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Abiotic variables in disease expression of a fungal pathogen in mountains under global change

Marilen Haver

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de Toulouse

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A. obstetricans in the mountains (Woodcut, Leipzig 2018, M.Haver)

Abstract

The health of mountain freshwater ecosystems, and the ecosystem services they provide, are threatened by global change impacts on mountain climate and hydrology. The disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), has been causing epidemics of amphibian populations globally, with hotspots of severe mortality events in mountain areas.

Emerging disease in freshwater species, such as caused by fungal pathogens, is seen as evidence of profound alterations in freshwater ecosystem functioning and ecosystem integrity. Pathogens, their hosts, but also the host microbiome and the plankton community respond to changes in abiotic variables. Observed disease and its severity is the expression of these responses and the complex interactions between species. A better understanding of drivers of disease in these ecosystems is needed, not only for the conservation of mountain freshwater biodiversity and their health, but also to be able to prevent further degradation in ecosystem quality and consequent loss of important ecosystem services to human societies.

In this thesis, the main abiotic variables, which can impact chytridiomycosis dynamics in mountains, are defined from the literature and discussed in a global change perspective. Further, *Bd* prevalence and disease dynamics in freshwater study sites along five elevation gradients in the Pyrenees mountains are analysed in their relationship to recent changes and variability in selected abiotic variables. Annual spring start timing (start of the annual growing season and activity of freshwater species such as amphibians, pathogens, plankton) and annual *Bd* prevalence in one highly infected Pyrenean study site were used to extrapolate infection risk to all study sites. The relationship between climate extremes and hydrology variables and this spring start related infection risk is tested. In addition, temperature conditions in the two months following annual spring start are characterized. As a result, both spring temperature, especially low average temperature, and hydroclimatic variables, which are pointing at snow drought conditions, are suggested as essential abiotic drivers in inter-annual chytridiomycosis dynamics in amphibians of the Pyrenees. In context of the predicted climate change effects on temperatures and water balance in the Pyrenees, these results may indicate increased future infection risk.

The presented framework and analysis may help in a better understanding of chytridiomycosis disease observed in mountains with snow regimes, anticipate changes in infection risk in a future climate and may allow to develop improved models of infection risk,

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that are transferable to other areas or species. The increasing availability of environmental data at high temporal resolution and at global spatial extent offers the potential of studying abiotic drivers of disease, such as those discussed here, on the scale of one mountain range and between mountain ranges.

In summary, this thesis suggests that abiotic variables in chytridiomycosis disease expression call for interdisciplinary research approaches. These will be crucial to inform on freshwater ecosystem integrity and help to formulate essential biodiversity variables with importance for mountain species under the impact of global change.

Keywords: Climate change; Freshwater ecosystems; *Batrachochytrium dendrobatidis*; Chytridiomycosis; Amphibians; Mountain hydrology; Pyrenees

Resumé

La santé des écosystèmes d'eau douce de montagne, et les services écosystémiques qu'ils fournissent, sont menacés par les impacts du changement global sur le climat et l'hydrologie des montagnes. La chytridiomycose, maladie causée par le champignon pathogène *Batrachochytrium dendrobatidis* (*Bd*), a provoqué des épidémies dans les populations d'amphibiens du monde entier, avec des foyers de mortalité grave dans les zones de montagne.

Les maladies émergentes chez les espèces d'eau douce, telles que celles causées par des pathogènes fongiques, sont considérées comme des preuves de profondes altérations du fonctionnement et de l'intégrité des écosystèmes d'eau douce. Les agents pathogènes, leurs hôtes, mais aussi le microbiome de l'hôte et la communauté planctonique réagissent aux changements des variables abiotiques. Les maladies observées et leur gravité sont l'expression de ces réponses et des interactions complexes entre les espèces. Une meilleure compréhension des moteurs de la maladie dans ces écosystèmes est nécessaire, non seulement pour la conservation de la biodiversité des eaux douces de montagne et de leur santé, mais aussi pour être en mesure de prévenir une nouvelle dégradation de la qualité des écosystèmes et la perte consécutive de services écosystémiques importants pour les sociétés humaines.

