollins-Smith and Woodhams 2012, Butler et al. 2013, Sonn et al. 2017). Higher temperatures, but still within the physiological limits of organisms, are considered beneficial for immune system activation (Maniero and Carey 1997, Jozkowicz and Plytycz 1998) and improved survival of Bd-exposed amphibians in higher temperatures is a pattern often encountered in the literature (Andre et al. 2008, Bustamante et al. 2010, Murphy et al. 2011)

Interestingly, the northern *R. arvalis* population, despite having higher mortality rate as controls, exhibited lower *Bd*-mediated mortality in all temperatures, i.e. the difference in survival between control and infected individuals in each temperature was lower in northern individuals.

Northern *B. bufo* individuals were very susceptible, with complete mortality of exposed groups in 18 and 22ºC, a pattern possibly driven by their lower body size compared to *R. arvalis* (Fig. 5). Meurling et al. (2024) had similar results in their infection experiment with both *B. bufo* and *R. arvalis* metamorphs from latitudinal populations in Sweden, and hypothesized that mass is an important predictor of survival probability in *Bd*-exposed amphibians. This protective effect of a higher mass, which is reported in other studies (Garner et al. 2009, Smith et al. 2022), may be the outcome of better water retention in larger individuals (Russo et al. 2018), an important function especially considering that chytridiomycosis may cause excessive skin sloughing and cutaneous water loss in amphibians (Voyles et al. 2009, Ohmer et al. 2015, Russo et al. 2018). Larger individuals may also have more resources to allocate to

immune system functions, as the trade-offs with somatic maintenance and development are harder to meet for smaller individuals (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Garner et al. 2009).

R. arvalis and *B. bufo* in our study exhibited a hotter-colder thermal adaptation (Angilletta 2009, Richter-Boix et al. 2015), with control individuals from northern populations growing faster in 14ºC and southern, more warm-adapted ones growing more in the warmer temperatures. *Bd* impacted the growth rate in a temperature dependent fashion, being more detrimental in the

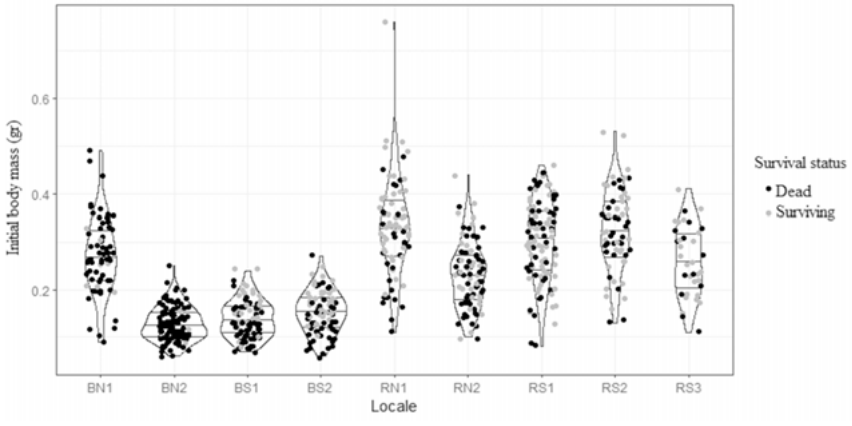

Figure 5. Initial body mass in grams, for northern and southern *B. bufo* and *R. arvalis* individuals. Mass is presented per sampling locality (BN & BS: northern and southern *B. bufo*, RN & RS: *R. arvalis*). Points represent individuals and are coloured based on whether the individual survived the 40-day *Bd* infection experiment (grey) or not (black).

lower temperatures compared to 22ºC (Fig. 4a-d). The negative impact of *Bd* on growth, which as remarked earlier can lead to amphibians suffering from lower overwintering survival (Earl and Whiteman 2015) and increased predation risk (Wilbur 1984, Werner 1986), was shared between the amphibian populations I tested in papers II and III. The lack of a strong impact of *Bd* on growth in 22ºC lends further support to the theory that the *Bd* strain in my experiment has a low temperature optimum.

Temperature effects on transcriptional responses (Paper IV)

Given the unexpected finding that the *Bd* strain used in the infection experiment had a low thermal optimum, it became very interesting to explore what type of transcriptomic responses exposure to *Bd* elicited. In **paper IV**, I characterized this response in both northern and southern populations of the two early breeders, *B. bufo* and *R. arvalis*.

The pattern of more pronounced gene expression differences between *Bd*-exposed and control individuals in skin compared to liver samples, which I observed in Paper I was also evident here (Fig. 6a-d). In this infection experiment however, southern individuals exhibited a generally more intense reaction to *Bd* exposure compared to northern ones, a pattern perhaps led by their genetic differences (Cortázar-Chinarro et al. 2017, Thörn et al. 2021).

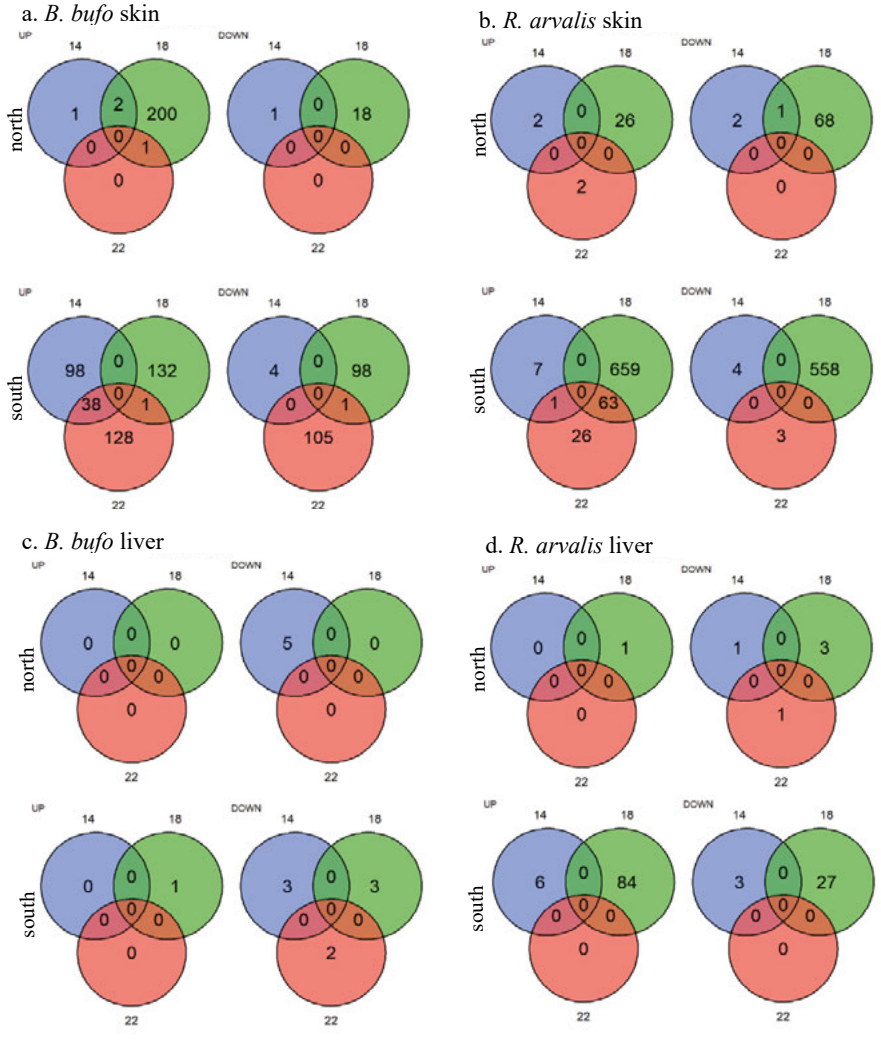

Figure 6. Number of differentially expressed genes between *Bd*-exposed and control samples from *B. bufo* and *R. arvalis*. Numbers are shown separately for the species, populations, up- and down- regulated genes and temperature.

