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"I thought it was gonna be like in the movies –
you know, inspirational music, a montage:
me sharpening my pencil, me reading, writing,
falling asleep on a big pile of books with my glasses all crooked,
'cause in my montage, I have glasses.
But real life is slow, and it's starting to hurt my occipital lobe."

Buffy Anne Summers

ABSTRACT

White-nose disease (WND) is a fungal disease of hibernating bats, caused by the psychrophilic fungus *Pseudogymnoascus destructans*. The disease was first discovered in 2006, when hibernating bats in upstate New York were found with white fungal growth around their ears, wings and muzzles. Since then, the disease has spread across North America, killing millions of bats, and resulting in population collapses in many affected areas, earning its reputation as one of the most detrimental wildlife diseases of modern times. The causative agent, *P. destructans*, is endemic to the Palearctic and was introduced to North America from Europe. The disease does not cause significant mortality in Palearctic bats, likely due to the long coevolution between the pathogen and the hosts, but the mechanisms behind the low mortality have remained largely unknown. It is also unclear, why the disease does not manifest (visible fungal growth, diagnostic cupping erosions on infected skin) throughout the distribution range of the pathogen. The purpose of this thesis is to provide a holistic view of the factors contributing to WND in the Palearctic by taking in to account all aspects of the disease triangle: the pathogen, the host, and the environment. In chapter I, I used hibernation site census data and open-source climate data from across Europe to determine how species composition within hibernacula and local climatic conditions affect the manifestation of the disease. I found three species/species groups that had a positive association with the occurrence of WND (*Myotis emarginatus, M. myotis/blythii* and *M. mystacinus/brandtii*), and two species that had a negative association with the disease (*Rhinolophus ferrumequinum, R. hipposideros*). In addition, I discovered a link between climatic conditions (mean annual surface temperature, annual precipitation) and the probability of disease manifestation, which enabled me to elucidate the potential global distribution of the disease and further create predictions on how climate change may shift these areas. In chapter II, I have illuminated the mechanisms facilitating the survival of the two main hosts, *M. myotis* in Europe and *M. lucifugus* in North America. The results show that *M. myotis*, a species that has coexisted with the pathogen for millennia, does not mount local immune reactions during infection, suggesting that the species is tolerant against WND. In contrast, *M. lucifugus* from a remnant population with short exposure history with the pathogen, showed signs of immunological resistance mechanisms. In chapters III and IV, I used passive acoustic monitoring to investigate the use of novel hibernation sites in an

area that lacks caves and reveal how weather conditions affect the activity of bats during the winter. The results show that rock outcrops with cracks and crevices are likely important hibernacula in areas without available caves, suggesting that these habitats may offer some protection against WND if climate change shifts the distribution range of the disease towards North. In addition, acoustic monitoring revealed new information on the expansion of the overwintering range of *Pipistrellus nathusii*, providing further evidence of the species' response to climate change. In addition, the finding highlights the potential future shifts in the distribution ranges of other hibernating bat species, including those that are affected by WND. The findings of this thesis emphasize the importance of host species composition and environmental conditions for the manifestation of the disease and raise concern for multiple areas that may be climatically suitable for the disease and are therefore at risk if the pathogen is introduced. In addition, the results highlight the massive potential that climate change may have for the distribution range of WND, and provide more evidence on the climate change related expansion of one hibernating bat species. The study presents two cost-efficient monitoring methods for WND. Visual assessment of WND (chapter I) is an effective way of monitoring the disease in areas where bats use traditional, accessible hibernacula, and passive acoustic monitoring (chapters III and IV) can be used to detect sickness behaviour in areas with novel hibernation sites such as rock crevices. The results of this thesis also draw attention for the need to gain more information on the mechanisms of tolerance and resistance in Nearctic and Palearctic bats. If the ability to tolerate infection allows bats to cope with WND without apparent costs, further studies could help predict the future of Nearctic and potentially other areas as well, if containing the pathogen in its current ranges fail.

KEYWORDS: bats, white-nose disease, hibernation, disease ecology, climate change, disease monitoring, emerging infectious disease

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TIIVISTELMÄ

Valkokuonosyndrooma on horrostavien lepakoiden sienitauti, jonka aiheuttaa kylmiin olosuhteisiin sopeutunut sieni *Pseudogymnoascus destructans*. Vuonna 2006 tutkijat löysivät New Yorkin osavaltiossa sijaitsevassa luolasta lepakoita, joiden korvat, siivet ja kuono olivat valkoisen sienirihmaston peitossa. Tauti nimettiin valkokuonosyndoroomaksi, ja se on sittemmin levinnyt läpi Pohjois-Amerikan tappaen miljoonia lepakoita ja romahduttaen talvehtivien lepakoiden populaatioita useilla alueilla. Nykyään valkokuonosyndrooma tunnetaankin yhtenä aikamme tuhoisimmista villieläimiä kohtaavista taudeista. Taudinaiheuttaja *P. destructans* on endeeminen Palearktiselle alueelle, ja se levisi Pohjois-Amerikkaan ihmisen siirtämänä Euroopasta. Tauti ei aiheuta palearktisissa lepakoissa merkittävää kuolleisuutta, mikä todennäköisesti johtuu taudinaiheuttajan ja lepakoiden välisestä pitkästä koevoluutiosta. Selviytymisen taustalla vaikuttavat mekanismit ovat kuitenkin edelleen pitkälti hämärän peitossa. On myös epäselvää, miksi taudin näkyviä merkkejä (valkoista sienikasvusta ja sienen aiheuttamia ihovaurioita) ei ilmaannu lepakoihin taudinaiheuttajan koko levinneisyysalueella. Tämän väitöskirjan tarkoituksena on muodostaa kokonaiskuva valkokuono-syndroomaan vaikuttavien tekijöiden välisistä suhteista Palearktisella alueella ottaen huomioon tautikolmion kaikki kolme osaa: taudinaiheuttajan, isännän ja ympäristön. Osatyössä I käytin euroopanlaajuista horroslaskenta-aineistoa sekä avointa ilmastoaineistoa tutkiakseni horrospaikkojen lajikoostumuksen ja paikallisten ilmasto-olosuhteiden merkitystä taudin esiintymiselle. Tulokset paljastivat positiivisen yhteyden taudin esiintymisen ja viiden lajin välillä (ruskosiippa (*Myotis emarginatus*); isosiippa (*M. myotis*) ja hiirenkorvasiippa (*M. blythii*); viiksisiippa (*M. mystacinus*) ja isoviiksisiippa (*M. brandtii*)) sekä negatiivisen yhteyden kahden lajin kanssa (isohevosenkenkäyökkö (*Rhinolophus ferrumequinum*); pikkuhevosen-kenkäyökkö (*R. hipposideros*)). Lisäksi löysin yhteyden ilmasto-olosuhteiden (vuoden keskilämpötila, vuotuinen sademäärä) sekä taudin esiintymisen kanssa, minkä avulla laadin ennusteen taudin nykyiselle maailmanlaajuiselle levinnei-syydelle, sekä sovitin mallin ilmastonmuutosskenaarioon osoittaakseni miten levin-neisyys voi muuttua tulevaisuudessa. Osatyössä II selvitimme valkokuono-syndrooman kahden pääasiallisen isännän, pohjoisamerikkalaisen pikkuruskosiipan (*Myotis lucifugus*) sekä eurooppalaisen isosiipan, selviytymisen taustalla vaikuttavia mekanismeja. Tulokset osoittavat, että isosiippa, joka on jakanut elinympäristönsä

taudinaiheuttajan kanssa vuosituhansia, ei käynnistä paikallisia immuunipuolustus-reaktioita infektion seurauksena, mikä viittaa lajin olevan taudille tolerantti. Sen sijaan Pennsylvaniassa sijaitsevaan selviytyjäpopulaatioon kuuluvat pikkurusko-siipat, jotka ovat olleet kontaktissa patogeenin kanssa vasta vähän aikaa, osoittivat merkkejä paikallisesta immunologisesta resistanssista. Osatöissä III ja IV käytin akustista seurantaa tutkiakseni lepakoiden käyttämiä luonnonhorrospaikkoja alueilla, joilta luolat puuttuvat, sekä selvittääkseni miten säätekijät vaikuttavat lepakoiden aktiivisuuteen talvella. Tulokset paljastivat, että halkeilleet kallio-paljastumat ovat todennäköisesti lepakoille tärkeitä horrospaikkoja luolattomilla alueilla. Onkin mahdollista, että nämä elinympäristöt saattavat suojata lepakoita luoliin sopeutuneelta taudinaiheuttajalta, mikäli ilmastonmuutoksen myötä valkokuonosyndrooman esiintymisalue siirtyy pohjoiseen. Lisäksi osatyössä IV paljastui, että pikkulepakko (*Pipistrellus nathusii*) on laajentanut talviaikaista levinneisyysaluettaan pohjoiseen, mikä viittaa siihen, että ilmastonmuutoksen aiheuttamat vaikutukset boreaalisten lepakoiden horroskäyttäytymiseen ovat jo käynnissä. Tämän väitöskirjan löydökset korostavat horrospaikan isäntälaji-koostumuksen sekä ympäristöolosuhteiden merkitystä valkokuonosyndrooman esiintymiselle. Lisäksi tulokset paljastavat eteläiseltä pallonpuoliskolta useita alueita, joiden ilmasto on suotuisa taudin ilmenemiselle. Näillä alueilla horrostaviin lepakoihin kohdistuu suuria riskejä, mikäli patogeeni jälleen ihmisen toiminnan seurauksena siirtyy niille. Lisäksi tulokset osoittavat, että ilmastonmuutoksella saattaa olla valtava vaikutus taudin maantieteelliseen esiintymiseen, ja antavat viitteitä siitä, että muutokset lepakoiden käyttämissä talvehtimisalueissa ovat jo käynnissä. Tämä väitöskirja esittelee kaksi tehokasta valkokuonosyndrooman seurantaan sopivaa menetelmää. Taudin näkyvien merkkien seuranta, jota sovelsin osatyössä I, soveltuu alueille, joilla lepakot horrostavat luolissa tai muissa sisään-pääsyn mahdollistavissa horrospaikoissa. Osatöissä III ja IV käyttämäni passiivinen akustinen seuranta sen sijaan mahdollistaa sairauskäyttäytymisen, kuten epä-normaalin talviaktiivisuuden, havaitsemisen. Lisäksi tämän väitöskirjan tulokset korostavat tarvetta selvittää tarkemmin toleranssi- ja resistenssimekanismeja, jotka mahdollistavat nearktisten ja palearktisten lepakoiden selviytymisen valkokuono-syndroomasta. Mikäli toleranssi mahdollistaa lepakoiden yhteiselon patogeenin kanssa ilman selviä kustannuksia lepakolle, lisätutkimukset voivat auttaa ennusta-maan nearktisen, ja mahdollisesti tulevaisuudessa myös muiden valkokuono-syndrooman esiintymisalueiden lepakoiden tulevaisuutta.

ASIASANAT: lepakot, valkokuonosyndrooma, horros, tautiekologia, ilmasto-muutos

Table of Contents

Abbreviations

WND   White-nose disease
MAST   Mean annual surface temperature

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I. Blomberg A. S., Lilley T. M., Fritze M., Puechmaille S. Climatic conditions and species composition in hibernacula drive susceptibility to fungal infection in hibernating Palearctic bats. *Manuscript*.