Dans cette thèse, les principales variables abiotiques, qui peuvent avoir un impact sur la dynamique de la chytridiomycose en montagne, sont définies à partir de la littérature et discutées dans une perspective de changement global. En outre, la prévalence de *Bd* et la dynamique de la maladie dans les sites d'étude d'eau douce le long de cinq gradients d'altitude dans les Pyrénées sont analysées dans leur relation avec les changements récents et la variabilité des variables abiotiques sélectionnées. Le début du printemps annuel (ici, début de la période de croissance après le dégel du lac) et la prévalence annuelle de *Bd* dans un site d'étude pyrénéen fortement infecté ont été utilisés pour extrapoler le risque d'infection à tous les sites d'étude. La relation entre les extrêmes climatiques et les variables hydrologiques et ce risque d'infection lié au début du printemps est testée. En outre, les conditions de température dans les deux mois suivant le début du printemps annuel sont caractérisées. En conséquence, la température printanière, en particulier la température moyenne basse, et les variables hydroclimatiques, qui indiquent des conditions de sécheresse nivale, sont suggérées comme des facteurs abiotiques essentiels dans la dynamique interannuelle de la

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chytridiomycose chez les amphibiens des Pyrénées. Dans le contexte des effets prévus du changement climatique sur les températures et le bilan hydrique dans les Pyrénées, ces résultats peuvent indiquer une augmentation du risque d'infection à l'avenir.

Le concept et l'analyse présentés peuvent aider à mieux comprendre la maladie de la chytridiomycose observée dans les montagnes avec des régimes de neige, anticiper les changements dans le risque d'infection dans un climat futur et permettre de développer des modèles améliorés du risque d'infection, qui sont transférables à d'autres zones ou espèces. La disponibilité croissante de données environnementales à haute résolution temporelle et à l'échelle spatiale mondiale offre la possibilité d'étudier les facteurs abiotiques de la maladie, tels que ceux discutés ici, à l'échelle d'une chaîne de montagne et entre les chaînes de montagne.

En résumé, cette thèse suggère que les variables abiotiques dans l'expression de la maladie de la chytridiomycose nécessitent des approches de recherche interdisciplinaires. Celles-ci seront cruciales pour informer sur l'intégrité des écosystèmes d'eau douce et aider à formuler des variables essentielles de biodiversité ayant une importance pour les espèces de montagne sous l'impact du changement global.

Mots-clés: Changement climatique; écosystèmes d'eau douce ; *Batrachochytrium dendrobatidis*; Chytridiomycosis; amphibiens ; hydrologie de montagne; Pyrénées.

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General introduction

Global biodiversity loss has triggered the idea of an ongoing sixth mass extinction event (Barnosky *et al.*, 2011; Ceballos *et al.*, 2015). Species declines or losses often occur due to a synergy of stressors, which can be of local or global origin. Major threats to ecosystem integrity and species survival are related to direct or indirect human impacts. Anthropogenic climate change is causing perhaps the most profound, complex, and certainly the most publicly debated, recent changes in global ecosystems, threatening species survival and health (Walther *et al.*, 2002). Climate change is exacerbating consequences of other stressors to biodiversity, such as habitat loss or fragmentation, environmental pollution, invasive species introduction and disease emergence, which often originate in human activities and globalization (Pecl *et al.*, 2017; Johnson *et al.*, 2017).

The emergence of wildlife disease can be seen as consequence and as well indicator of ecosystem degradation (Daszak, Cunningham, & Hyatt, 2001; Jones *et al.*, 2008). Multiple species, including humans, are affected by emerging wildlife disease (Daszak, Cunningham, & Hyatt, 2000). As one dramatic example, the panzootic decline of amphibian species has, in many locations globally, been related to emerging disease caused by a pathogen (Daszak *et al.*, 1999; Fisher & Garner, 2020). Species declines and losses due to disease are not only a conservation challenge. Human economies and human health are dependent on the integrity and health of natural ecosystems. Thus, due to the understanding that there is only “One health” (Cunningham, Daszak, & Wood, 2017)(Figure 1), as human, wildlife and environmental health are inseparable, it is crucial to identify drivers of emerging wildlife disease.

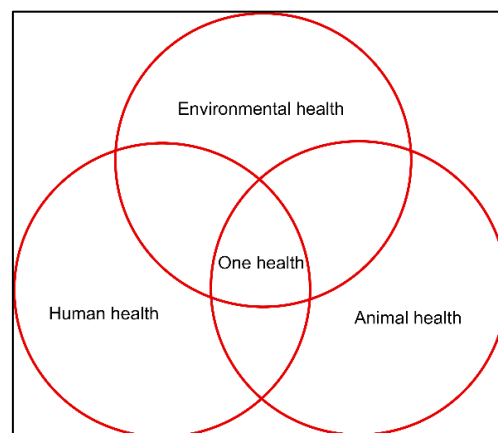


Figure 1: Simplified illustration of the OneHealth-concept, which underlines the inseparability of environmental, animal and human health.