In contrast to the infection experiment in Paper I, there was a number of both *B. bufo* and *R. arvalis* individuals that managed to clear *Bd* from their skin,

especially in higher temperatures as I showed in paper III. This generated the potential to extend the comparisons from *Bd*-exposed vs control individuals, to exposed individuals with a positive *Bd* test at the experimental endpoint ("*Bd*-positive"), exposed individuals negative for *Bd* ("cleared") and the controls (Fig. 7), which showed different results depending on both temperature and population. Below I discuss some of the key results that emerge for skin tissue, as liver samples presented a much more muted response.

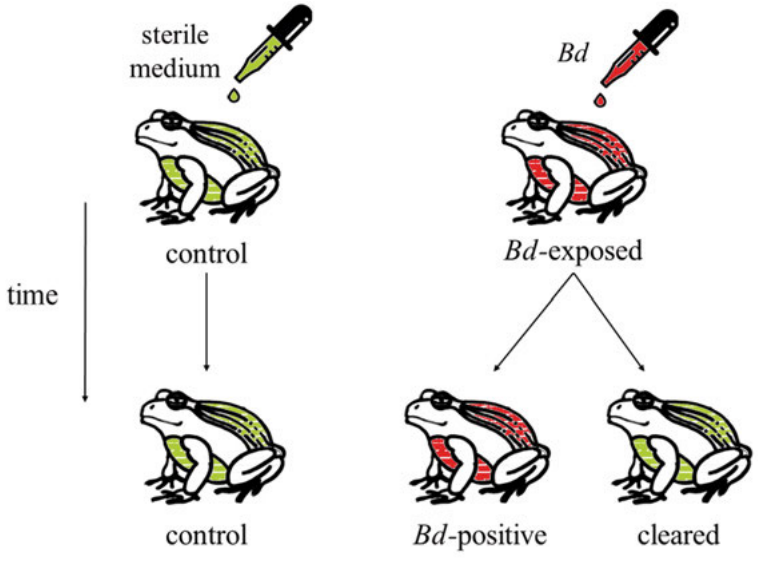

Figure 7. Illustration of the three categories of individuals in the beginning or the experiment (top) and at the experimental endpoint (bottom): control = exposed to sterile culture medium, *Bd*-exposed = exposed to *Bd*, *Bd*-positive = detectable *Bd* load at the experimental endpoint, cleared = no detectable *Bd* load at the experimental endpoint.

In northern individuals, only 18°C elicits a strong response in *Bd*-exposed individuals, and some of the differentially expressed genes that are upregulated in exposed amphibians are related to immune system functions, many more in *B. bufo* than *R. arvalis* (Fig. 6a, 6c, 8a). Despite that, mortality in *Bd*-exposed *B. bufo* was complete in the subset of individuals used for the transcription analysis. This result corroborates earlier findings by Savage et al. (2020) who observed that maintaining the immune system active for a prolonged time after infection was in fact detrimental for *Bd*-exposed *Rana yavapaiensis*, perhaps due to the high energy cost associated with an immune reaction (Sears et al. 2011). The lack of immune response in 22°C indicates two possibilities: either *Bd* has a lower growth rate at this temperature, creating imperceptible differences between exposed and control individuals, or that any immune-assisted clearance of the fungus happened earlier during the infection and was not captured by my set-up.

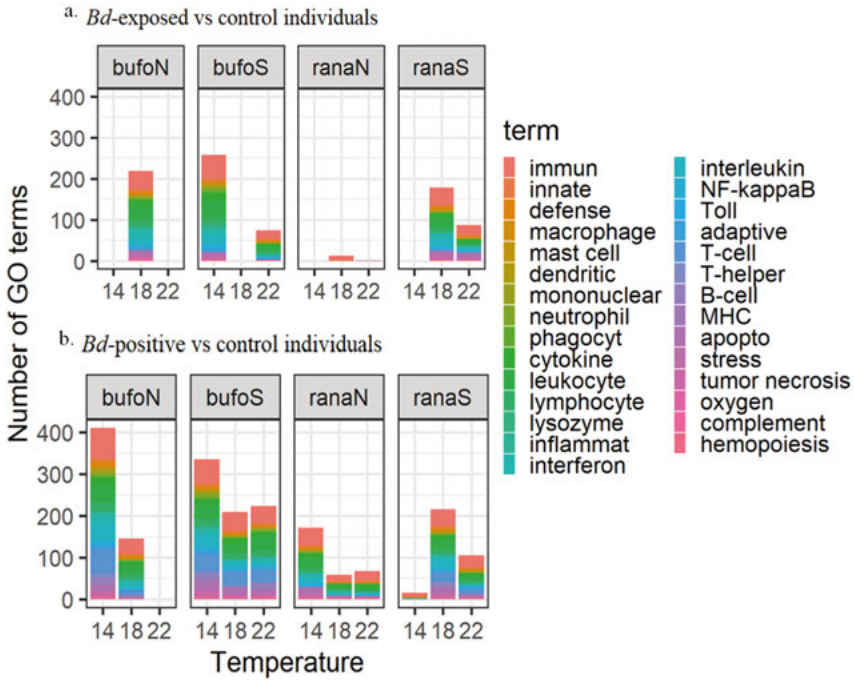

Figure 8. Number of immune-related gene ontology terms enriched in sets of differentially expressed genes in skin tissues of *B. bufo* and *R. arvalis* (N = northern, S = southern populations). Enriched terms are shown for upregulated genes in all Bd-exposed individuals compared to controls.

The lack of difference in 14ºC was more puzzling, so I decided to separately analyse and compare the two categories of *Bd*-exposed individuals, *Bd*-positive and cleared ones, to controls. Indeed, differences in gene expression between *Bd*-positive and control individuals were evident in both populations and species, not only in 14ºC but also in 22ºC (Fig. 9). The lowest temperature generated the strongest immune response, except in the southern *R. arvalis* samples (Fig. 8b). Moreover, in 14ºC, the immune related terms expressed were more in the north than in the south for both species, and in 18ºC the opposite was true, with more immune terms being expressed in southern individuals (Fig. 8b). As immune system function is temperature dependent and reflects the thermal adaptations of the hosts (Butler et al. 2013, Rollins-Smith 2017), this pattern may indicate that 18ºC is an already warm temperature for northern individuals. For northern *R. arvalis*, the immune reaction in 14ºC was in fact protective, as *Bd*-positive individuals survived best in that temperature (Fig. 3b), while southern *R. arvalis* add to the observations of individuals with a high immune response and lowest survival (Fig. 3d).