II. Lilley T. M., Prokkola J. M., Blomberg A. S., Paterson S., Johnson J. S., Turner G. G., Bartonička T., Bachorek E., Reeder D. M., Field K. A. Resistance is futile: RNA-sequencing reveals differing responses to bat fungal pathogen in Nearctic *Myotis lucifugus* and Palearctic *Myotis myotis*. *Oecologia*. 2019; 191; 295–309

III. Blomberg A. S., Vasko V., Meierhofer M. B., Johnson J. S., Eeva T., Lilley T. M. Winter activity of boreal bats. *Mammalian Biology.* 2021; 101: 609–618

IV. Blomberg A. S., Vasko V., Salonen S., Pētersons G., Lilley T. M. First record of Nathusius' pipistrelle (*Pipistrellus nathusii*) hibernating at a latitude above 60ºN. *Mammalia*. 2020; 85: 74–78

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1   Introduction

1.1   White nose disease: emergence and current distribution

The number of emerging fungal diseases has been increasing globally for the past 20 years, posing a serious threat to plants, wildlife, food production, and human health (Fisher et al., 2012, 2016). For instance, the pathogenic fungus *Batrachochytrium dendrobatidis* is responsible for the rapid global declines of a number of amphibian populations (Fisher et al., 2009), and *Phytophthora ramorum*, the causative agent of sudden oak death, has caused massive devastation for natural forests and plantations (Grünwald et al., 2012). In addition, *Candida auris*, described in 2009, causes severe candidiasis in humans and is particularly concerning due to its wide resistance to antifungals (Satoh et al., 2009). Among emerging diseases, fungal pathogens are particularly concerning due to the low number of available antifungal agents, and the saprophytic nature of many fungi that allows them to grow and produce often resilient spores even in the absence of their primary hosts, resulting in long-lasting environmental reservoirs (Nnadi and Carter, 2021). Indeed, the high virulence and reproductive potential, resilient environmental stages, and often generalist nature, increase the risks fungal diseases pose to biodiversity (Fisher et al., 2012).

One of the most detrimental wildlife diseases of modern times is white-nose disease, a fungal disease that affects bats during their prolonged hibernation period in the winter. The disease was first described in 2006, when bats hibernating in a cave in upstate New York were observed with white fungal growth around their ears and muzzles (Blehert et al., 2009). Since then, the disease has spread across North America, resulting in the deaths of millions of bats, often causing population collapses in affected areas (Blehert et al., 2009; Frick et al., 2015). The pathogen, *Pseudogymnoascus destructans*, is endemic to the Palearctic and was likely introduced to North America from Europe (Leopardi et al., 2015). While the pathogen is widespread in the Palearctic, with records ranging from Europe (Wibbelt et al., 2010; Puechmaille et al., 2011) and Russia (Kovacova et al., 2018) all the way to North-Eastern China (Hoyt et al., 2016b) and Japan (Hoyt et al., 2020), it does not cause significant mortality in affected bats in these areas, likely due to the long co-evolution between the pathogen and the hosts (Martínková et al., 2010; Fritze and

Puechmaille, 2018). In addition, the area in which the disease manifests (i.e., white fungal growth is visible on hibernating bats and the diagnostic cupping erosions can be found on their wing membrane) is smaller than the distribution range of the pathogen (Barlow et al., 2015).

While the vast majority of literature uses the term white-nose syndrome to describe the infection caused by *P. destructans*, for this thesis I have chosen to use the term white-nose disease instead. The word "syndrome" refers to a set of symptoms, which in the case of white-nose disease as described in North American bats include a multitude of behavioural and physiological changes in infected individuals. These symptoms, triggered by the severe wing damage as a result of the fungus invading the skin, range from increased arousal frequency and abnormal day-time activity during the hibernation period, to electrolyte imbalance, dehydration, weight-loss, and often mortality (Blehert et al., 2009; Warnecke et al., 2013; Carr et al., 2014). However, in Palearctic bats, which make up the majority of the host species of the fungus, the infection is not generally associated with these symptoms (Martínková et al., 2010; Puechmaille et al., 2011; Zukal et al., 2016b). This thesis focuses mainly on these Palearctic species, and therefore I consider the term white-nose disease to be more accurate.

1.2 The pathogen and pathology

The causative agent of white-nose disease is *Pseudogymnoascus destructans* (Ascomycota: Pseudeurotiaceae; previously *Geomyces destructans*; Figure 1), a psychrophilic (i.e., cold-adapted) fungus that takes advantage of bats' predisposition to infection during hibernation, when they have lowered their body temperature and immune functions (Gargas et al., 2009). While related to other saprophytes belonging to genera *Geomyces* and *Pseudogymnoascus* that are commonly found in the ground sediments of hibernation sites, *P. destructans* appears to be specialized as a pathogen of hibernating bats (Minnis and Lindner, 2013). The long co-evolution and subsequent specialization are supported by the annual fluctuations in the germination rate of *P. destructans*, which peaks during the winter when hosts are available (Fischer et al., 2020), and the unique enzymatic characteristics of the pathogen that allow it to invade and digest bat skin tissue (Veselská et al., 2020).

In several Nearctic bat species, the infection disrupts the normal hibernation patterns of bats, causing them to deplete their fat stores prior to their spring emergence, resulting in starvation and ultimately death (Reeder et al., 2012). While hibernation is an extremely effective energy saving mechanism allowing bats to survive the scarcity of insects over the winter, it also includes severe physiological costs, such as dehydration and build-up of metabolic waste. Therefore, hibernation in bats, similarly to other mammals, consists of bouts of torpor interrupted with brief

arousals, during which bats are able to reduce the costs of torpor (Humphries et al., 2003; Boyles et al., 2020). These arousals are energetically costly, amounting to over 80 % of the entire winter energy budget of bats (Thomas et al., 1990). Therefore, bats must balance the costs of torpor with available energy resources (Humphries et al., 2003; Boyles et al., 2020). Hence, the increased arousal frequency and duration seen in susceptible species during late-stage infection is often lethal for the bats (Reeder et al., 2012; Warnecke et al., 2012).

While the underlying reasons for the increased arousals observed in susceptible species is likely not yet fully understood, one important factor appears to be the damage *P. destructans* causes to the wing tissue of bats, as the fungus invades and digests the membrane and forms the diagnostic cupping erosions (Meteyer et al., 2009). Wings have an important role in retaining homeostasis during hibernation, as the majority of gas exchange during torpor occurs through the wing membrane (Makanya and Mortola, 2007). The wing damage and resulting disrupted gas-exchange causes to chronic respiratory acidosis, hyperkalaemia, and increased energy expenditure, which force the bats to arouse from torpor and hyperventilate in order to normalize their CO_2 and pH levels (Warnecke et al., 2013; Verant et al., 2014). Hyperventilation and disruption of normal gas-exchange through the wing membrane increase evaporative water loss, which further increase the arousal frequency. In addition, *M. lucifugus* responds to infection through inflammation and the production of cytokines (Field et al., 2015; Lilley et al., 2017). The pain and itchiness caused by these inflammatory reactions likely further contribute to more frequent arousals (Field et al., 2015). Indeed, infected Nearctic *Myotis lucifugus* arouse three or four times more often than their healthy conspecifics, resulting in emaciation, and ultimately death (Warnecke et al., 2012).

Individuals that survive the hibernation period usually heal quickly, and are able to purge the pathogen loads within a few weeks after the end of the hibernation period (Fuller et al., 2011, 2020), although signs of wing damage, such as scarring and necrosis can in some cases be found during breeding season (Reichard and Kunz, 2009). However, in some individuals, an intense inflammatory response known as the immune reconstitution inflammatory syndrome (IRIS) takes place after the emergence, causing increased pathology of the wing membranes, and often resulting in death (Meteyer et al., 2012). The increased energetic costs caused by excess arousals during the winter, as well as amplified metabolic rates during the healing process (Meierhofer et al., 2018), and potentially reduced foraging efficiency due to damaged wing membranes may also have considerable carry-over effects into the active season of the bats and negatively affect the reproduction success of females that have survived the infection (Davy et al., 2017b).

The spores of *P. destructans* are environmentally persistent and, in suitable conditions, can survive viable for several years even in the absence of hosts (Hoyt et

al., 2015b; Vanderwolf et al., 2016). In addition, the fungus is capable of infecting a plethora of bat species and can therefore proliferate and persist in hibernacula despite possible population collapses of its main host species (Zukal et al., 2014). The environmental persistence and wide host range has made it difficult to manage or slow down the spread of the disease in the Nearctic after the pathogen was introduced.

Figure 1. **A**) A closeup of cultured *Pseudogymnoascus destructans* showing the recognizable curved conidia. Sampled from a hibernating *Myotis brandtii* in Salpalinja, Virolahti, Finland. **B**) *P. destructans* cultured on a glucose-chloramphenicol agar plate. Sampled from a hibernating bat in Skuterud, Norway.

1.3   Hosts and host responses

So far, cupping erosions, which are considered to be the clinical sign of WND, have been recorded on 12 Nearctic and 31 Palearctic bat species (Hoyt et al., 2020). In addition, the causative agent has been detected on eight Nearctic and seven Palearctic species, but without clinical signs of disease. The most notably affected species in North America are *Myotis septentrionalis* (northern long-eared bat), *M. lucifugus* (little brown bat), and *Perimyotis subflavus* (tricolored bat), which have experienced declines of over 90 % in affected hibernacula (Cheng et al., 2021). In addition, *Eptesicus fuscus* (big brown bat) and *M. sodalis* (Indiana bat) have been impacted by the disease, but the effects on winter colony sizes have been less drastic (Cheng et al., 2021). While the future of the most affected species may seem grim, populations are still persisting ten years after the initial arrival of the disease (Lilley et al., 2016), and have even been reported to show signs of recovery (Frank, 2019). In Europe, the disease has been largely associated with *M. myotis* (greater mouse-

eared bat). Compared to individuals of other species sharing the same hibernacula, *M. myotis* have higher pathogen loads, as well as higher prevalence and density of lesions on their wing membranes (Zukal et al., 2014, 2016a). Yet, the species is able to hibernate without any apparent harm from the infection, and no mortality is associated between the disease and *M. myotis* (Fritze and Puechmaille, 2018).

In general, hosts have three options for responding to diseases: resistance, tolerance and avoidance (Roy and Kirchner, 2000; Schneider and Ayres, 2008; Klemme et al., 2020). Resistance mechanisms, such as immunological resistance, aim to reduce the negative consequences of diseases by limiting the growth of the pathogen. In contrast, tolerance strategies allow the host to tolerate the infection without apparent negative fitness consequences. Thus, resistance traits in host populations should lead to lower infection incidence and pathogen loads, while tolerance traits allow the pathogen to proliferate freely (Roy and Kirchner, 2000). Given that tolerance is also beneficial to the host organism, as it does not require any resources to be allocated for resisting the infection, tolerance should be the favoured evolutionary outcome for host-pathogen relationships (Roy and Kirchner, 2000). Indeed, there is wide support for tolerance as the survival mechanism of *M. myotis*, a species with a long evolutionary history with *P. destructans* (Hecht et al., 2015; Hecht-Höger et al., 2020; Fritze et al., 2021). In contrast, the Nearctic *M. lucifugus,* newly introduced to the pathogen, attempts to fight the infection through an inefficient immunological resistance strategy (Langwig et al., 2017; Lilley et al., 2017; Hecht-Höger et al., 2020). However, bats may also find refuge from the disease in other ways. There is evidence for antifungal microbes being present on the wing tissue of some bat species, possibly able to limit the infection by *P. destructans* (Hoyt et al., 2015a; Lemieux-Labonté et al., 2017, 2020; Li et al., 2022). In addition, bats may avoid the disease by selecting environments unsuitable for the infection (Turner et al., 2022) or increase their chances of survival by accumulating large enough fat storages to count for the excess arousals caused by the disease (Cheng et al., 2019). These mechanisms add to the complexity of potential host responses as a part of white-nose disease dynamics.