Disease in mountain freshwater ecosystems

Disease is one rapidly evolving threat to global freshwater biodiversity, where many species declines have occurred in recent decades (Reid *et al.*, 2019). Devastating amphibian epidemics due to the fungal pathogen/ chytridiomycete fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) have been observed in freshwater ecosystems globally (Scheele *et al.*, 2019; Lambert *et al.*, 2020; Fisher & Garner, 2020) (Figure 2). Amphibians infected with *Bd* can develop the potentially lethal disease chytridiomycosis, which, in case of high susceptibility of hosts to disease, may cause epidemics in amphibian populations in nature (Berger *et al.*, 1998; Daszak *et al.*, 1999; Rachowicz & Vredenburg, 2004). Recent findings suggest that *Bd* is an invasive pathogen in most of its occurrence sites and has its origin in Asia, from where it has most likely spread by globalized human trade (O’Hanlon *et al.*, 2018).

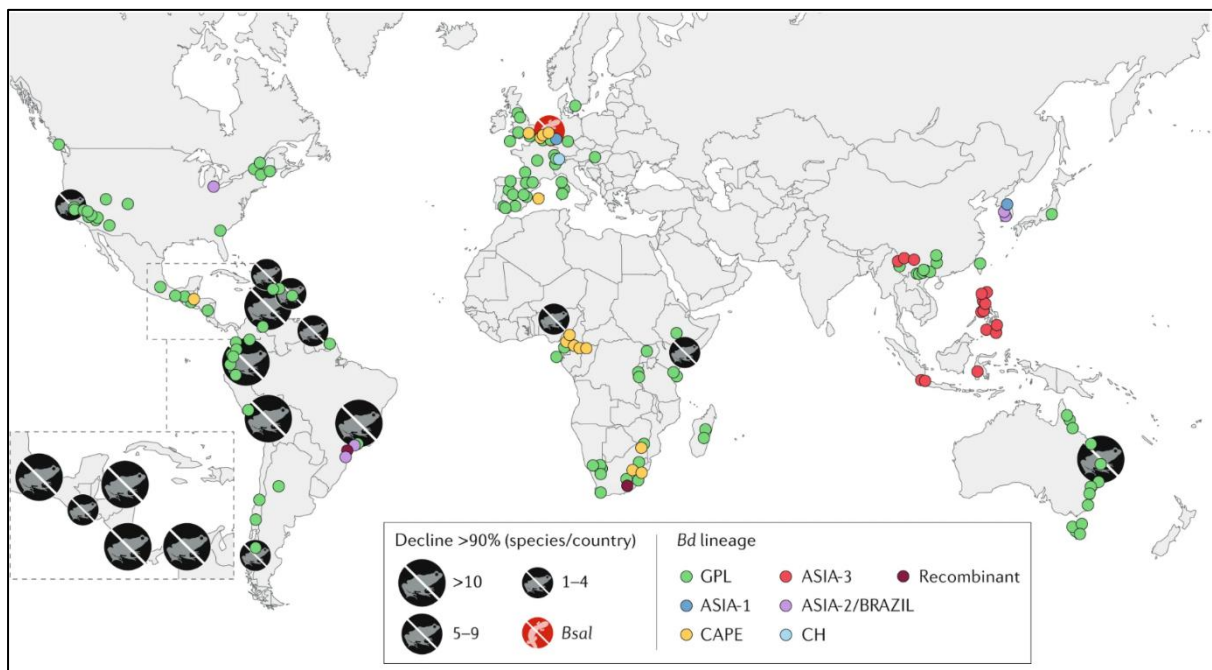


Figure 2: Map of global amphibian declines and *Bd* lineages, that were related to these extinctions, as published in Fisher & Garner, 2020, Fig.1.

Once introduced into an area, the establishment, spread and virulence of the pathogen and infection outcomes in amphibians will depend on a complex interplay of abiotic and biotic variables – thus, variables related to the environment and to the involved species or species interactions (Fisher & Garner, 2020). Recently, the so called *disease pyramid* concept was formulated, in order to stress the importance of including interactions between the pathogen,

host, host microbiome and the environment on the expression of disease (Bernardo-Cravo *et al.*, 2020).