In cleared individuals, gene expression was intermediate to both *Bd*-positive and control individuals, with few differentially expressed genes in either comparison (Fig. 9), possibly another artifact of my experimental setup which did not include samples from a few days after *Bd* exposure (Grogan et al. 2018a). I found some residual immune gene up-regulation in northern *B. bufo* skin samples in 18ºC and southern in 22ºC. The low survival of *Bd*-exposed individuals, especially in the lower temperatures and often despite immune system activation, is evidence of the ineffectiveness of the amphibian immune response to prevent *Bd*-mediated mortality (Ellison et al. 2014, Eskew et al. 2018, Savage et al. 2020). On a more positive note, the strains of *Bd* used here and in paper I, do not demonstrate the ability to immunosuppress the experimental individuals, as has been shown in other studies (Ribas et al. 2009, Ellison et al. 2014, 2015), at least not when analysing the data using the *Homo sapiens* gene ontology database as a reference.

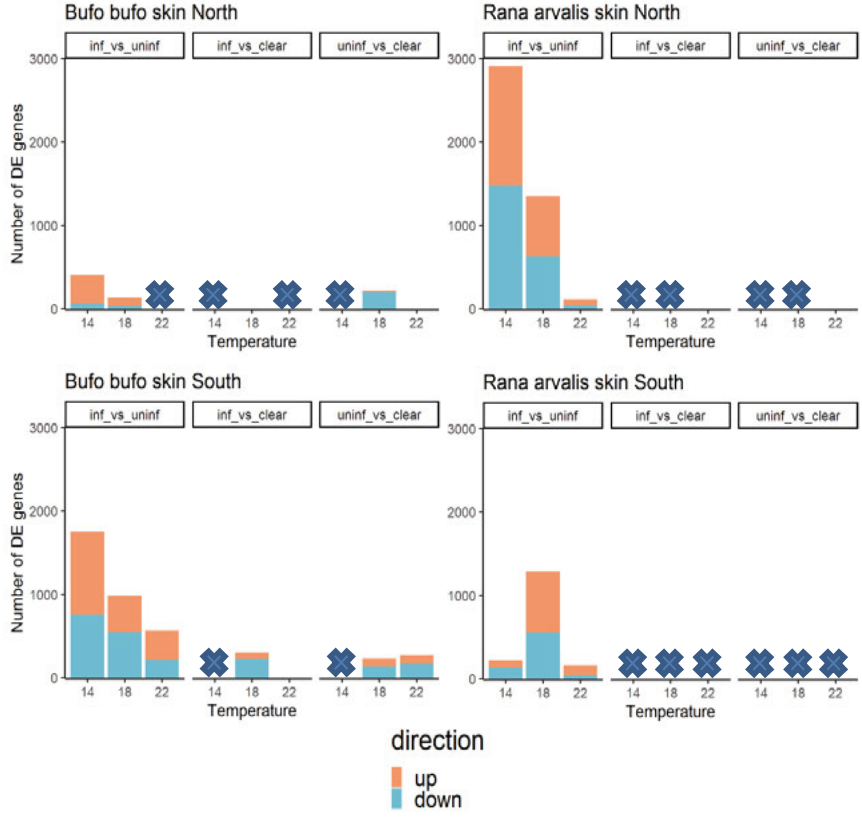

Figure 9. Number of differentially expressed genes per experimental tempera-ture in *R. arvalis* and *B. bufo* skin samples from northern (top) and southern (bottom) individuals. Comparisons are made between "inf" = infected individuals (i.e *Bd*-positive at the experimental endpoint), "clear" = cleared individuals, and "uninf" = control individ-uals). Blue crosses denote comparisons that were not performed due to lack of samples (< 3) in some category (infected, uninfected, cleared).

Conclusion and future directions

Temperature is an abiotic factor affecting all life on earth, with big repercussions on how organisms function on the physiological level, and how this affects their fitness and the types of environments they can live in. Therefore, temperature also affects the interactions of parasites and their hosts, a balance that can easily be disturbed by the rise in temperature that is a direct effect of climate change.

The main conclusion of my thesis is that temperature does play a significant role in deciding the outcome of host-parasite interactions, but not always in a predictable way.

Throughout the results I got some evidence that the thermal adaptations of the parasite, *Bd*, are important. The *Bd* strain I used in the infection experiments in papers II, III and IV, may have a lower thermal optimum as a result of adaptation to the cooler climate in the higher latitude it was isolated from. This could explain why it does not elicit a strong immune responses in amphibians exposed to the highest temperature, and why survival and growth of control and infected frogs do not differ in 22ºC. Elucidating if this is the case could be done through culturing the *Bd* strain used on the infection experiment in vitro in the experimental temperatures and comparing in vivo patterns (as in Raffel et al. 2013). An exciting alternative would be to perform infection experiments with *Bd* isolates prior to and after they have undergone experimental evolution in the laboratory under varying thermal regimes (as in Voyles et al. 2014).

As the laboratory is an approximation of field conditions, some pieces of the puzzle are missing, like the effects of temperature variability on infection outcome (Raffel et al. 2013), and the potential of the amphibian skin microbiome to protect against *Bd*, a function that appears to be temperature-dependent (Robak and Richards-Zawacki 2018, Robak et al. 2023). Using mesocosms would remove the precise control of temperature conditions, but closer mimic field conditions, especially if hosts also have the chance to come into contact with each other, allowing one to study other aspects of the puzzle, including reinfection patterns and pathogen spread in a community.

A hasty conclusion that could be drawn from the results of this thesis is that rising temperature and *Bd* infection are not acute conservation concerns in high latitude systems. While it is true that high temperature may help amphibians clear the fungus, it is also very detrimental for the survival of most of the

populations I examined. Moreover, the demonstrated ability of *Bd* to persist in amphibian habitats, either in the water (Johnson and Speare 2003) or on amphibian (Briggs et al. 2010, Kärvemo et al. 2019) and non-amphibian (Garmyn et al. 2012, McMahon et al. 2013) reservoirs, enables the parasite to reinfect and spread in a community even after the original hosts have recovered or been eliminated. It thus maintains the potential to reinfect amphibians, with perhaps dire consequences, especially if reinfection occurs in the beginning of the breeding season, when the temperatures are still low and the amphibian immune system has not yet recovered its full functionality after hibernation (Rollins-Smith 2017). Lastly, rising temperatures may push both the amphibians and *Bd* to higher thermal optima in a Red Queen race fashion (Van Valen 1973), which *Bd* is likely to "win", due to its smaller size and shorter generation time, which help it adapt faster (Gillooly et al. 2001). As temperature seems to greatly affect the outcome of host-parasite interactions, a potential direction for future research is to increase the range of temperatures under which this outcome is tested. For example, temperatures that approximate winter conditions would elucidate whether high latitude amphibians suffer from *Bd*-mediated mortality during hibernation, which has been shown for some amphibian species (Hammond et al. 2021), but rejected for others (Kásler et al. 2023).