In the Palearctic, the colony sizes of hibernating bats tend to be lower than in the Nearctic (McManus, 1974; Caceres and Barclay, 2000; Brack, 2007; Siivonen and Wermundsen, 2008; Uhrin et al., 2010). In addition, Palearctic bats hibernate more often solitarily compared to Nearctic species (Boratyński et al., 2012; Czenze and Willis, 2015; Martínková et al., 2020). Interestingly, remnant bat populations affected WND in North America show changes in their hibernation behaviour that resemble those currently seen in Palearctic bats. For instance, the proportion of *M. lucifugus* hibernating solitarily has increased considerably in some populations after the arrival of the disease (Kurta and Smith, 2020), and colony sizes of hibernating bats in affected areas have reduced to a level that resembles those in the Palearctic

(Frick et al., 2015). While it is impossible to determine whether the hibernation behaviour seen today in the Palearctic is a result of the historical emergence of WND in the Palearctic, it is possible that the disease has indeed had a remarkable role in shaping the overwintering strategies of Palearctic bat communities.

1.4 Role of environment

For any disease to manifest, a pathogen and a host must be present in a suitable environment (Scholthof, 2007). Many hibernating bat species and *P. destructans* share an environmental optimum in cool, humid hibernacula (Thomas and Cloutier, 1992; Marroquin et al., 2017; Martínková et al., 2018). This overlap of the environmental preferences of the pathogen and naïve hosts has resulted in a perfect "deadly storm" in the Nearctic (Johnson et al., 2014). Optimal environmental conditions contribute to the persistence of the spores, promote infection, and consequently increase the proliferation rate of the fungus, adding to the pathogen load in environmental reservoir, which is also the primary source of infection (Fischer et al., 2020, 2022; Hoyt et al., 2020; Meierhofer et al., 2021).

Ambient temperature is an important variable for the growth rate of *P. destructans*. While the optimal temperature for maximal growth rate in laboratory settings is around $15\,^{\circ}C$ (Verant et al., 2012), it is significantly lower on bats, peaking between 5–$6\,^{\circ}C$ (Martínková et al., 2018). This inconsistency is due to the effect ambient temperature has on the torpor patterns of bats: low ambient temperature promotes longer bouts of torpor (Geiser and Broome, 1993), thus giving the bats fewer opportunities to groom themselves or mount responses to fight off the infection. On the other hand, hibernating at temperatures lower than $5\,^{\circ}C$ will slow down the growth of the fungus despite the increase in the duration of the torpor bouts (Johnson et al., 2014). In addition to suitable temperature, the growth of *P. destructans* also requires sufficient humidity. The growth rate of the fungus is reduced when relative humidity is below $81.5\,\%$ in $13\,^{\circ}C$, although the study did not find differences in spore production between humidity treatments (Marroquin et al., 2017). Most bat species prefer hibernacula with high humidity, as it reduces evaporative water loss during hibernation and consequently reduces the necessity to utilize energetically expensive arousals for rehydration (Thomas and Cloutier, 1992).

Environmental conditions may also have indirect, lesser known, effects for the disease dynamics of white-nose disease. For instance, the environmental conditions may play an important role in shaping the structure and function of the microbial communities on the skin of the bats. As mentioned in the previous chapter, the skin tissue of bats can sometimes harbour microbes that have antifungal properties, and could therefore limit the growth of *P. destructans* and provide protection from the

infection (Hoyt et al., 2015a; Lemieux-Labonté et al., 2020; Li et al., 2022). Given that the microbial composition of the hibernacula wall is an important factor determining the skin microbiota of bats, it is likely that the environmental conditions of the hibernation site are critical for the ability of bats to gain protection from the infection via antifungal microbes (Lemieux-Labonté et al., 2017).

1.5   Climate change, bats, and the future of white-nose disease

Climatic factors, such as rainfall and temperature, play an important role in disease dynamics and host abundance (Aune et al., 2021; Dabaro, 2021; Xu et al., 2022). Indeed, climate change is predicted to have a notable effect on the incidence of many wildlife diseases, including multiple fungal diseases (Nnadi and Carter, 2021). The environmental conditions in underground sites, such as hibernacula, correlate with the local climate. For instance, the mean annual surface temperature (MAST) resembles the ambient temperature within underground hibernacula (Dwyer, 1971; Martínková et al., 2018; McClure et al., 2020), and a similar correlation can be found between outside annual precipitation and humidity within underground sites (Perry, 2013). Given that these environmental factors are important for the manifestation of the disease (Verant et al., 2012; Lilley et al., 2016; Martínková et al., 2018), it is inevitable that changes in ambient temperature and rainfall will have an effect for the incidence of WND as well.

Climate change has the potential to introduce diseases to new areas through shifts in the range distributions of host organisms (Mills et al., 2010). The same may be true for WND, as there is already evidence of range expansion linked to climate change by some bat species (Lundy et al., 2010; Ancillotto et al., 2016; Uhrin et al., 2016; Sachanowicz et al., 2019). Indeed, changes in the abundance and species composition inside hibernation sites has been considered to be a good indicator of the effects of climate change (Newson et al., 2009). Bats, in general, are highly mobile, and use climatic cues to time their seasonal movements (Dechmann et al., 2017). Therefore, migratory species are most likely to be the first to respond to climate change through range expansion. The most important limiting factor for the hibernation ranges of bats is the length of hibernation period, as bats must survive the winter often solely with the fat they have accumulated prior to hibernation (Humphries et al., 2002). Given that climate change will inevitably shorten the hibernation period in many areas, it is likely that bats will respond to this by expanding their hibernation range towards areas that are currently too cold. However, as nocturnal animals, their potential for northward expansion may depend on the species' ability to tolerate the lack of darkness during summer at higher latitudes.

1.6 Aims of the study

The aim of this thesis is to provide a holistic view of the factors contributing to WND by taking into account all aspects of the disease triangle: the pathogen, the host, and the environment (Figure 2). For instance, the distribution of the pathogen in Europe is far wider than the area where the disease manifests (i.e., visible signs of fungal growth and diagnostic cupping erosions can be found on infected bats), but the reasons behind this remain unclear. Studying WND dynamics in the Palearctic, where the fungus has coexisted with the bats for millennia, may offer a window to the future of bats in North America, where the two have just begun their coevolution.

1. Investigate **the host-pathogen interactions** of WND by identifying how species composition within hibernacula affect the manifestation of the disease (chapter I) and the mechanisms facilitating the survival of the two main hosts, *M. myotis* in Europe and *M. lucifugus* in North America (chapter II)

2. Examine **the relationship between the pathogen and the environment** by determining how climatic conditions affect the manifestation of the disease, and generate predictions for the current and future potential global distribution of the disease (chapter I)

3. Investigate **the host-environment relationship** through identifying novel hibernation sites in an area that lacks caves and may be affected by WND in the future (chapter III), understanding how weather conditions affect the winter activity of bats (chapter III), and how climate change may alter the host composition of hibernacula in the future (chapter IV)

4. Present cost-efficient **monitoring methods** for disease manifestation and sickness behaviour, as well as changes in hibernation ranges of species in areas with traditional and novel hibernacula (chapters I, III, IV)
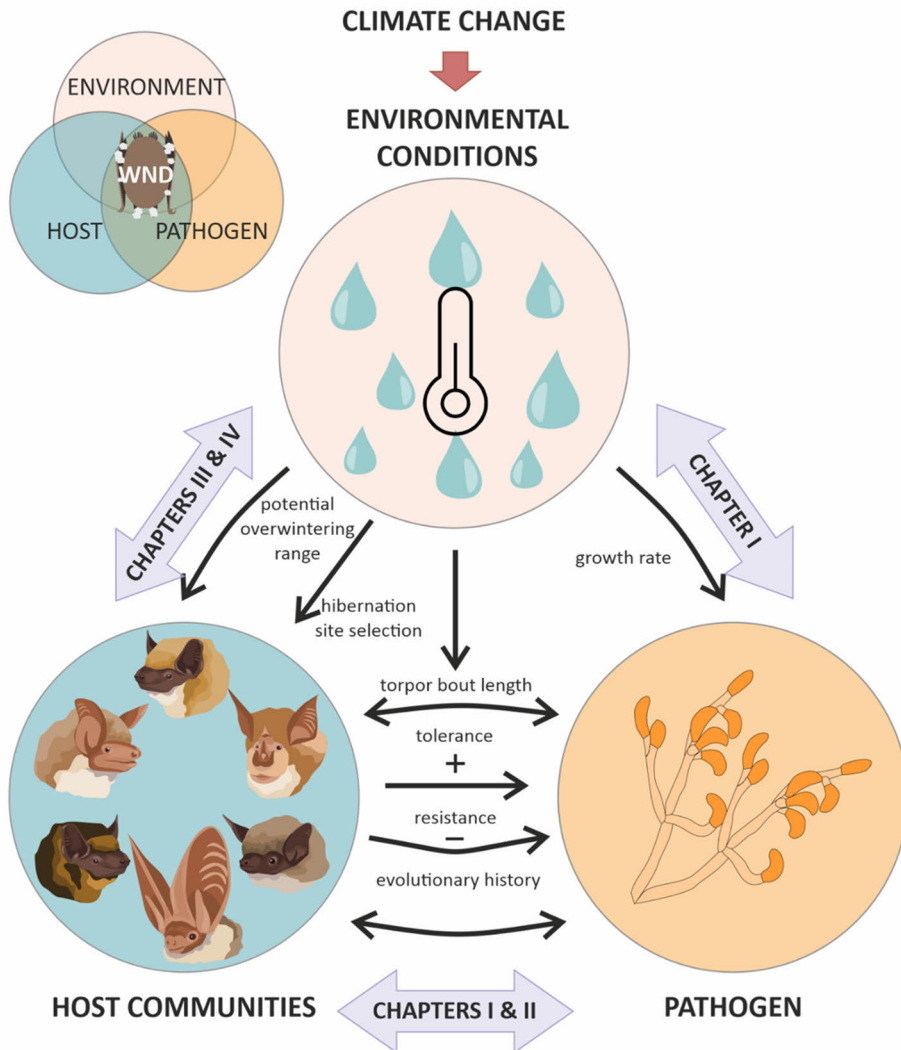
Figure 2. White-nose disease occurs when the pathogen and a host encounter in a suitable environment. However, there are multiple interactions between all three sides of the disease triangle. Climatic conditions alter the environmental conditions in hibernacula, which on the other hand determine the host species composition and growth rate of the fungus. In addition, they affect the torpor bout length of the bats during hibernation, which consequently affects the ability of the pathogen to invade their tissue through increased or reduced possibilities to mount immune reactions and groom themselves. The evolutionary history of the hosts, their survival mechanisms, and their susceptibility to the pathogen on the other hand alters the propagation success of the pathogen, and therefore the environmental pathogen load inside hibernacula.

2 Materials and Methods

2.1 Hibernation site census data and visual assessment for white-nose disease

Hibernation site censuses are a cost-efficient, widely used method for monitoring hibernating bat populations (Uhrin et al., 2010; European Environment Agency., 2014; Van der Meij et al., 2015; Kurta and Smith, 2020). Due to the high fidelity of bats to their hibernation sites, and immobility during torpor, hibernation site censuses allow relatively precise counts that have produced valuable long-term data on population trends across large geographical areas (Van der Meij et al., 2015). However, bats are sensitive to disturbance during the hibernation season, and therefore counts should be performed efficiently and with minimal disturbance for the bats (Battersby, 2010; Olson et al., 2011). Each arousal is energetically costly and disruptions during torpor may decrease the chances of survival or successful breeding (Czenze et al., 2017). In addition, arousal of one bat may lead to a cascade of arousals (Turner et al., 2015). However, when done appropriately, the benefits of winter censuses may outweigh the potential harm for the bats (Kilpatrick et al., 2020).