Many hotspots of chytridiomycosis occurrence and related extinctions are situated in mountain areas (Figure 2, (Fisher & Garner, 2020). Mountains are often considered pristine environments because they are remote from human settlements and direct (visible) human impact. Despite, global stressors, such as climate and land use changes and the introduction of pollutants, have been imprinting on mountain freshwater ecosystems, which is evidenced by changes in their functioning and health (Le Roux, Hansson, & Claustres, 2016; Le Roux *et al.*, 2020; Schmeller *et al.*, 2018). Among the severe consequences of climate change and other global changes in mountains may be the loss of freshwater habitat and freshwater species and the degradation of water quality. This further endangers important ecosystem services freshwaters deliver to human societies, such as for example the provision of clean water (Vári *et al.*, 2021). Amphibians are an important part of freshwater ecosystems, and contribute essentially to ecosystem services, for example by predation of insects or by nutrient recycling (Hocking & Babbitt, 2014). Because amphibians as ectotherms and with aquatic and terrestrial life stages are very sensitive to changes in their abiotic environment (e.g. temperature, water availability, pollution), they are often seen as indicator species for the quality of aquatic ecosystems (Hopkins, 2007).

Global change impacts in mountains cause changes in abiotic variables in the mountain atmosphere, cryosphere, hydrosphere and biosphere. Recent research efforts, such as by the IPCC, summarize these observed changes (IPCC, McDowell, & Barr, 2019). Variables, representative of climate and its changes, that are feasible and cost-efficient to observe and globally available, have been formulated by the scientific community as so-called *essential climate variables* (ECVs) (Bojinski *et al.*, 2014). Recently, such indicators were suggested for mountain areas, as *essential mountain climate variables* (EMCVs) (Thornton *et al.*, 2021). Building on the ECV-concept, *essential biodiversity variables* (EBVs) have been defined as multi-scale variables (with a time, space and biological component) intended to offer a standardized framework that link primary observational data on a biodiversity variable to temporal changes in this variable (Pereira *et al.*, 2013; Schmeller *et al.*, 2017). Due to the importance of mountain-specific global change impacts, a set of *essential mountain biodiversity variables* (EMBV) are currently discussed and defined by experts (Kulonen, Adler, & Palazzi, 2020).

General introduction

In order to understand and mitigate global change impacts that threaten mountain ecosystem integrity, such as loss of amphibian biodiversity due to disease, mountain-specific variables and processes need to be accounted for. Changes in species abundance (e.g., abundance of amphibians, pathogens, plankton and microbiome species), which are leading to changes in species community composition, are examples of EBV-classes that can inform on indicators for conservation policy (Jetz *et al.*, 2019). Indicators are for example the impact of species invasions (e.g., invasive pathogens), species extinction risk (e.g., for amphibian species), ecosystem condition and ecosystem services (Jetz *et al.*, 2019).

As we consider amphibians indicator species for ecosystem health, *Bd* prevalence and chytridiomycosis in mountains might point at the presence of general stressors, risking health of other mountain species as well as human health. Understanding drivers of biodiversity change or biodiversity loss is crucial also for mitigating negative effects for human health and human economies, as the underlying degradation of ecosystem integrity threatens human health and well-being through loss of ecosystem services (Schmeller *et al.*, 2018).

Aim and scope

The aim of the work presented in this thesis is to contribute to a better understanding of abiotic environmental drivers of disease in mountain freshwater ecosystems. Abiotic variables with relevance for host-pathogen dynamics and the microbiome (thus for the “mountain” disease pyramid, Figure 3) are defined and discussed in the context of mountains under global change.

The three main research questions are formulated as follows:

1. Which variables can be seen as main abiotic drivers of chytridiomycosis disease in mountain freshwaters and how are they affected by global change? *See introductory Chapters 1 and 2.*
2. How are freshwater ecosystems in the Pyrenees affected by global change? *See methods, results and discussion in Chapters 3 to 6.*
3. Which abiotic variables of the categories climate and hydrology may be essential for chytridiomycosis disease dynamics in freshwaters of the Pyrenees and how do they relate to climate change? *This is the focus in Chapter 6.*