The knowledge that high temperature may be beneficial for *Bd*-infected frogs does also open up avenues for conservation efforts. As theoretically outlined by Hettyey et al. (2019) and demonstrated by Waddle et al. (2024), offering amphibians the chance to use heated shelters in the field helps them clear *Bd* infection and perhaps become resilient against the fungus. Maintaining habitat heterogeneity, such as both shaded and unshaded spots around ponds, which allow frogs to behaviourally thermoregulate (Becker et al. 2012, Roznik et al. 2015) may also be helpful, as it is not feasible to build heated shelters everywhere.

This study also marked the first attempts to study the transcriptional responses in high-latitude amphibians. Previous studies in other hosts have shown that, while both the innate and adaptive part of the immune system are involved in the response to chytridiomycosis, there is variation in the effectiveness of the immune reaction. Here, responses to *Bd* infection differed both between species and populations and included some immune system activity, which could be both protective and detrimental depending on the context, illustrating the need for further investigations. One axis along which future studies in high latitude amphibians should move is deciphering what gene expression looks like in the crucial early days of infection (Grogan et al. 2018a). Another crucial research direction, is the development of more complete transcriptome annotations for more amphibian species which would enable us to more accurately characterize their reactions. Thirdly, working with adults instead of metamorphs, which, due to the immune responses being delayed or reduced around metamorphosis (Rollins-Smith 1998, Grogan et al. 2018b),

may be the life stage most susceptible to *Bd* (Rachowicz and Vredenburg 2004, Briggs et al. 2010), would help better predict the effects of chytridiomycosis in ecological communities.

The pathogen transcriptomic responses may also vary with temperature, though this was not studied here. Ellison et al. (2017) has demonstrated that there is differential gene expression of *Bd* genes in culture and in two amphibian hosts, and that some of those genes are likely involved in host exploitation pathways. The possibility that *Bd* can alter its gene expression warrants further investigation, including the possibility that changes are dependent on temperature. Moreover, since RNA can be extracted from swabs, it would be interesting to follow changes at least in skin gene expression during disease progression in the same host, as the need for invasive sampling is eliminated. This could be combined with the *Bd* expression profiling outlined above, and result in a better understanding of the interplay between the host defence and *Bd* host exploitation mechanism, and whether those are affected by temperature.

The outlook of climate change looks dire at least for high latitude amphibians and the hope that *Bd* will not adapt to the temperature changes looks slim. The best course of action is always giving nature space, removing other potential amphibian threats that we have control over, like habitat degradation or destruction, and taking actions to halt and ultimately reverse the effects of climate change.

Svensk sammanfattning

Framväxande infektionssjukdomar och deras interaktioner med global uppvärmning och klimatförändringar är ett akut hot mot vilda djur. Den termiska mismatchningshypotesen förutspår att temperaturskiftningar bort från värdens termiska optima kommer att resultera i högre värdmottaglighet, eftersom parasiter har möjlighet att snabbare acklimatisera sig och anpassa sig till skiftet. Köldanpassade värddjur kommer att vara mottagliga när temperaturerna stiger, vilket innebär att i höga breddgrader där klimatförändringarna också är snabbare, de är mer sårbara.

En av de parasiter som kan utgöra ett betydande hot mot värddjur på hög latitud om temperaturen fortsätter att stiga är amfibiehudsvampen *Batrachochytrium dendrobatidis* (*Bd*) som orsakar chytridiomykos, en ledande orsak till att amfibiepopulationen minskar och utrotas över hela världen. *Bd*-stammar uppvisar ett brett spektrum av termiska preferenser och grader av patogenicitet. Svårighetsgraden av chytridiomykos skiljer sig mellan amfibiepopulationer och arter, med värdsvar som sträcker sig från fullständig resistens till total mottaglighet för sjukdomen.

Min avhandling har drivits av viljan att utforska interaktioner mellan värd och parasit på nordliga breddgrader och har den extra fördelen att fokusera på ett system där bevarande är mycket angeläget, amfibier, de mest hotade ryggradsdjuren i världen och deras parasitiska hudsvamp *Bd*, som orsakar den hittills värsta dokumenterade panzootin. Mitt mål är att ta itu med den övergripande frågan: Är temperaturen en betydande faktor som påverkar resultatet av värd-parasitinteraktioner på höga breddgrader?

Under mitt examensarbete utförde jag en serie infektionsexperiment med sex populationer av fyra groddjursarter från Sverige, den vanliga paddan *Bufo bufo*, åkergrodan *Rana arvalis*, den ätliga grodan *Pelophylax esculentus* och den grönfläckiga paddan *Bufotes viridis*. För experimenten använde jag en *Bd*-stam isolerad i södra Sverige, och tre temperaturregimer, 14, 18 och 22°C och analyserade amfibiernas överlevnad, tillväxt och transkriptomiska svar. Jag analyserade också transkriptomiska data från ett infektionsexperiment på två populationer av vanliga paddor exponerade till två stammar av *Bd* med olika ursprung.

I artikel I analyserade jag hud- och levertranskriptomiska profiler av *B. bufo*-metamorfer från två populationer från olika breddgrader exponerade för två stammar av *Bd*. Genuttrycket skilde sig mycket mellan vävnadstyperna,

vilket kan förklaras av de två organens olika funktioner. Huden är viktig för syreintag och osmoreglering och har många aktiva immunologiska mekanismer, medan levern är involverad i energi- och proteinmetabolism samt glykogenlagring. Överlevnad påverkade också genuttryck, och jag fokuserade på överlevande individer för att förstå vilka genuttrycksmönster som är förknippade med överlevnad hos arten, och om de skiljer sig åt med *Bd*-stam.

I huden skilde sig genuttrycket mestadels mellan kontrollindivider och de som exponerades för *Bd* med minimala skillnader mellan de två *Bd*-stammarna. Differentiellt uttryckta gener berikades funktionellt i relation till både de medfödda (t.ex. cytokiner, makrofager) och den adaptiva delen (t.ex. B- och T-celler) av immunsystemet. En anmärkningsvärd skillnad mellan stammarna var aktiveringen av en komplementkomponent som endast förekom i huden på individer som exponerats för den svenska stammen av *Bd*, vilket delvis kan förklara de högre överlevnadsfrekvenserna för dessa individer. Fler gener i de norra proverna jämfört med de södra proverna uttrycktes annorlunda mellan kontrollindivider och de som exponerats för *Bd*. I de norra proverna var immunrelaterade berikande faktorer mer talrika.