In chapter I, I used hibernation site census data from 448 hibernacula across Europe (Figure 3), collected over the years 2014–2022, mostly during annual routine censuses. In addition to the information on species composition and abundance, the data included information of whether visible signs of WND (defined as visible fungal growth on the muzzle, ears, or the wing tissue) has ever been recorded in any of the bats occupying the hibernacula, either during the census, or prior. Visual signs of WND correlate with the fungal load, making it a reliable proxy for infection severity (Janicki et al., 2015; Langwig et al., 2016). Therefore, observing WND on bats instead of using invasive methods, such as swabbing the skin for *P. destructans*, offers a method for monitoring the disease presence and severity without causing any additional disturbance for the bats. However, censuses should be carried out during the late hibernation season if WND surveillance is included, as the fungus usually only becomes visible near the end of hibernation period (Martínková et al., 2010). The data set we used partially overlaps with the data published by Fritze and Puechmaille (Fritze and Puechmaille, 2018), which also contains the detailed protocol for performing the counts.
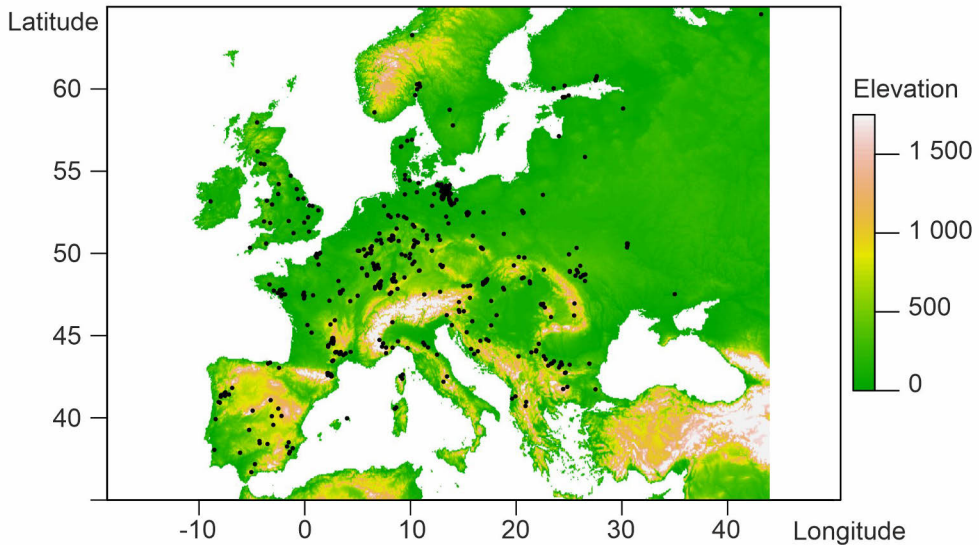
Figure 3. Map of 448 censused hibernation sites.

2.2 Whole-transcriptome sequencing

In chapter II, we used whole-transcriptome RNA-sequencing and differential gene expression analysis to compare the mechanisms underlying the survival of two bat species: Palearctic *M. myotis,* a species with long exposure history with the pathogen, and Nearctic *M. lucifugus*. The *M. lucifugus* were from a remnant population in Pennsylvania, first exposed to WND in 2009. The population collapsed quickly after the arrival of the disease but has since begun to show signs of recovery. We were also able to compare the responses to those seen in naïve *M. lucifugus* during their first contact with the pathogen in a previous study (Field et al., 2018). We conducted the study using free-ranging individuals, as the success rate in studying infection in laboratory settings has been varying (Davy et al., 2017a; Field et al., 2018; Moore et al., 2018).

We expected that by comparing the differences in the transcriptomic responses of *M. myotis* and *M. lucifugus*, we would be able to shed light on the mechanisms behind the reduced susceptibility of *M. myotis*, as well as investigate whether the remnant Pennsylvanian population of *M. lucifugus* show dampened immunological responses to the infection compared to the *M. lucifugus* from Wisconsin, newly introduced to the disease. To achieve this, paired wing tissue samples were collected from five *M. myotis* and five *M. lucifugus*. From each bat, one biopsy punch was taken from a non-infected location on the right wing membrane, and the second from an infected location on the left wing membrane. The diagnostic cupping erosions are visible in long-wave UV-light due to the fluorescence of riboflavin, a metabolite

extracted by the fungus as it invades the skin membrane (Turner et al., 2014), which allowed locating infected and non-infected locations on the membrane. The number of lesions correlates with infection intensity (Pikula et al., 2017), and therefore they were also counted from photographs of the wings taken under UV-light. Prior to sampling, bats were allowed to arouse from torpor for 60–120 minutes to mount immune responses.

From these paired tissue samples, RNA was extracted in mixed batches (however, with both tissue samples from same individuals extracted in the same batch). The illumina RNA sequencing was carried out by the University of Liverpool Centre for Genomic Research. The amount of *P. destructans* transcripts was quantified to determine the level of infection in each sample to ensure that the levels of local infection were similar despite the lower number of lesions observed in *M. myotis* compared to *M. lucifugus*. The paired samples allowed us to conduct differential expression analysis, where we compared the levels of expressed genes in infected and non-infected samples. A gene ontology analysis was further conducted to determine the functional categories of differentially expressed genes.

2.3   Acoustic monitoring

Acoustic monitoring is a powerful, cost-efficient, and non-invasive tool for monitoring bat activity. Recording the echolocation calls of bats provides information on species composition, habitat use, and the spatiotemporal variation in the activity of bats (Vasko et al., 2020; Lundberg et al., 2021). In addition, acoustic monitoring generally allows more accurate estimates on species richness of an area compared to traditional trapping methods (O'Farrell and Gannon, 1999; Mancini et al., 2022).

In chapters III and IV, we used passive acoustic monitoring to investigate bat activity in potential hibernation sites for two consecutive winters to gain information on 1) the use of natural rock formation as hibernation sites, 2) species composition in natural hibernation sites, and 3) to examine the effect of weather conditions on bat activity during the winter. Our monitoring sites included three types of natural formations: four rock outcrops with cracks and crevices, three ancient shores, and three glacial erratics/boulder fields. In addition, we monitored two types of control sites: three root cellars, where we knew bats to hibernate in, and three sites without any apparent potential hibernacula. The criteria for site selection were our estimation on their suitability as hibernation sites, as well as their accessibility, as we had to visit the sites every other week for battery exchange during the period with the coldest and longest nights. All 16 sites were located in South-Western Finland (Figure 4) and monitored from 12th November 2017 to 31st April 2019. We used 11 SongMeter SM2+ BAT (Wildlife Acoustics) and 4 AnaBat SD2 (Titley Scientific)

passive detectors, with the two types of equipment evenly distributed among monitoring site classes to avoid bias. We programmed the SongMeters to start recording half an hour before sunset and end recording half an hour after sunrise, and adjusted the recording schedule of the AnaBats monthly to match with the SongMeters. We identified the species manually but pooled the recordings of *Myotis* spp. for the analyses to gain more power and due to uncertainty in identification between these species.
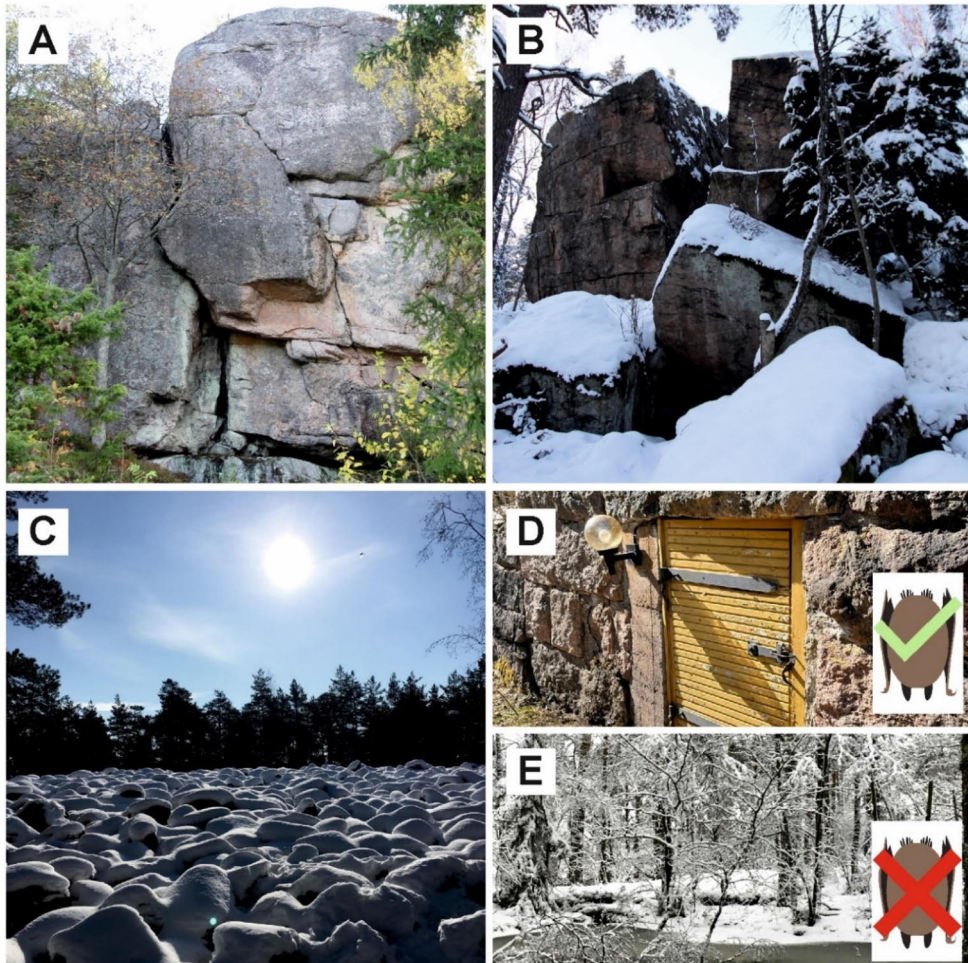

Figure 4. Types of monitored habitats. **A**) Rock outcrops with deep cracks and crevices. **B**) The group consisting of glacial erratics/boulder fields, including two glacial erratics (i.e., glacially deposited rocks), and one anthropogenic yet similar boulder field. **C**) Ancient shores, i.e., post-glacial formations, where land rising has caused the sea to retreat, leaving rocks and pebbles where the seashore historically resided. **D**) Root cellars used by bats. **E**) Feeding sites used by bats in the summer, without any apparent potential as hibernation sites.

2.4 Statistical methods

In chapter I, we used logistic regression analysis (package stats in R (R Core Team, 2020)), with binomial distribution ("logit" link function) to investigate whether species composition in hibernacula and the local climatic conditions (MAST and annual precipitation) can be used to predict the probability of WND being visually detectable in any of the bats in the hibernacula (1= WND visible in bat(s), 0= WND not visible in any of the bats; data collection described in section 2.1). To take into account the expected non-linear effect of MAST and precipitation, we included them in the model as quadratic terms. As it is unlikely that rare species would be important for the occurrence of WND, we only included species that were present in at least 10% of the hibernacula to decrease the number of variables and to avoid type I error (Burnham and Anderson, 2002). Due to the uncertainty in identifying some bat species correctly to species level, we pooled some of them for the analysis. We used model averaging to select the best model (package MuMIn in R) and obtain the regression coefficients of the predictor variables and their 85 % confidence intervals. We considered variables significant if their 85% confidence interval did not include zero, as recommended by Arnold (Arnold, 2010). We then used the averaged regression coefficients of MAST and precipitation to predict the current (worldclim version 2.1 database) and future (2061–2080; under ACCESS-CM2, Shared Socio-economic Pathway 3-7.0) possible global distributions of WND. We conducted all analyses in chapter I using R version 4.0.2 (R Core Team, 2020).