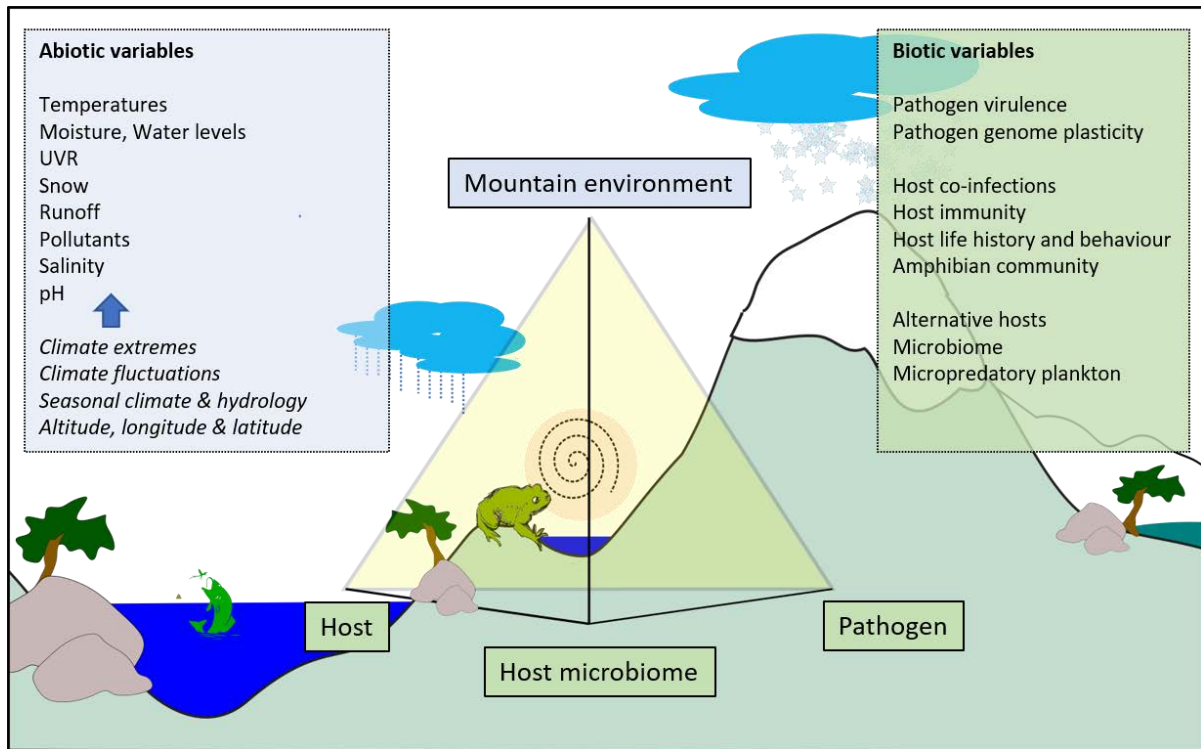


Figure 3: The disease pyramid in a mountain context, modified after the disease pyramid concept as defined and published in Bernardo-Cravo *et al.* 2020 and for abiotic and biotic variables in disease after Fisher & Garner, 2020, Figure 3.

The thesis Chapters 1 and 2² give a general theoretical background by describing the complexity of the mountain habitat of amphibians and pathogen as well as by introducing the current state of knowledge in abiotic variables driving chytridiomycosis disease expression together with their importance in mountains. Chapters 3 to 6, cover the study of climate extremes and hydrology in 25 freshwater sites in the Pyrenees. The study sites are situated along 5 elevation gradients and a longitudinal gradient in the Pyrenees, and *Bd* occurs in currently 8 of the study sites. The focus of discussion in Chapters 3 to 6 is on climatic and hydrologic variables, which are used to derive spatiotemporal infection risk for two amphibian species heavily affected by chytridiomycosis in the Western Pyrenees. Past infection risk (1950-2018) is simulated and mapped for all study sites and discussed in terms of climate change, which is likely to already have and will continue to have important impacts on observed infection dynamics in the Pyrenees, and in other mountains globally.

² Chapter 2 is a literature review article, submitted to STOTEN on 21/07/2021 (and previously submitted to *Biological Reviews* on 20/01/2021, rejected by *Biological Reviews* on 24/05/2021). For more information, please see Chapter 2.

General introduction

A better knowledge of drivers of disease will inform the definition of EMBVs and help in mountain freshwater species conservation. The framework presented here is intended to inspire more interdisciplinary research and conservation policies that can benefit mountain biodiversity and mountain ecosystem integrity.