I levern producerade de två *Bd*-stammarna olika svar. Det fanns fortfarande skillnader i uttryck mellan prover från kontrollindivider och de som exponerats för den svenska *Bd*-stammen men den brittiska *Bd*-stammen medförde nästan inget svar i levern. Det är möjligt att det brittiska isolatet som används i denna studie har ackumulerat mutationer under odling som lett till att leverns försvarsmekanismer inte upptäcker det. Hos *Bd*-SWE-exponerade individer berikades differentiellt uttryckta gener för immunrelaterade faktorer, och, som i hudproverna, mer när de härstammar från nordliga jämfört med sydliga individer. Populationsskillnaderna representerar sannolikt genetiska skillnader mellan populationerna, som har formats av deras olika återkoloniseringsvägar till Skandinavien.

I artikel II undersökte jag temperaturens effekter på överlevnaden och tillväxten av två kall- och två varmanpassade groddjur från södra Sverige. Med undantag för *P. esculentus* var exponering för *Bd* skadlig för alla arter, som led av överdödlighet och minskad tillväxt. Den varmaste temperaturregimen, 22°C, tycks dock vara i utkanten av det använda *Bd*-isolatets termiska optimum, vilket framgår av flera experimentella resultat. De tre arterna lyckades återhämta sig från infektion med högre hastighet vid 22°C och hos *Bd*-positiva individer var infektionsbelastningen lägre. Överlevnad och tillväxt av *Bd*-exponerade individer förbättrades också signifikant jämfört med de lägre temperaturerna.

Detta resultat var oväntat, eftersom de köldanpassade arternas, *B. bufo* och *R. arvalis*, termiska optima är under 22°C, och eftersom parasiter generellt har bredare termiska optimum jämfört med sina värddjur. *Bd*-isolatet som användes i detta experiment kan ha utvecklat en lägre termisk tolerans antingen som en anpassning till det kallare klimatet i Sverige, eller som ett resultat av lång-

varigt hållande under laboratorieförhållanden. Alternativt, kan den observerade avvikelsen från den termiska mismatchningshypotesen antyda en bredare termisk bredd hos värddjur på hög latitud.

Hos *P. esculentus* påverkades inte överlevnad och tillväxt lika mycket av *Bd*-exponering och endast två individer testade positivt för *Bd* i slutet av experimentet, vilket antyder att arten är *Bd*-tolerant.

I artikel III tittar jag närmare på de två arterna med en bredare utbredning, *B. bufo* och *R. arvalis*, genom att lägga till individer från nordliga populationer, som har en unik genetisk bakgrund och är mer köldanpassade än deras sydliga motparter.

Jag bekräftade resultaten i artikel II angående det lägre termiska optimumet för *Bd*, eftersom de nordliga populationerna av *R. arvalis* också uppvisade lägre överlevnadsskillnader och jämförbar tillväxthastighet mellan kontroll och infekterade individer i 22°C. Hos båda arterna och populationerna var förekomsten av infektionsbelastning av *Bd* också lägst vid 22ºC. Förutom förutsägelsen från hypotesen om ett lägre termiskt optimum för *Bd*, kan detta fynd tillskrivas temperaturberoendet av immunsystemets funktioner hos amfibier eftersom högre temperaturer, men fortfarande inom organismernas fysiologiska gränser, anses vara fördelaktiga för immunsystemets aktivering. Intressant nog uppvisade den nordliga *R. arvalis*-populationen, trots att de hade högre dödlighet som kontroller, lägre *Bd*-medierad dödlighet i alla temperaturer

Nordliga *B. bufo*-individer var mycket mottagliga, med fullständig dödlighet av exponerade grupper i 18 och 22ºC, ett mönster som möjligen berodde på deras mindre kroppsstorlek. Den skyddande effekten av en högre massa kan vara resultatet av bättre vattenretention hos större individer, eller mindre avvägningar mellan immunitet och somatiskt underhåll.

Bd påverkade tillväxthastigheten på ett temperaturberoende sätt och var mer skadligt i de lägre temperaturerna jämfört med 22°C. Den negativa effekten av *Bd* på tillväxten kan leda till lägre övervintringsöverlevnad för amfibier och öka deras predationsrisk.

I artikel IV karakteriserade jag det transkriptomiska svaret i de nordliga och södra populationerna hos de två köldanpassade arterna.

I detta infektionsexperiment uppvisade sydliga individer en generellt intensivare reaktion på *Bd*-exponering jämfört med nordliga. I motsats till det tidigare infektionsexperimentet fanns det ett antal individer av bägge arterna som lyckades bli av med *Bd* från sin hud, speciellt i högre temperaturer, så jag kunde separat jämföra *Bd*-positiva, *Bd*-negativa och kontrollindivider. Jag fokuserade min analys på huden, eftersom levern hade ett mycket mer dämpat svar.

Hos nordliga individer framkallar 18°C ett starkt svar hos *Bd*-exponerade individer och några av de uppreglerade generna hos exponerade amfibier är relaterade till immunsystemets funktioner, många fler hos *B. bufo* än *R. arvalis*. Trots det var dödligheten i *Bd*-exponerade *B. bufo* fullständig, ett resultat som bekräftar tidigare upptäckter att bibehållande av immunsystemsaktivitet

under en längre tid efter infektion är skadligt, kanske på grund av den höga energikostnaden förknippad med en immunreaktion. Avsaknaden av immunsvar i 22°C indikerar två möjligheter: antingen har *Bd* en lägre tillväxthastighet vid denna temperatur, eller att någon immunassisterad eliminering av svampen inträffade tidigare under infektionen och inte fångades upp av min experimentuppställning.

Vid analys av *Bd*-positiva individer var genetiska skillnader uppenbara mellan både populationer och arter. Den lägsta temperaturen genererade det starkaste immunsvaret, förutom i de sydliga *R. arvalis*-proverna., De immunrelaterade faktorerna uttrycktes dessutom mer i norr än i söder för båda arterna i 14°C, och i 18°C gällde motsatsen. Detta kan tyda på att redan 18°C är en varm temperatur för nordliga individer. För norra *R. arvalis* är immunreaktionen i 14°C faktiskt skyddande, eftersom *Bd*-positiva individer överlever bäst i den temperaturen, medan södra *R. arvalis* kompletterar observationerna av individer med högt immunsvar och lägst överlevnad. Hos individer som återhämtat sig från infektion var genuttrycket mellanliggande för både *Bd*-positiva och kontrollindivider, med få differentiellt uttryckta gener i båda jämförelserna.

Utsikterna för klimatförändringar ser alvarliga ut åtminstone för amfibier på hög nordlig latitud och hoppet om att *Bd* inte kommer att anpassa sig till temperaturförändringarna ser litet ut. Det bästa tillvägagångssättet är att ge naturen utrymme, ta bort andra potentiella groddjurshot som vi har kontroll över, som livsmiljöförändringar eller habitatförluster, och vidta åtgärder för att stoppa och i slutändan vända effekterna av klimatförändringarna.

Σύνοψη στα ελληνικά

Η παρούσα διδακτορική διατριβή εξετάζει τις επιπτώσεις της θερμοκρασίας στην επιβίωση, την ανάπτυξη και τη γονιδιακή έκφραση αμφιβίων εκτεθειμένων στον χυτριδιομύκητα *Batrachochytrium dendrobatidis* (*Bd*), σε μια προσπάθεια να ρίξει φως στις σχέσεις μεταξύ παρασίτων και των ξενιστών τους σε μεγάλα γεωγραφικά πλάτη.