In chapter II, we investigated differential gene expression between infected and uninfected tissue samples using edgeR v.3.22.3 (Robinson et al., 2010). We fitted a generalized linear model to determine whether the gene expression in infected and uninfected samples differed. Prior to running the model, the transcript counts were normalized using the TMM-method (Robinson and Oshlack, 2010). To measure the statistical power of our differential expression analysis, we used Scotty (Busby et al., 2013) in Matlab R2018a (9.4.0.813654). Based on Scotty, our sampling design (paired samples from 5 individuals in both species) allowed us to identify at least 50 % of differentially expressed genes. We compared the differences in the amounts of *P. destructans* transcripts between UV-positive *M. lucifugus* and UV-positive *M. myotis* samples using Welch two-sample t test. In addition, we compared the amounts of *P. destructans* transcripts in UV-positive and UV-negative samples using paired t tests. We also conducted a principal component analysis to compare the gene expression patterns in *M. myotis* and *M. lucifugus*.

In chapter III, we used a general linear model (Proc GLIMMIX in SAS, SAS Institute Inc. 2013) using version SAS 9.4 of SAS to investigate the differences in winter bat activity between habitat types, species, and sites, using normal error distribution with an identity link function. Prior to the analysis, we calculated monthly activity indexes for each species (*E. nilssonii* , *P. auritus*, pooled *Myotis*

spp.) by dividing the number of minutes with bat activity by the total number of hours the equipment had been recording. We used these activity indexes with a $\log_{10}$-transformation as the response variable in the analyses, and species, habitat type and site nested under habitat type as fixed effects. In addition, we investigated the differences between habitats and species using the Tukey-Kramer method. We also fit four generalized linear models (package glm in R) in chapter III to investigate how weather conditions (ambient temperature and barometric pressure) affect bat activity (total bat activity, activity of *E. nilssonii*, activity of *P. auritus* and activity of *Myotis* spp.). In these analyses, we measured bat activity as minutes with activity/night, and the ambient temperature and barometric pressure were means of the previous 24 hours. In addition, we included the interaction between ambient temperature and barometric pressure as explanatory variables. To select the best models, we used backwards stepwise model selection and retained the models with the lowest AICs.

3 Overview of the results

3.1 Host-pathogen interactions

In chapter I, we showed that the composition of host species communities can predict the manifestation of WND in hibernacula. We found significant positive association between the probability of WND ever having been observed in bat(s) occupying the hibernacula and the abundance of *Myotis emarginatus, M. myotis/blythii* and M. *mystacinus/brandtii (*Figure 5 A, B, C*)*. In addition, our results also revealed a negative relationship between the probability of WND and *Rhinolophus ferrumequinum* and *R. hipposideros* (Figure 5 D, C).

The results from chapter II suggest that the Palearctic *M. myotis* and Nearctic *M. lucifugus* rely on different mechanisms to cope with infection, likely reflecting their evolutionary history with the pathogen. The comparisons of transcriptomic responses in infected and non-infected tissue samples from the wo species revealed that only one gene was downregulated in *M. myotis*, while samples of *M. lucifugus* showed 1016 upregulated and 510 downregulated genes. Most of the differentially expressed genes in *M. lucifugus* were related to immune responses, such as leukocyte migration and activation, and inflammatory responses. The overall number of lesions was significantly lower in *M. myotis* compared to *M. lucifugus*, although the samples from both species included similar levels of *P. destructans* transcripts. We also found some differences between the gene expression in the remnant Pennsylvanian *M. lucifugus* population and the Wisconsin population that had come in to contact with the pathogen only recently. For instance, some of the genes associated in immune responses in Wisconsin *M. lucifugus* showed more considerable local responses, while some responses were attenuated compared to the Pennsylvanian population. In addition, the Pennsylvanian population showed more variation in gene expression among individuals.
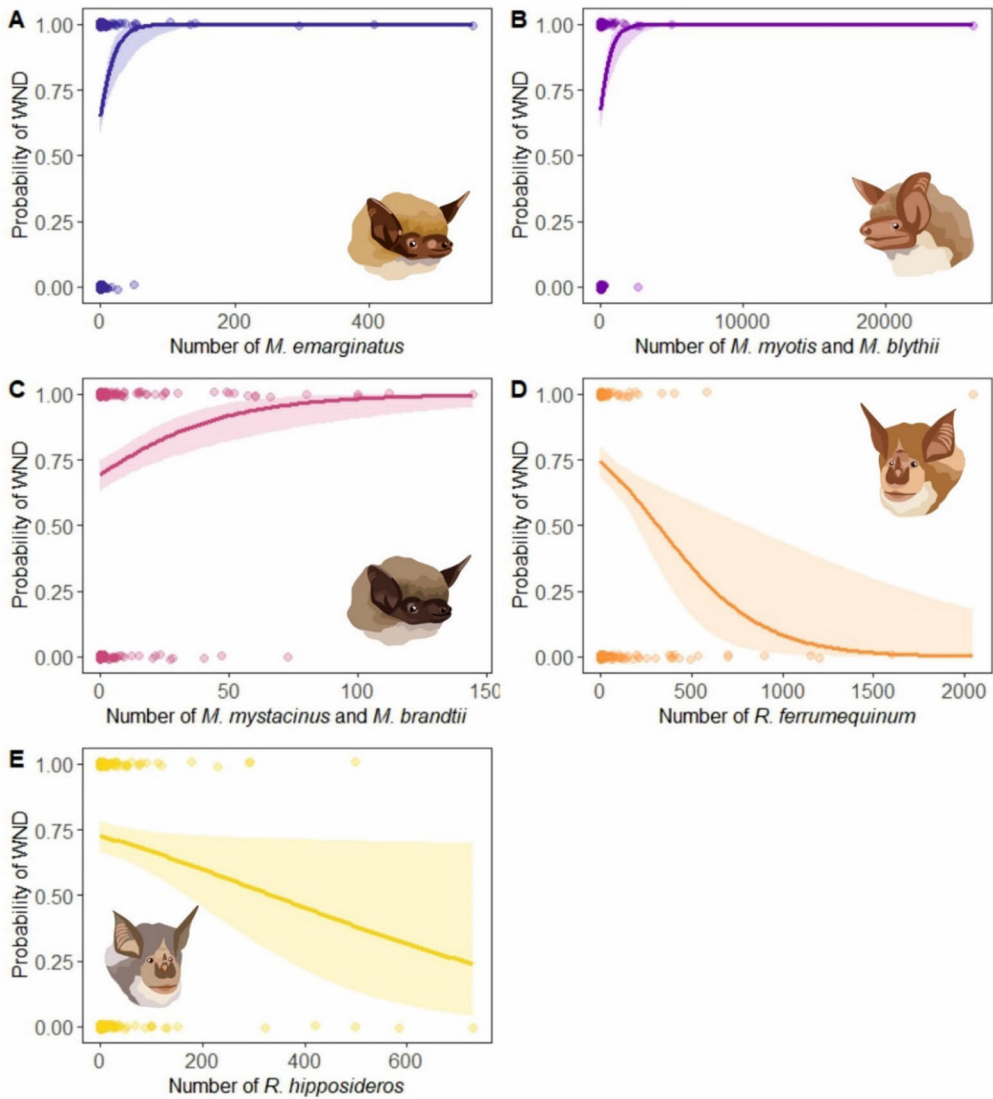
Figure 5. Effects of the number of inhabiting **A**) *Myotis emarginatus*, **B**) *M. myotis/blythii*, **C**) *M. mystacinus/brandtii*, **D**) *Rhinolophus ferrumequinum*, and **E**) *R. hipposideros* on the probability of observing WND at a hibernation site. Coloured areas represent the 85% confidence intervals. Points represent the observed infection status in hibernacula (1 = bat(s) with WND, 0 = no bats with WND; jitter added for clarity).

3.2 Environment and the pathogen: climatic conditions affecting the manifestation of WND

In chapter I, our results revealed significant non-linear relationships between climatic conditions (MAST, precipitation) and the probability of observing WND in hibernacula (Figure 6). The probability of observing WND in hibernation sites was highest in areas where MAST is 8.3 °C (predicted probability of WND = 0.73, 85% CI 0.67—0.79).
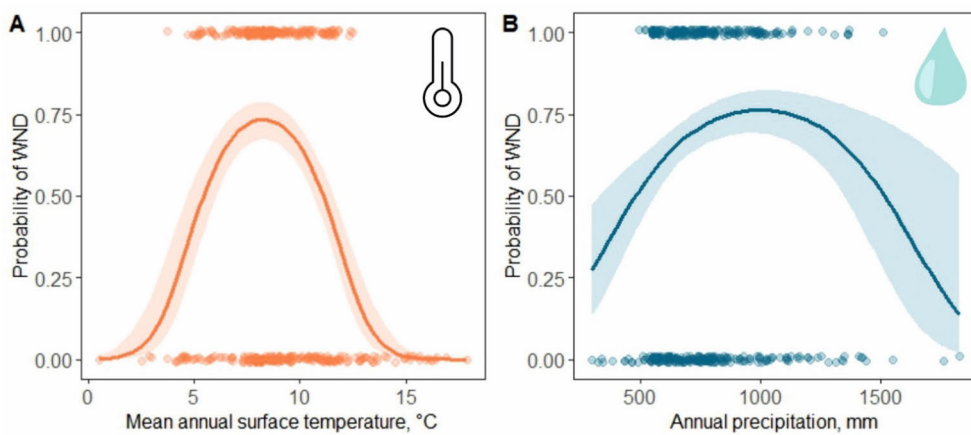

Figure 6. Effects of **A**) mean annual surface temperature and **B**) annual precipitation on the probability of observing WND on any bat at a hibernation site. Coloured areas represent the 85% confidence intervals. Points represent the observed infection status in hibernacula (1 = bat(s) with WND, 0 = no bats with WND; jitter added for clarity).

We used an additional dataset to further examine the correlation between MAST and hibernation site temperature by using Pearson's correlation coefficient, confirming the positive correlation between the two (r = 0.61, 95% CI 0.54–0.67, N = 356). According to the regression line, MAST of 8.3 °C corresponds to 7.1 °C inside hibernacula, although the variation in measured temperatures is considerable (Figure 7).

Based on our model, we generated predictions on the current potential global distribution range for the disease (Figure 8 A, B), elucidating areas in South America, Australia, and New Zealand, that may be under risk if the pathogen is introduced. Furthermore, applying the model to a climate change scenario for years 2061–2080 resulted in a remarkable shift in the potential global range for the disease (Figure 8 C, D).
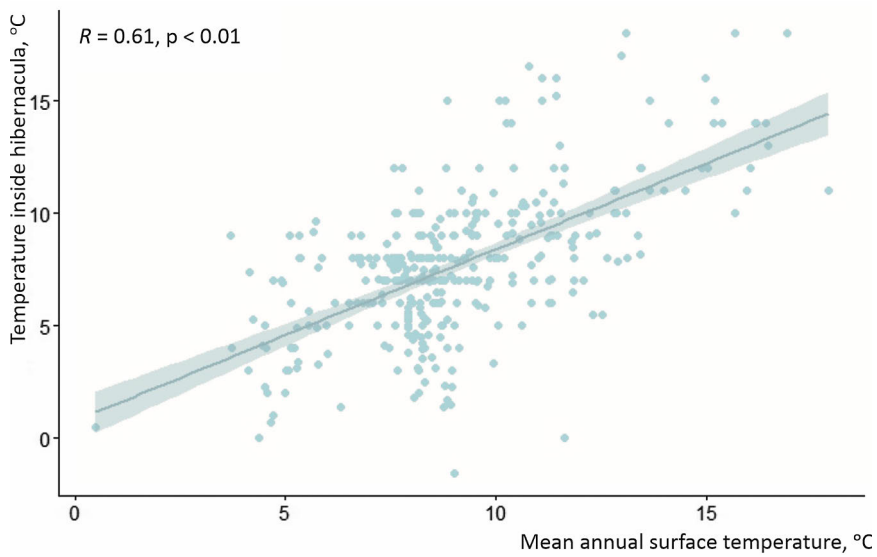
Figure 7. Correlation between mean annual surface temperature and temperature measured inside hibernacula (Pearson's correlation coefficient r = 0.61, 95% CI 0.54–0.67, N = 356).