Introduction générale en français

La perte de biodiversité à l'échelle mondiale a fait naître l'idée d'une sixième extinction massive en cours (Barnosky *et al.*, 2011 ; Ceballos *et al.*, 2015). Les déclin ou les pertes d'espèces sont souvent dus à une synergie de facteurs de stress, qui peuvent être d'origine locale ou mondiale. Les principales menaces pour l'intégrité des écosystèmes et la survie des espèces sont liées aux impacts humains directs ou indirects. Le changement climatique anthropique est à l'origine des changements récents les plus profonds, les plus complexes et certainement les plus débattus publiquement dans les écosystèmes mondiaux, menaçant la survie et la santé des espèces (Walther *et al.*, 2002). Le changement climatique exacerbe les conséquences d'autres facteurs de stress sur la biodiversité, tels que la perte ou la fragmentation des habitats, la pollution environnementale, l'introduction d'espèces invasives et l'émergence de maladies, qui trouvent souvent leur origine dans les activités humaines et la mondialisation (Pecl *et al.*, 2017; Johnson *et al.*, 2017).

L'émergence de maladies de la faune sauvage peut être considérée comme une conséquence et comme un indicateur de la dégradation des écosystèmes (Daszak, Cunningham, & Hyatt, 2001 ; Jones *et al.*, 2008). De multiples espèces, dont l'Homme, sont affectées par les maladies émergentes de la faune sauvage (Daszak, Cunningham, & Hyatt, 2000). À titre d'exemple dramatique, le déclin panzootique des espèces d'amphibiens a été, dans de nombreux endroits du monde, lié à une maladie émergente causée par un agent pathogène (Daszak *et al.*, 1999a ; Fisher & Garner, 2020). Les déclin et les pertes d'espèces dus aux maladies ne constituent pas seulement un défi pour la conservation. Les économies humaines et la santé humaine dépendent de l'intégrité et de la santé des écosystèmes naturels. Ainsi la santé des écosystèmes et la santé et le bien-vivre des humains ne font qu'un (Cunningham, Daszak, & Wood, 2017)(Figure 1), il est crucial d'identifier les moteurs des maladies émergentes de la faune.

Les maladies dans les écosystèmes d'eau douce de montagne

Les maladies constituent une menace en évolution rapide pour la biodiversité mondiale des eaux douces, où de nombreux déclin d'espèces ont eu lieu au cours des dernières décennies (Reid *et al.*, 2019). Des épidémies dévastatrices d'amphibiens dues au champignon pathogène/chytridiomycète *Batrachochytrium dendrobatidis* (ci-après *Bd*) ont été observées dans les écosystèmes d'eau douce du monde entier (Scheele *et al.*, 2019; Lambert *et al.*, 2020;

Fisher & Garner, 2020) (Figure 2). Les amphibiens infectés par *Bd* peuvent développer la chytridiomycose, une maladie potentiellement mortelle, qui, en cas de forte sensibilité des hôtes à la maladie, peut provoquer des épidémies dans les populations d'amphibiens dans la nature (Berger *et al.*, 1998; Daszak *et al.*, 1999 ; Rachowicz & Vredenburg, 2004). Des découvertes récentes suggèrent que *Bd* est un pathogène invasif dans la plupart de ses sites d'occurrence et qu'il est originaire d'Asie, d'où il s'est très probablement propagé par l'augmentation globale des échanges commerciaux (O'Hanlon *et al.*, 2018).

Une fois introduit dans une zone, l'établissement, la propagation et la virulence de l'agent pathogène et les résultats de l'infection chez les amphibiens dépendent d'une interaction complexe de variables abiotiques et biotiques - donc de variables liées à l'environnement et aux espèces impliquées ou aux interactions entre espèces (Fisher & Garner, 2020). Récemment, le concept de pyramide des maladies a été formulé afin de souligner l'importance des interactions entre l'agent pathogène, l'hôte, le microbiome de l'hôte et l'environnement dans l'expression de la maladie (Bernardo-Cravo *et al.*, 2020).

De nombreux points chauds de l'apparition de la chytridiomycose et des extinctions qui y sont liées sont situés dans des zones montagneuses (Figure 2, (Fisher & Garner, 2020). Les montagnes sont souvent considérées comme des environnements vierges car elles sont éloignées des établissements humains et de l'impact humain direct (visible). Malgré cela, les facteurs de stress mondiaux, tels que les changements climatiques et d'utilisation des terres et l'introduction de polluants, ont laissé des traces dans les écosystèmes d'eau douce de montagne, comme en témoignent les changements dans leur fonctionnement et leur santé (Le Roux, Hansson, & Claustres, 2016 ; Le Roux *et al.*, 2020 ; Schmeller *et al.*, 2018). Parmi les graves conséquences du changement climatique et d'autres changements globaux dans les montagnes, on peut citer la perte d'habitats d'eau douce et d'espèces d'eau douce ainsi que la dégradation de la qualité de l'eau. Cela met davantage en danger les services écosystémiques importants que les eaux douces fournissent aux sociétés humaines, comme par exemple l'approvisionnement en eau potable (Vári *et al.*, 2021). Les amphibiens sont un élément important des écosystèmes d'eau douce, et contribuent essentiellement aux services écosystémiques, par exemple par la prédation des insectes ou par le recyclage des nutriments (Hocking & Babbitt, 2014). Parce que les amphibiens, en tant qu'ectothermes et avec des stades de vie aquatiques et terrestres, sont très sensibles aux changements dans leur environnement abiotique (par exemple, la température, la disponibilité de l'eau, la pollution),