Στις μέρες μας, πολλά παράσιτα αναγνωρίζονται ως σημαντική απειλή για τη βιοποικιλότητα. Οι συνέπειες των μολυσματικών ασθενειών περιπλέκονται με την έλευση της κλιματικής αλλαγής και ακραίων καιρικών φαινομένων, που μπορούν να αλλάξουν την ισορροπία στις σχέσεις μεταξύ ξενιστών και των παρασίτων τους. Η θεωρία της θερμικής ασυμφωνίας (thermal mismatch hypothesis) προβλέπει ότι μια αλλαγή της θερμοκρασίας μακριά από το θερμικό βέλτιστο του ξενιστή θα έχει ως αποτέλεσμα υψηλότερη ευαισθησία του ξενιστή, καθώς τα παράσιτα μπορούν, χάρη στο μικρό μέγεθος και τη γρήγορη αναπαραγωγή τους, να εγκλιματιστούν ταχύτερα στη νέα θερμοκρασία. Μάλιστα, καθώς τα παράσιτα συνήθως αντέχουν σε μεγαλύτερο εύρος θερμοκρασιών, ξενιστές που είναι προσαρμοσμένοι σε υψηλές θερμοκρασίες θα είναι πιο ευάλωτοι όταν η θερμοκρασία μειώνεται ενώ προσαρμοσμένοι στο κρύο ξενιστές θα είναι ευαίσθητοι όταν η θερμοκρασία αυξάνεται. Σε μεγάλα γεωγραφικά πλάτη λοιπόν, είναι εξαιρετικά ευάλωτοι στην υπερθέρμανση, καθώς εκεί είναι προσαρμοσμένοι σε χαμηλές θερμοκρασίες ενώ και η κλιματική αλλαγή είναι έως και τέσσερις φορές ταχύτερη σε σχέση με τις τροπικές περιοχές.

Ένα από τα παράσιτα που θα μπορούσε να αποτελέσει σημαντική απειλή για τους ξενιστές σε μεγάλα γεωγραφικά πλάτη εάν η θερμοκρασία συνεχίσει να αυξάνεται είναι ο δερματικός μύκητας *Batrachochytrium dendrobatidis* (*Bd*) που προκαλεί χυτριδιομυκητίαση στα αμφίβια, μια αρρώστια που ευθύνεται για πολλές μειώσεις πληθυσμών και εξαφανίσεις ειδών παγκοσμίως. Ο μύκητας αυτός έχει πολλά στελέχη που παρουσιάζουν ένα ευρύ φάσμα θερμικών προτιμήσεων καθώς και βαθμών επικινδυνότητας για τα αμφίβια. Τα αποτελέσματα της μόλυνσης με *Bd* διαφέρουν μεταξύ πληθυσμών, με τις αποκρίσεις των αμφιβίων να κυμαίνονται από πλήρη αντίσταση έως πλήρη ευαισθησία στη νόσο.

Σκοπός της διατριβής μου είναι η εξερεύνηση των αλληλεπιδράσεων ξενιστών-παρασίτων σε μεγάλα γεωγραφικά πλάτη και πώς επηρεάζονται από τη θερμοκρασία. Για αυτό το σκοπό διάλεξα ένα σύστημα ξενιστών-

παρασίτου με μεγάλο ενδιαφέρον και από τη σκοπιά της διατηρησης της βιοποικιλότητας, και εστιάζω στα αμφίβια, που αποτελούν τα πιο απειλούμενα σπονδυλωτά στον κόσμο και στον παρασιτικό μύκητα *Bd*, που προκαλεί τη χειρότερη ζωική επιδημία (όσον αφορά τον αριθμό των ειδών που επηρεάζει) που έχουμε μελετήσει έως σήμερα.

Κατά τη διάρκεια της διατριβής μου, πραγματοποίησα μια σειρά πειραματικών μολύνσεων σε έξι συνολικά πληθυσμούς τεσσάρων ειδών αμφιβίων από τη Σουηδία, τον κοινό φρύνο, *Bufo bufo*, το καφέ βάτραχο *Rana arvalis* (που δεν συναντάται στην Ελλάδα), τον «εδώδιμο» βάτραχο *Pelophylax esculentus* (που επίσης δεν συναντάται στην Ελλάδα) και τον πράσινο φρύνο *Bufotes viridis*. Για τα πειράματα, χρησιμοποίησα ένα στέλεχος *Bd* που απομονώθηκε στη νότια Σουηδία, και τρεις θερμοκρασίες, 14, 18 και 22^{o}C, και ανέλυσα την επιβίωση, την ανάπτυξη και τις μεταγραφικές αποκρίσεις των αμφιβίων. Ανέλυσα επίσης μεταγραφικά δεδομένα από ένα πείραμα σε δύο πληθυσμούς κοινών φρύνων που εκτέθηκαν σε δύο στελέχη *Bd* με διαφορετική προέλευση.

Η διατριβή μου στηρίζεται σε τέσσερα άρθρα τα οποία παρατίθενται στο τέλος της εργασίας.

Στο **άρθρο Ι**, ανέλυσα τη γονιδιακή έκφραση στο δέρμα και το ήπαρ νεαρών κοινών φρύνων από δύο πληθυσμούς, έναν στη βόρεια και έναν στη νότια Σουηδία, που εκτέθηκαν σε δύο στελέχη του μύκητα *Bd*, από τη Σουηδία και το Ηνωμένο Βασίλειο. Η γονιδιακή έκφραση διέφερε πολύ ανά ιστό, κάτι που μπορεί να οφείλεται στις διαφορετικές λειτουργίες των δύο οργάνων. Το δέρμα είναι σημαντικό για την πρόσληψη οξυγόνου και την ωσμορύθμιση ενώ έχει επίσης πολλές ανοσοποιητικές λειτουργίες, ενώ το ήπαρ εμπλέκεται στον μεταβολισμό ενέργειας και πρωτεϊνών και στην αποθήκευση γλυκογόνου. Εάν τα άτομα επιβίωσαν ή όχι μέχρι το τέλος του πειράματος (το οποίο διήρκησε 40 ημέρες) επηρέασε επίσης τη γονιδιακή τους έκφραση. Επικεντρώθηκα λοιπόν στα επιζώντα άτομα για να μελετήσω ποια γονίδια σχετίζονται με την επιβίωση στο είδος και αν η έκφρασή τους διαφέρει ανάλογα με το στέλεχος *Bd*.