Figure 8. A) The current potential global distribution of WND according to our model predictions. **B**) Closeup for Europe. **C**) Predicted global distribution of WND during the years 2061–2080 under climate change model ACCESS-CM2, Shared Socio-economic Pathway 3-7.0. **D**) Closeup for Europe.

3.3 Environment and hosts

3.3.1 Climate and host distributions

The data collected for chapter I revealed bats utilizing hibernacula in wide ranges of climatic conditions. Of the species we discovered had signifi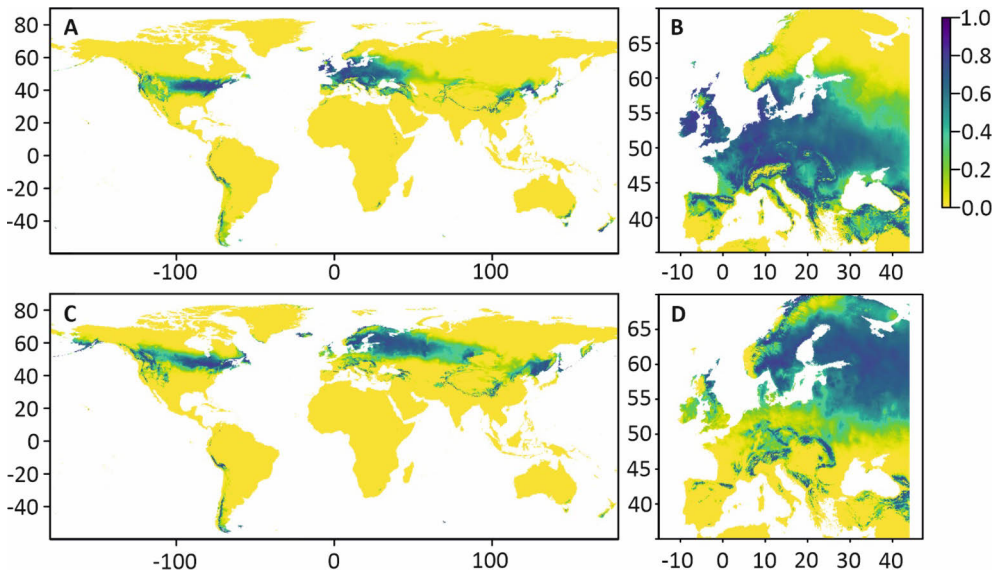cant associations with the probability of observing WND in hibernacula in chapter I, *M. myotis/blythii* and *M. mystacinus/brandtii* (positive association) used hibernacula that were located in colder climates than *M. emarginatus* (positive association), *R. ferrumequinum*, and *R. hipposideros* (negative association) (Figure 9 A). In contrast, the species without significant associations with the probability of WND had no notable differences in their distributions regarding the climate (Figure 9 B).
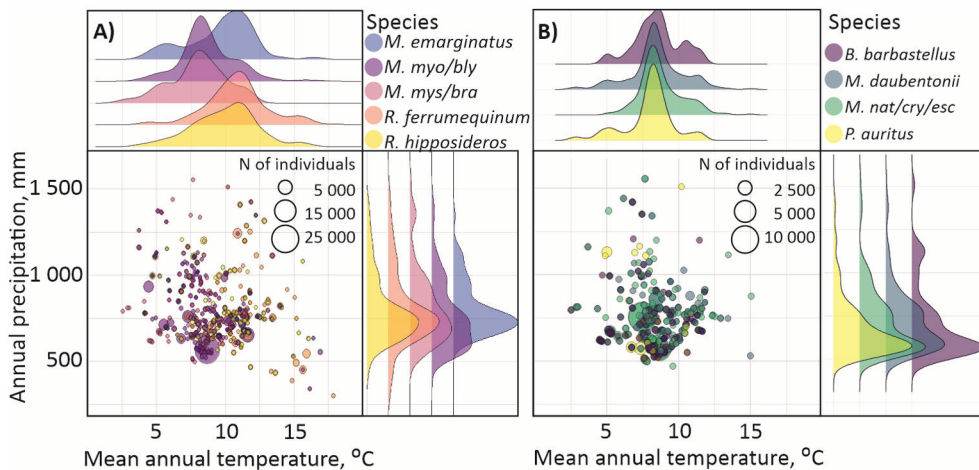
Figure 9. Bubble plot depicting the abundance of **A**) five bat species (*M. emarginatus* (63 sites), *M. myotis/blythii* (262 sites), *M. mystacinus/brandtii* (118 sites), *R. ferrumequinum* (153 sites), *R. hipposideros* (145 sites)) that had a statistically significant association with the probability of WND in hibernacula, and **B**) four bat species/species groups (*B. barbastellus* (53 sites), *M. daubentonii* (221 sites), *M. nattereri/crypticus/escalerai* (179 sites) *and P. auritus* (142 sites)) that had no significant association with the occurrence of WND, hibernating across a range of climatic conditions. The size of the bubble is relative to the number of bats occupying a given hibernacula. The density plots represent the distribution of hibernacula where the species is present.

3.3.2 Weather and winter activity of bats

In chapter III, we discovered that the mean ambient temperature and barometric pressure of the previous 24 hours had a positive effect on the activity of *E. nilssonii* during the winter (Figure 10 A, B). For *M. mystacinus/brandtii* and *P. auritus*, the effect of temperature was dependant on barometric pressure: the activity of *P. auritus* increased with temperature when barometric pressure was low, and, in contrast, the activity of *M. mystacinus/brandtii* increased with temperature when barometric pressure was high (Figure 10 B, D).
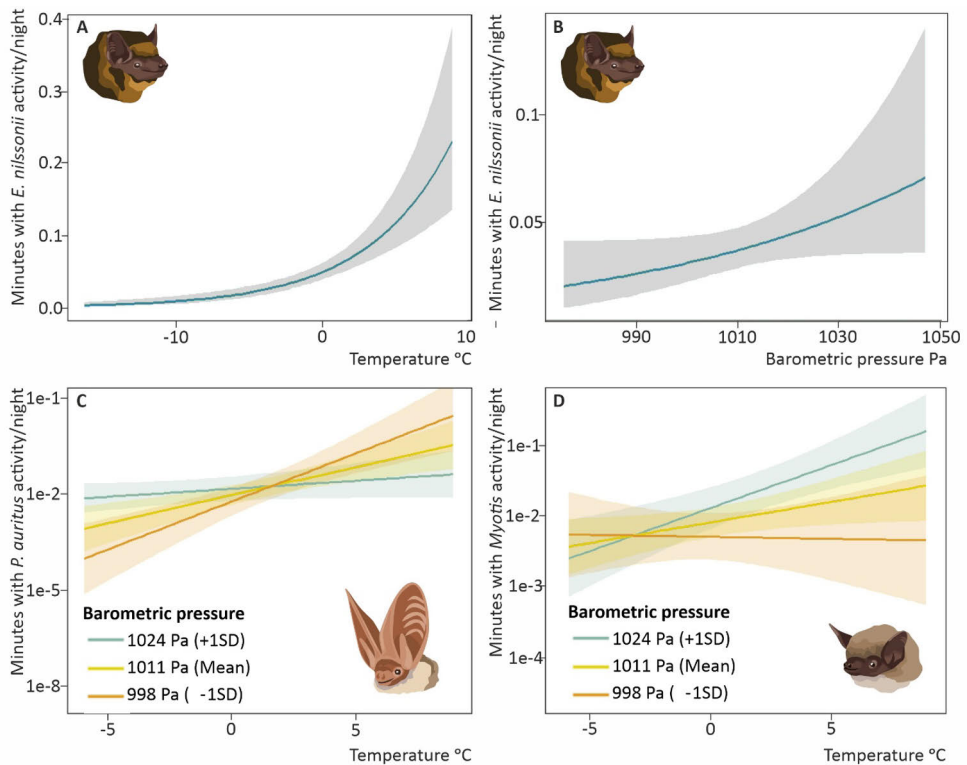

Figure 10. A) The effect of ambient temperature and **B**) barometric pressure on the activity of *E. nilssonii*. **C**) The effect of temperature on the activity of *P. auritus* in different barometric pressures and **D**) The effect of temperature on the activity of *M. mystacinus/brandtii* in different barometric pressures.

3.3.3 Use of novel hibernacula in the future range of WND

In chapter III, we found significant differences in winter activity between habitats ($F_{(4, 405)} = 9.91$; $P < 0.01$), species ($F_{(2, 405)} = 19.29$; $P < 0.01$) and sites ($F_{(11, 405)} = 2.11$; $P = 0.02$). Activity was highest in the vicinity of rock outcrops, which had significantly more activity than other habitats (glacial erratics, ancient shores, feeding areas), root cellars excluded. In addition, bat activity near root cellars was significantly higher than at feeding areas. Estimates for activity indexes in all habitats are presented in Table 1, and differences between habitats are shown in Figure 11 A.

During the two consecutive winters (November–March considered as winter months), we recorded 182 minutes of *E. nilssonii* activity, 66 minutes of *P. auritus* activity, and 35 minutes of *Myotis* spp. activity. The recordings were relatively evenly distributed throughout the winter months (Figure 12). The pooled *Myotis* species included *M. daubentonii*, *M. brandtii*, *M. mystacinus* and *M. nattereri*. We recorded significantly more *E. nilssonii* activity than *P. auritus* or *Myotis* spp. activity. Estimates for activity indexes for the three species are presented in Table 2, and differences between species are shown in Figure 11 B.

In addition, we recorded the echolocation calls of *P. nathusii* in one of the rock outcrops throughout the winter 2018–2019, suggesting that the species is expanding its' overwintering range northwards (chapter IV).

Table 1. LS-means for bat activity indexes measured at different habitats during the winter. Bat activity is measured at each site with a monthly activity index (minutes with recordings/number of recorded hours). Monthly activity indexes were calculated for 5 months (November-March) for two consecutive winters, for three species/species groups: E. nilssonii (182 minutes with activity), P. auritus (66 minutes with activity) and Myotis spp. (35 minutes with activity), for all 16 sites. We excluded 57 monthly activity indexes due to malfunctions in equipment (month considered successful when the equipment recorded more than half of the nights). Total N = 423 monthly activity indexes.

Habitat	N sites	N monthly indexes / habitat	Estimate	SE mean	Lower mean	Upper mean
Ancient shores	4	87	$7.998*10^{-7}$	1.467	$3.765*10^{-7}$	$1.699*10^{-6}$
Bedrocks	3	117	$7.831*10^{-6}$	1.392	$4.090*10^{-6}$	$1.499*10^{-5}$
Glacial erratics	3	78	$8.652*10^{-7}$	1.505	$3.873*10^{-7}$	$1.932*10^{-6}$
Root cellars	3	66	$4.370*10^{-6}$	1.660	$1.613*10^{-6}$	$1.184*10^{-5}$
Feeding sites	3	75	$4.857*10^{-7}$	1.544	$2.067*10^{-7}$	$1.140*10^{-6}$

Table 2. LS-means for activity indexes of different species during the winter. Bat activity is measured at each site with a monthly activity index (minutes with recordings/number of recorded hours). Monthly activity indexes were calculated for 5 months (November-March) for two consecutive winters, for three species/species groups: *E. nilssonii* (180 minutes with activity), *P. auritus* (66 minutes with activity) and *Myotis* spp. (35 minutes with activity), for all 16 sites. Total N = 423 monthly activity indexes.