ils sont souvent considérés comme des espèces indicatrices de la qualité des écosystèmes aquatiques (Hopkins, 2007).

Les impacts du changement global en montagne entraînent des modifications des variables abiotiques dans l'atmosphère, la cryosphère, l'hydrosphère et la biosphère de la montagne. Des efforts de recherche récents, comme ceux du GIEC, résument ces changements observés (GIEC, McDowell et Barr, 2019). Des variables, représentatives du climat et de ses changements, qu'il est possible d'observer de manière faisable et rentable et qui sont disponibles à l'échelle mondiale, ont été formulées par la communauté scientifique comme des *variables climatiques dites essentielles* (ECVs) (Bojinski *et al.*, 2014). Récemment, de tels indicateurs ont été suggérés pour les zones de montagne, en tant que *variables climatiques essentielles de montagne* (EMCVs) (Thornton *et al.*, 2021). S'appuyant sur le concept des ECVs, les *variables essentielles de la biodiversité* (EBVs) ont été définies comme des variables multi-échelles (avec une composante temporelle, spatiale et biologique) destinées à offrir un cadre normalisé qui relie les données d'observation primaires sur une variable de la biodiversité aux changements temporels de cette variable (Pereira *et al.*, 2013 ; Schmeller *et al.*, 2017). En raison de l'importance des impacts du changement global spécifiques aux montagnes, un ensemble de *variables essentielles de la biodiversité des montagnes* (EMBV) est actuellement discuté et défini par des experts (Kulonen, Adler, & Palazzi, 2020).

Afin de comprendre et d'atténuer les impacts du changement global qui menacent l'intégrité des écosystèmes de montagne, comme la perte de biodiversité des amphibiens due aux maladies, il faut tenir compte des variables et des processus spécifiques aux montagnes. Les changements dans l'abondance des espèces (par exemple l'abondance des amphibiens, des agents pathogènes, du plancton et des espèces du microbiome), qui entraînent des changements dans la composition des communautés d'espèces sont des exemples de classes d'EBV qui peuvent informer sur les indicateurs pour la politique de conservation (Jetz *et al.*, 2019). Les indicateurs sont par exemple l'impact des invasions d'espèces (par exemple, les agents pathogènes envahissants), le risque d'extinction des espèces (par exemple, pour les espèces d'amphibiens), l'état des écosystèmes et les services écosystémiques (Jetz *et al.*, 2019).

Comme nous considérons les amphibiens comme des espèces sentinelles de la santé des écosystèmes, la prévalence de *Bd* et de la chytridiomycose en montagne pourrait indiquer la présence de facteurs de stress généraux, risquant la santé d'autres espèces de montagne ainsi

que la santé humaine. Il est également essentiel de comprendre les facteurs de changement ou de perte de biodiversité pour atténuer les effets négatifs sur la santé et l'économie humaines, car la dégradation sous-jacente de l'intégrité des écosystèmes menace la santé et le bien-être de l'Homme en raison de la perte des services écosystémiques (Schmeller *et al.*, 2018).

Objectif et portée

L'objectif du travail présenté dans cette thèse est de contribuer à une meilleure compréhension des facteurs environnementaux abiotiques des maladies dans les écosystèmes d'eau douce de montagne. Les variables abiotiques pertinentes pour la dynamique hôte-pathogène et le microbiome (donc pour la pyramide des maladies «de montagne», Figure 3) sont définies et discutées dans le contexte des montagnes sous changement global.