Στο δέρμα, η γονιδιακή έκφραση διέφερε κυρίως μεταξύ των ατόμων που εκτέθηκαν σε *Bd* και των αρνητικών μαρτύρων (δηλαδή ατόμων που δεν εκτέθηκαν στο μύκητα και χρησιμοποιούνται ως «επίπεδο αναφοράς»), ενώ υπήρχαν ελάχιστες διαφορές μεταξύ των δύο στελεχών *Bd*. Τα διαφορικά εκφρασμένα γονίδια αφορούσαν λειτουργίες τόσο του έμφυτου (π.χ. κυτοκίνες, μακροφάγα) όσο και με του επίκτητου (π.χ. Β και Τ κύτταρα) ανοσοποιητικού συστήματος. Μια αξιοσημείωτη διαφορά μεταξύ των στελεχών ήταν η ενεργοποίηση ενός συστατικού του συμπληρώματος μόνο στο δέρμα ατόμων που εκτέθηκαν στο σουηδικό στέλεχος *Bd*, το οποίο θα μπορούσε εν μέρει να ευθύνεται για τα υψηλότερα ποσοστά επιβίωσης αυτών των ατόμων. Περισσότερα γονίδια στα βόρεια σε σύγκριση με τα νότια δείγματα παρουσίασαν διαφορές στο επίπεδο γονιδιακής έκφρασης μεταξύ των ατόμων μαρτύρων και εκείνων που εκτέθηκαν στο μύκητα και οτα

γονίδια που σχετίζονται με το ανοσοποιητικό ήταν περισσότερα στα βόρεια δείγματα.

Στο ήπαρ, τα δύο στελέχη *Bd* παρήγαγαν διαφορετικές αποκρίσεις. Ανίχνευσα και πάλι διαφορές στη γονιδιακή έκφραση μεταξύ μαρτύρων και ατόμων που εκτέθηκαν στο σουηδικό στέλεχος *Bd*, αλλά το στέλεχος από το Ηνωμένο Βασίλειο δεν προκάλεσε σχεδόν καμία απόκριση στο ήπαρ. Είναι πιθανό το στέλεχος από το Ηνωμένο Βασίλειο που χρησιμοποίησα σε αυτή τη μελέτη να έχει συσσωρεύσει μεταλλάξεις κατά τη διάρκεια της καλλιέργειας που του επιτρέπουν να διαφεύγει την ανίχνευσης από τους μηχανισμούς απόκρισης του ήπατος. Σε άτομα που εκτέθηκαν με το σουηδικό στέλεχος του μύκητα, πολλά από τα γονίδια με διαφορές στην έκφραση σχετίζονται με το ανοσοποιητικό, και, όπως και στα δείγματα δέρματος, είχαν περισσότερα γονίδια όταν προέρχονταν από δείγματα από το βόρειο πληθυσμό. Οι πληθυσμιακές διαφορές πιθανότατα αντιπροσωπεύουν γενετικές διαφορές μεταξύ των πληθυσμών, οι οποίες έχουν διαμορφωθεί από τις διαφορετικές οδούς επανααποικισμού του είδους προς τη Σκανδιναβία μετά την τελευταία εποχή των παγετώνων.

Στο **άρθρο ΙΙ**, διερεύνησα τις επιπτώσεις τριων θερμοκρασιών (14, 18 και 22°C) στην επιβίωση και την ανάπτυξη τεσσαρων αμφιβίων από τη νότια Σουηδία, δύο προσαρμοσμένων σε χαμηλές και δύο σε υψηλότερες θερμοκρασίες. Η έκθεση στο μύκητα ήταν επιζήμια για όλα τα είδη, τα οποία υπέφεραν από υψηλή θνησιμότητα και μειωμένη ανάπτυξη, με εξαίρεση τον εδώδιμο βάτραχο. Ωστόσο, η υψηλότερη θερμοκρασία, 22°C, πιθανόν βρίσκεται στο θερμικό όριο του σουηδικού στελέχους του μύκητα που χρησιμοποίησα σε αυτό το πείραμα, όπως φαίνεται από πολλαπλά πειραματικά αποτελέσματα. Τα τρία είδη κατάφεραν να απαλλαγούν από το μύκητα σε μεγαλύτερο ποσοστό στους 22°C και στα άτομα που παρέμειναν θετικά στο μήκυτα μέχρι το τέλος του πειράματος, το μολυσματικό φορτίο ήταν χαμηλότερο σε αυτή τη θερμοκρασία. Η επιβίωση και η ανάπτυξη των ατόμων που εκτέθηκαν στο μύκητα στους 22°C ήταν επίσης σημαντικά βελτιωμένες σε σχέση με τις χαμηλότερες θερμοκρασίες, και συγκρίσιμες με την επιβίωση και ανάπτυξη των αρνητικών μαρτύρων στους 22°C.

Αυτό το αποτέλεσμα ήταν απροσδόκητο, καθώς το θερμικό βέλτιστο των προσαρμοσμένων στο κρύο είδος (κοινός φρύνος και καφέ βάτραχος), βρίσκεται κάτω από τους 22°C, και επειδή τα παράσιτα έχουν γενικά ευρύτερα θερμικά όρια σε σύγκριση με τους ξενιστές τους. Το στέλεχος του μύκητα που χρησιμοποιήθηκε σε αυτό το πείραμα μπορεί να έχει αναπτύξει χαμηλότερη θερμική ανοχή είτε ως προσαρμογή στο ψυχρότερο κλίμα στη Σουηδίας, είτε ως αποτέλεσμα παρατεταμένης συντήρησης σε εργαστηριακές συνθήκες. Εναλλακτικά, για την παρατηρούμενη απόκλιση από τη θεωρία της θερμικής ασυμφωνίας που ανέπτυξα στην εισαγωγή, μπορεί να ευθύνεται το ευρύτερο θερμικό εύρος των ξενιστών από μεγάλα γεωγραφικά πλάτη.

Στον εδώδιμο βάτραχο, η επιβίωση και η ανάπτυξη δεν επηρεάστηκαν τόσο από την έκθεση στο μύκητα και μόνο δύο άτομα βρέθηκαν θετικά στο

τέλος του πειράματος, αποτέλεσμα που συμφωνεί με παλαιότερες παρατηρήσεις που χαρακτηρίζουν αυτό το είδος ανθεκτικό στον μύκητα *Bd*.

Στο **άρθρο ΙΙΙ**, εστιάζω περισσότερο στα δύο είδη με ευρύτερη κατανομή, τον κοινό φρύνο και τον καφέ βάτραχο, προσθέτοντας άτομα από βόρειους πληθυσμούς, που έχουν, όπως προαναφέρθηκε, διαφορετικό γενετικό υπόβαθρο και είναι πιο προσαρμοσμένα στο κρύο από τους αντίστοιχους νότιους πληθυσμούς.