Species	N activity minutes	N monthly indexes / species	Estimate	SE mean	Lower	Upper
E. nilssonii	182	141	$7.464*10^{-6}$	1.361	$4.072*10^{-6}$	$1.368*10^{-5}$
Myotis spp.	35	141	$7.005*10^{-7}$	1.361	$3.820*10^{-7}$	$1.284*10^{-6}$
P. auritus	66	141	$8.281*10^{-7}$	1.361	$4.518*10^{-7}$	$1.518*10^{-6}$
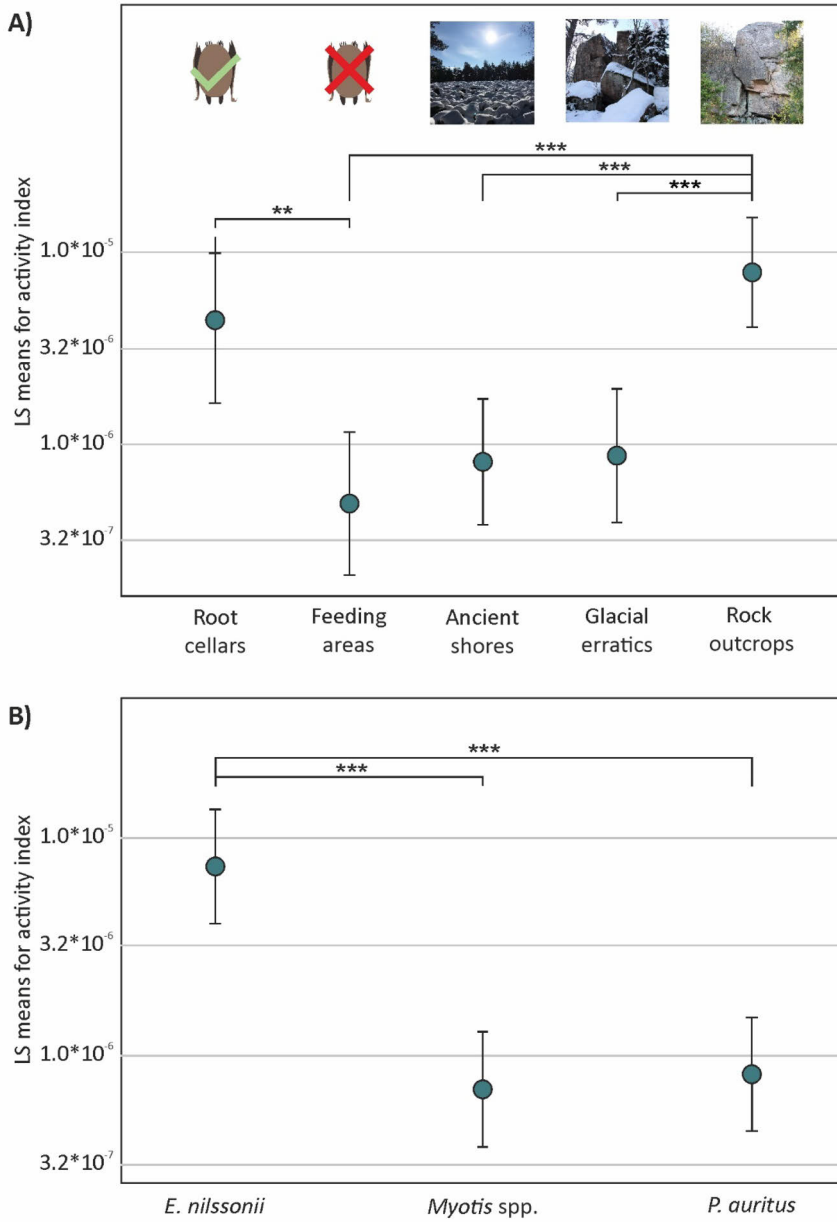
Figure 11. A) LS-means for monthly activity indexes in different habitats and **B**) LS means for monthly activity indexes of different species. Monthly activity index = number of minutes with recordings / number of total recorded hours at a site. Asterisks represent statistically significant differences between habitats (** $P \leq 0.01$; *** $P \leq 0.001$), Tukey-Kramer adjusted for multiple comparisons. Monthly activity indexes were calculated for 5 months (November-March) for two consecutive winters, for three species/species groups: *E. nilssonii* (180 minutes with activity), *P. auritus* (66 minutes with activity) and *Myotis spp.* (35 minutes with activity), for all 16 sites. N = 423 monthly activity indexes.

Figure 12. Temporal distribution of recorded activity of **A**) *E. nilssonii*, **B**) *P. auritus* and **C**) *Myotis* spp.

4   Discussion

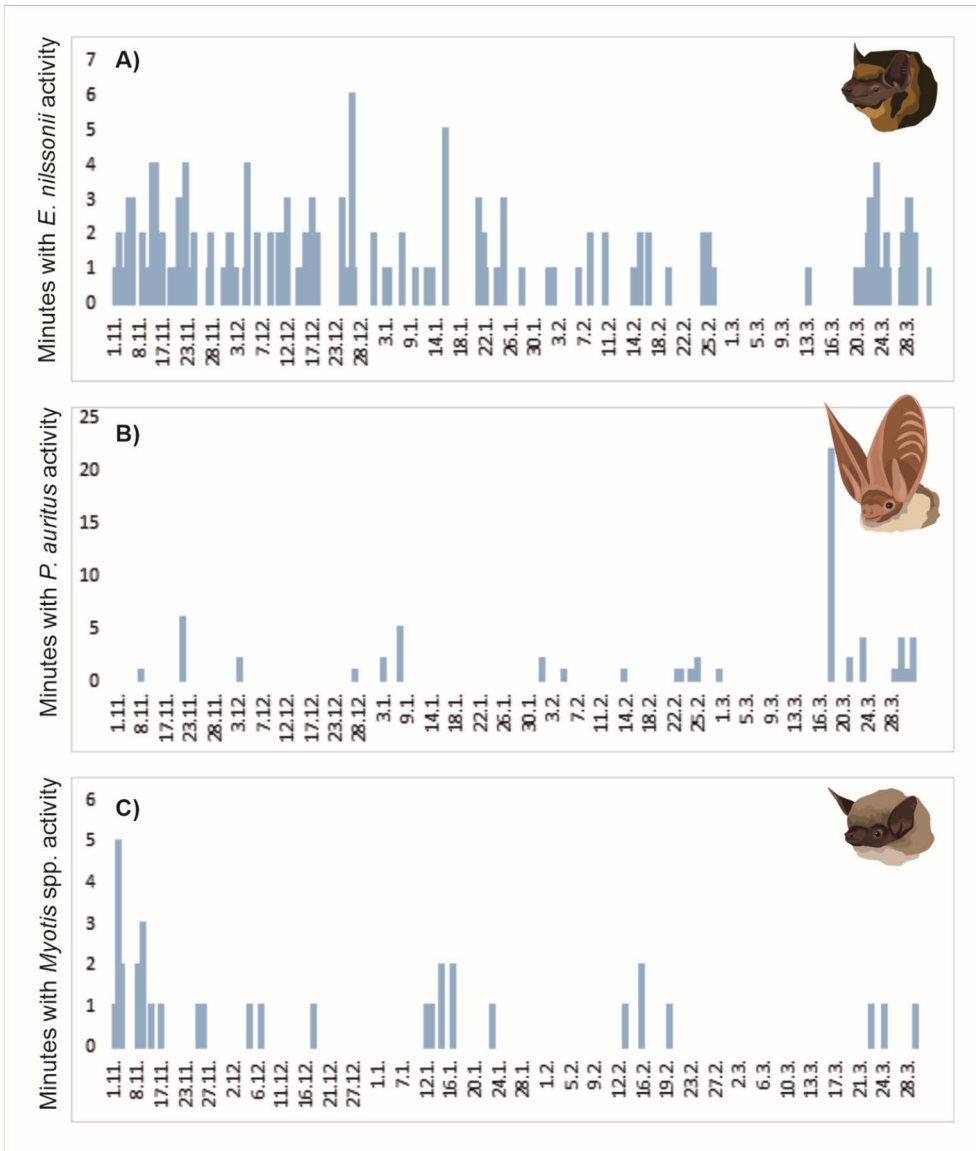The purpose of this thesis was to build a holistic view of the interactions between different factors affecting the onset of WND: the pathogen, the host, and the environment. Studying WND dynamics in the Palearctic, where the fungus has coexisted with its hosts for a considerable time, allowing coevolution between the two, can help illuminate the future of bats in newly affected areas. In addition, in-depth understanding of the resistance, tolerance and avoidance mechanisms in the Palearctic bat communities can be valuable in conservation planning.

In chapter I, I discovered that three species/species groups (*M. emarginatus*, *M. myotis/blythii* and *M. mystacinus/brandtii*) positively correlated with the occurrence of WND in hibernacula, and, in contrast, two species (*R. ferrumequinum* and *R. hipposideros*) had a negative association with the disease. These results could indicate tolerance in species with positive effects on the disease, or resistance shown by the species with negative associations: tolerance, in general, leads to elevated pathogen loads in the environment, whereas resistance should decrease the load through lowered proliferation of the pathogen. However, these results may also reflect the differing environmental preferences of these species and how these conditions limit or boost the onset of WND. For instance, while there is some support for resistance in *R. ferrumequinum* for WND (Hoyt et al., 2016a), our data indicates that at least some of the ability of *R. ferrumequinum* and *R. hipposideros* to avoid infection could be due to environmental factors, i.e., selecting hibernacula that are warmer than would be optimal for disease manifestation. Ambient temperature affects the torpor bout length of bats (Ransome, 1971), and bats hibernating in warmer environments arouse more often and as a consequence show milder signs of infection (Fritze et al., 2021). Interestingly, the environmental preferences of *M. emarginatus*, which we discovered had a positive association with WND, appear to be quite similar to those preferred by *R. ferrumequinum* and *R. hipposideros*. The relationship between *M. emarginatus* and WND therefore requires more attention in the future.

Of the species with a positive correlation with WND, *M. myotis* has been most often associated with the disease (Puechmaille et al., 2011). In chapter II, we demonstrated that *M. myotis* does not show local immune responses as a response to WND infection, i.e., the species appears to be tolerant to *P. destructans*, possibly

explaining the strong association between the species and the disease in Europe that was also evident in our results in chapter I. In contrast, the remnant population of *M. lucifugus* attempted to resist the infection by mounting intense local immune responses. In light of the exposure histories of these species to *P. destructans*, this result is fitting: tolerance should eventually be favoured by natural selection, as it benefits both the host and the pathogen through exempting the host from energetically costly resistance mechanisms while allowing the pathogen to proliferate freely (Roy and Kirchner, 2000). Indeed, it appears that *M. myotis*, a species that has coexisted with *P. destructans* for millennia, has evolved a commensal relationship with the fungus. Similarly to other studies (Field et al., 2015, 2018; Davy et al., 2017a), the transcriptomic responses we found in the Nearctic *M. lucifugus* were related to immune responses, such as immune cell activation, migration and inflammatory pathways. While transcriptomic data does not necessarily always reflect the final protein products (Maier et al., 2009), similar results suggesting tolerance in *M. myotis* and attempted resistance in *M. lucifugus* have been obtained from a study comparing the plasma proteomic profiles of infected and non-infected individuals (Hecht-Höger et al., 2020), giving further credence to our result. Interestingly, we discovered that despite the lack of immune responses, *M. myotis* showed a lower amount of cupping lesions compared to *M. lucifugus*. While the mechanism behind this observation is not clear, possible explanations include environmental conditions, such as humidity and temperature in hibernacula (Johnson et al., 2014), as well as antifungal microbes (Cornelison et al., 2014; Hoyt et al., 2015a; Micalizzi et al., 2017), or protecting fatty esters of the skin membrane (Frank et al., 2018). Given that our pair-wise sampling method could only account for differences in gene expression between infected and noninfected samples, we also cannot rule out the possibility of a systemic response in *M. myotis*. Indeed, there is evidence of severely infected *M. myotis* utilizing a passive base line immune response during torpor (Fritze et al., 2021), which could explain the low number of lesions in *M. myotis* in our study.