Επιβεβαίωσα τα αποτελέσματα του άρθρου ΙΙ σχετικά με το χαμηλότερο θερμικό όριο του *Bd*, καθώς και οι βόρειοι πληθυσμοί του καφέ βάτραχου είχαν παρόμοια ποσοστά επιβίωσης και συγκρίσιμο ρυθμό ανάπτυξης μεταξύ μαρτύρων και των μολυσμένων ατόμων στους 22ºC. Εκτός από το χαμηλότερο θερμικό όριο του μύκητα, αυτό το εύρημα θα μπορούσε να αποδοθεί και στην εξάρτηση από τη θερμοκρασία των λειτουργιών του ανοσοποιητικού συστήματος στα αμφίβια: υψηλότερες θερμοκρασίες, αλλά εντός των φυσιολογικών ορίων των οργανισμών, θεωρούνται ευεργετικές για την ενεργοποίηση του ανοσοποιητικού συστήματος. Είναι ενδιαφέρον ότι ο βόρειος πληθυσμός του καφέ βατράχου, παρά το γεγονός ότι είχε υψηλότερο ποσοστό θνησιμότητας στους μάρτυρες, εμφάνισε χαμηλότερη θνησιμότητα λόγω του μύκητα σε όλες τις θερμοκρασίες.

Οι βόρειοι κοινοί φρύνοι ήταν πολύ ευαίσθητοι, με πλήρη θνησιμότητα των εκτεθειμένων ατόμων στους 18 και 22ºC, κάτι που πιθανώς οφείλεται στο μικρό σωματικό τους μέγεθος. Η προστατευτική επίδραση του μεγάλου μεγέθους μπορεί να είναι το αποτέλεσμα καλύτερης κατακράτησης νερού σε μεγαλύτερα άτομα ή μικρότερης ανάκης για διαμοίρασμα της σωματικής ενέργειας μεταξύ ανοσίας και σωματικής συντήρησης.

Η έκθεση στο μύκητα επηρέασε επίσης αρνητικά τον ρυθμό ανάπτυξης των αμφιβίων, ιδιαίτερα στις χαμηλές θερμοκρασίες. Ο αρνητικός αντίκτυπος του μύκητα στην ανάπτυξη μπορεί να οδηγήσει σε χαμηλότερη επιβίωση των αμφιβίων κατά τη διάρκεια του χειμώνα και να αυξήσει τον κίνδυνο θήρευσης.

Στο **άρθρο ΙV**, μελέτησα τη γονιδιακή έκφραση των ατόμων τους άρθρου ΙΙΙ.

Σε αυτό το πείραμα, άτομα από τους νότιους πληθυσμούς παρουσίασαν μια γενικά πιο έντονη αντίδραση στην έκθεση στο μύκητα σε σύγκριση με τους βόρειους πληθυσμούς.

Σε αντίθεση με πειραματικές μολύνσεις του άρθρου Ι, σε αυτό το πείραμα ένα μεγάλος αριθμός ατόμων και από τα δύο είδη κατάφεραν να απαλλαγούν από το μύκητα μέχρι το τέλος του πειράματος. Αυτό μου επέτρεψε να συγκρίνω τη γονιδιακή έκφραση μεταξύ τριων κατηγοριών, των θετικών ή αρνητικών στο μύκητα ατόμων, και των μαρτύρων. Εδώ, εστίασα την ανάλυσή μου στο δέρμα, καθώς το ήπαρ είχε λιγότερο έντονες αποκρίσεις.

Στα βόρεια άτομα, μόνο οι 18ºC προκαλούν ισχυρή απόκριση σε άτομα που εκτέθηκαν στο μύκητα. Ορισμένα από τα γονίδια που υπερεκφράστηκαν στα εκτεθειμένα αμφίβια σχετίζονται με τις λειτουργίες του ανοσοποιητικού συστήματος, και είναι μάλιστα πολύ περισσότερα στους κοινούς φρύνους σε

σχέση με τους καφέ βατράχους. Παρόλα αυτά, η θνησιμότητα στους εκτεθειμένους φρύνους ήταν πλήρης, ένα αποτέλεσμα που αντικατοπτρίζει ευρήματα άλλων ερευνητών, ότι η διατήρηση ενεργής ανοσοποιητικής απόκρισης για παρατεταμένο χρονικό διάστημα μετά τη μόλυνση είναι επιζήμια, ίσως λόγω του υψηλού ενεργειακού κόστους που σχετίζεται με την ανοσολογική αντίδραση. Η έλλειψη ανοσοαπόκρισης στους 22°C υποδεικνύει δύο πιθανότητες: είτε ο μύκητας έχει χαμηλότερο ρυθμό ανάπτυξης σε αυτή τη θερμοκρασία, είτε ότι η πιθανή ανοσοαπόκριση συνέβη κατά τις αρχικές μέρες της μόλυνσης και δεν καταγράφηκε από την πειραματική μου διάταξη.

Κατά την ανάλυση ατόμων θετικών στο μύκητα, διαφορές στη γονιδιακή έκφραση ήταν εμφανείς τόσο ανάμεσα στους πληθυσμούς όσο και στα είδη. Η χαμηλότερη θερμοκρασία γενικά δημιούργησε την ισχυρότερη ανοσολογική απόκριση, εκτός από τα δείγματα από το νότιο πληθυσμό καφέ βατράχων. Επιπλέον, στους 14°C, γονίδια που σχετίζονται με το ανοσοποιητικό υπερεκφράστηκαν στους βόρειους παρά στους νότιους πληθυσμούς και στα δύο είδη, ενώ το αντίθετο ίσχυε στους 18°C. Αυτό μπορεί να υποδηλώνει ότι οι 18°C είναι μια ήδη ζεστή θερμοκρασία για τα βόρεια άτομα. Σε άτομα που απαλλάχθηκαν από το μύκητα κατα τη διάρκεια του πειράματος, η γονιδιακή έκφραση ήταν ενδιάμεση μεταξύ των θετικών στο μύκητα και των μαρτύρων.

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Ramin, älskling, αγάπη μου, I place you here among the people that I hold closest to my heart and you fit right in ♡ You have been a big part of this accomplishment, with all the care you showed for the frogs and the people that work with them, and you made this better with your ideas and enthusiasm. And more importantly, you make life more fun and exciting and interesting, calm and easy with your empathy and thoughtfulness and beautiful with your smile. I love you Ramin!

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Κοιτάξτε τι έκανα! Σας περιμένω! Και σας αγαπώ πολύ ♡

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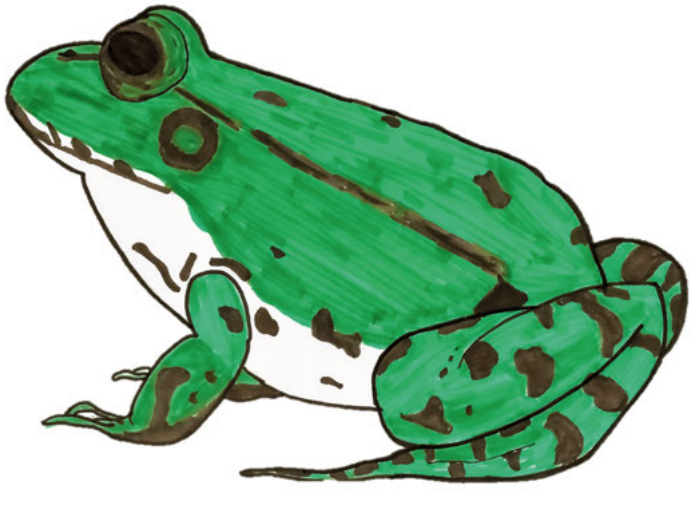

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