In addition to the striking differences in the local immune responses between *M. myotis* and *M. lucifugus*, we found evidence that there may have already been some selection for responses in populations that were among the first to come into contact with the disease in North America. However, it is still evident that the Pennsylvanian bat populations, while recovering, are still not out of danger, and surviving populations of *M. lucifugus* suffer from elevated mortality. Bats are sensitive to disturbance, and reproduce slowly, and are therefore vulnerable to stochastic events. For instance, as the survival of the remnant populations is likely to be highly dependent on the ability of the bats to accumulate fat prior to the hibernation season to fuel their arousals and immune responses, threats such as global insect decline and climate change may put these populations in danger. In addition, there may also be

possible carry-over effects for reproduction in populations suffering from WND (Davy et al., 2017b). Females require energy for gestation after the hibernation period, and therefore the depletion of their fat reserves due to WND may reflect on their reproduction success (Jonasson and Willis, 2011; Czenze et al., 2017). Hence, conservation measures should be implemented to protect the populations outside the hibernation season as well.

In addition to available hosts and their responses to infection, environmental conditions are another important factor contributing to WND dynamics. In chapter I, we showed that the occurrence of WND is also dependent on climatic factors, namely the mean annual surface temperature (MAST) and annual rainfall. Temperature in underground sites correlate with MAST (McClure et al., 2020), and a similar connection exists between rainfall and underground humidity (Perry, 2013). Both temperature and humidity within hibernacula are important environmental components for WND (Verant et al., 2012; Marroquin et al., 2017; Martínková et al., 2018). Our model showed that the probability of WND being visible in hibernacula was highest in areas where MAST was 8.3 °C. When we compared this to available temperature data measured inside hibernacula, we discovered that MAST of 8.3 °C corresponded to approximately 7 °C within hibernation sites. Previous research suggests that the optimal temperature for disease onset is between 5–6 °C (Martínková et al., 2018). The environmental conditions within hibernacula, as well as the availability and variability of microclimates are affected by a plethora of factors besides MAST, and the slight discrepancy could be due to bats selecting colder microclimates within hibernation sites to reduce their energetic costs. Our model supports the possibility of utilizing open-source climate data to predict WND risk on a regional scale, instead of environmental measures from hibernation sites, despite MAST not being an accurate measure of predicting environmental conditions in specific hibernacula.

Applying our model to current global climatic conditions revealed areas that are likely suitable for the manifestation of the disease, and may therefore be under risk if the pathogen is accidentally introduced. In addition to the current known distribution of the disease in the Palearctic and North America, these areas include southern parts of South America, parts of Southern Australia, and New Zealand, giving further credence to previous research that has raised concern for the introduction of the disease to these areas (Turbill and Welbergen, 2019; Lilley et al., 2020). This result highlights the importance of proper hygiene when visiting caves or other suitable hibernacula in these areas, and the need for local monitoring programs so that the possible pathogen introduction can be discovered early on, possibly enabling the prevention of further spread. Our predictions on the current possible distribution for WND in the Palearctic resemble closely to the recorded range of the disease, excluding the absence of the visible disease from the UK and

southern Scandinavia, likely due to being outside of *M. myotis* hibernation range. In contrast, the predicted range of the disease in North America is smaller than what has been seen in reality. We suggest that the high availability of extremely susceptible hosts may compensate for the less optimal environmental conditions, as susceptible hosts increase the proliferation of the fungus, resulting in high pathogen loads in the environment and consequently increased risk of infection. Interestingly, according to our model the area of the original introduction in New York is optimal in terms of climatic conditions for the manifestation of the disease. This could help explain, why despite the rampant tourism and non-existent hygiene procedures the disease only took hold in North America in 2006. It is possible that the introduction of the pathogen in New York was not the first on the continent, but due to the local optimal environment, the fungus was able to take hold and proliferate. Therefore, while the areas deemed to be at high risk according to our model may not be the only ones where the disease can manifest, focusing monitoring efforts on new disease epicentres in areas with optimal climatic conditions is advisable.

Applying our model to a climate change scenario resulted in dramatic changes in the predicted global distribution range of WND. This result highlights the massive potential climate change has for shifting the geographic range of the disease and put new bat populations at risk of infection. For instance, our climate change scenario model predicted that by the years 2061–2080 the potential range of WND will have moved considerably towards north in the northern hemisphere, with large parts of Fennoscandia suitable for the disease manifestation. Due to the lack of caves, the hibernation sites in these areas have been poorly known, and monitoring of hibernating bats has mainly focused on anthropogenic structures, such as mines, root cellars and bunkers. However, the censused individuals at these sites only account for a fraction of the actual bat populations. Therefore, it has long been assumed that bats in these areas are using other sites over the winter as well. In chapters III and IV, we applied acoustic monitoring to reveal natural hibernation sites and their species compositions in South-Western Finland. We discovered that rock outcrops with cracks and crevices had similar levels of bat activity over the winter compared to the vicinity of root cellars that were confirmed to be occupied by bats. These sites are very distinct from the traditional cave hibernacula usually associated with the occurrence of WND, as they are in general more exposed to the outside environment, for instance water flow resulting from rain or melting snow. Therefore, these hibernation habitats may provide protection from the infection in the future.

Our climate change prediction in chapter I is based on a model that presumes the species composition is constant. Given the importance of species composition for the disease manifestation according to our results, the eventual range of WND will depend on how species respond to climate change by shifting their hibernation ranges. Climate change has already caused shifts in the distribution ranges of several

bat species (Lundy et al., 2010; Ancillotto et al., 2016; Uhrin et al., 2016). During the present study, we recorded one new hibernating species for Finland, *Pipistrellus nathusii*, a species that has shown considerable range expansion often attributed to climate change (Lundy et al., 2010; Ancillotto et al., 2016). To this date, this is also the northernmost record of overwintering for this species. As a long distant migrant, *P. nathusii* is exceptionally mobile, and therefore likely well adapted to utilizing new suitable areas. While this species has not been recorded with WND, the range expansion of *P. nathusii* could be considered a sign that environmental conditions have already been altered by climate change in a way that affects the species composition in hibernacula.

The present study demonstrates two methods for WND surveillance. Visual assessment of white-nose syndrome (chapter I) is possible in areas where bats hibernate in sites that are accessible to humans, whereas acoustic monitoring (chapters III and IV) can be used to recognize unusual winter activity, a sign of sickness behaviour (Carr et al., 2014), in areas where bats use hibernation sites that are covert or less accessible. However, baseline information on the hibernacula and the prevalence and drivers of winter activity are needed to distinguish possible sickness behaviour from usual activity due to foraging or roost switching. Furthermore, as we show in chapter IV, acoustic monitoring can be used to effectively monitor changes in hibernation ranges of species. In addition, given the difficulty of identifying hibernating bats to species level, acoustic monitoring can help determine the species composition of hibernacula more precisely than visual censuses. While we chose to pool all *Myotis* spp. for the analyses in chapter III to gain more power, most of the recordings were identifiable to species level. For instance, we confirmed the occurrence of *M. nattereri*, a species classified as endangered in Finland, at two of the study sites. As the ecology of this elusive species in Finland is poorly known, with only a few previously known hibernation sites, these findings have also value for conservation.

In addition to novel hibernation sites, in chapter III we illuminated relationships between winter activity and weather conditions, namely mean ambient temperature and barometric pressure of the previous 24 hours. By timing their activity to warm nights, bats can save energy during relocation, or replenish fat reserves through foraging as insects are more likely to be available (Avery, 1985; Zahn and Kriner, 2016). Several mammalian species, including some bats, have been confirmed to be able to sense changes in barometric pressure, which can be used a as proxy for weather (Paige, 1995; Czenze and Willis, 2015). In Finland, during the winter, high barometric pressure systems usually arrive from the east, bringing cold yet clear weather. In contrast, southwestern low barometric pressure systems generally corresponds to cloudy and rainy, yet mild weather in the winter (Similä, 1981). We predicted that bat activity during the winter would be increased by warm

temperature, and, presuming bats are capable of utilizing barometric pressure as a cue, bats would be more active when barometric pressure was low. We found that ambient temperature had a positive effect on the activity of *E. nilssonii*, but unlike we predicted, we also found a positive association between the activity of the species and barometric pressure. In contrast, our results revealed that the effect of temperature on the activity of *Myotis* spp. was different depending on present barometric pressure: *Myotis* spp. activity increased with temperature when barometric pressure was high, but during low barometric pressure there was no association between temperature and activity. Interestingly, for *P. auritus*, the relationship between the effect of ambient temperature and barometric pressure on their activity was the opposite: the activity of *P. auritus* increased with ambient temperature when barometric pressure was low, but the positive effect did not exist during high barometric pressure. This result indicates that *P. auritus* may indeed use barometric pressure as a proxy to time their activity to mild nights. Most *Myotis* species, as well as *E. nilssonii*, are aerial hawkers, which means that they predominantly hunt flying insects. Therefore, the increase in the activity of these species during high barometric pressure could be the result of these species avoiding activity in rainy weather. In contrast, *P. auritus* utilizes a different foraging strategy and gleans prey from surfaces, increasing their chances of finding prey over the winter even when it is too cold or rainy for flying insects to be active. Therefore, being able to use barometric pressure as a proxy would improve their overwintering success. A better understanding of the drivers of winter activity and the overwintering strategies of bats can help distinguish unusual behaviour in these species, and evaluate their future in the changing climate. In addition, being able to replenish fat reserves over the winter can help hibernating bats to limit the harm from WND due to the possibility for more arousals.

This study presents new information on the complex interactions contributing to WND in the Palearctic. The results emphasize the importance of host species composition and climatic conditions for the manifestation of the disease and raise concerns on the potential introduction of the pathogen to areas that are climatically suitable for the disease. In addition, the results highlight the massive potential that climate change may have for the areas affected by the disease and provide more evidence on the climate change related expansion of one hibernating bat species. It also shows that acoustic monitoring can be an effective tool for locating novel, unknown hibernacula, and investigate their species composition and the drivers of winter activity. Our results also draw attention for the need to conduct more research on the mechanisms of tolerance and resistance in both Nearctic and Palearctic bats. If the ability to tolerate infection allows bats to cope with WND without apparent costs, further studies could help predict the future of Nearctic and potentially other areas as well, if containing the pathogen in its current ranges fail.

5   Conclusions

1. Species composition and local climatic conditions can be used to predict the probability of WND being manifested in hibernacula.

2. The Palearctic *Myotis myotis* does not mount local immune responses as a consequence of WND infection, indicating that the species is tolerant towards the fungus. In contrast, the Nearctic *M. lucifugus* shows massive differences in gene expression between infected and non-infected tissue, mainly in genes associated with immune functions, suggesting that the current mechanism for survival in this species is resistance.

3. Several geographical areas, mainly in southern South America, southern Australia, and New Zealand, have climatic conditions that are suitable for the manifestation of the disease. This result calls for attention in hygiene procedures when visiting hibernacula, and the need for monitoring.

4. Due to the correlation between climatic conditions and the abiotic conditions inside hibernacula, climate change can have massive effects for the potential global distribution of the disease.

5. Novel hibernacula in the non-karst regions of Fennoscandia may provide protection for bats from the disease in the future, as the climatic conditions become more optimal for the manifestation of the disease due to climate change.

6. Weather conditions are an important driver for winter activity of bats in South-Western Finland. Ambient temperature has a positive effect on activity, likely due to the increased foraging possibilities when insects are active. In addition, *Plecotus auritus* may be using low barometric pressure as a cue for mild outside temperatures during hibernation season.

7. Expansion of hibernation ranges are already apparent in the mobile, migratory bat species *Pipistrellus nathusii*.

8. Visual assessment of WND status is an effective way of monitoring the disease in areas where bats rely on traditional, accessible hibernacula such as caves and anthropogenic structures. In contrast, acoustic monitoring can help locate novel hibernation sites in areas where these traditional hibernacula are rare, and reveal signs of possible sickness behaviour.

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