Les trois principales questions de recherche sont formulées comme suit :

1. Quelles variables peuvent être considérées comme les principaux facteurs abiotiques de la maladie de la chytridiomycose dans les eaux douces de montagne et comment sont-elles affectées par le changement global ? *Voir les chapitres introductifs 1 et 2.*
2. Comment les écosystèmes d'eau douce des Pyrénées sont-ils affectés par le changement global? *Voir les méthodes, les résultats et la discussion dans les chapitres 3 à 6.*
3. Quelles variables abiotiques des catégories climat et hydrologie peuvent être essentielles pour la dynamique de la maladie chytridiomycose dans les eaux douces des Pyrénées et comment sont-elles liées au changement climatique? *C'est l'objet du chapitre 6.*

Les chapitres 1 et 2 de la thèse fournissent un contexte théorique général en décrivant la complexité de l'habitat de montagne des amphibiens et des agents pathogènes et en présentant l'état actuel des connaissances sur les variables abiotiques qui déterminent l'expression de la maladie de la chytridiomycose ainsi que leur importance en montagne. Les chapitres 3 à 6 couvrent l'étude des extrêmes climatiques et de l'hydrologie dans 25 sites d'eau douce des Pyrénées. Les sites étudiés sont situés le long de 5 gradients d'altitude et d'un gradient longitudinal dans les Pyrénées, et *Bd* est actuellement présente dans 8 des sites étudiés. Les chapitres 3 à 6 sont consacrés aux variables climatiques et hydrologiques, qui sont utilisées pour calculer le risque d'infection spatio-temporel pour deux espèces d'amphibiens fortement touchées par la chytridiomycose dans les Pyrénées occidentales. Le risque d'infection passé

Introduction générale en français

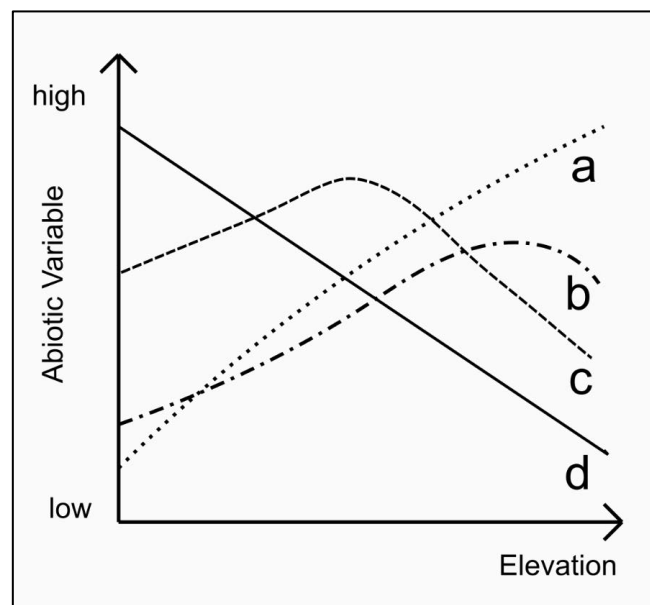
(1950-2018) est simulé et cartographié pour tous les sites d'étude et discuté en termes de changement climatique, qui est susceptible d'avoir déjà et de continuer à avoir des impacts importants sur la dynamique d'infection observée dans les Pyrénées, et dans d'autres montagnes au niveau mondial.

Une meilleure connaissance des moteurs de la maladie permettra de définir les EMBVs et contribuera à la conservation des espèces d'eau douce en montagne. Le cadre présenté ici vise à inspirer davantage de recherches interdisciplinaires et de politiques de conservation susceptibles de bénéficier à la biodiversité des montagnes et à l'intégrité de leurs écosystèmes.

Chapter 1: Abiotic variables in mountains & their response to global change

1.1 Introduction

Mountains are defined by pronounced terrain ruggedness and climate that distinguish them from lowlands, and that differ between each mountain range (Körner *et al.*, 2017). Elevation gradients are responsible for steep gradients in abiotic variables in mountains (Figure 4). Gradients and terrain complexity create diverse habitats for mountain species (McCain & Grytnes, 2010) and make mountains global biodiversity hotspots (Swanson *et al.*, 1988; Rahbek *et al.*, 2019). The geographic location of a mountain range is the main control on moisture gradients or seasonality in climate (Körner, 2007; McCain & Grytnes, 2010). Large-scale synoptic patterns, proximity to oceans and longitudinal or latitudinal orientation of a



mountain range are further controls on mountain climate (Del Barrio, Creus, & Puigdefabregas, 1990).

Figure 4: Elevation gradients of ecologically relevant abiotic variables in mountains. Examples are: a) increasing with altitude, e.g.: UVR, snow cover area and duration, ice cover duration, exposure to long-range transported atmospheric pollutants (orographic deposition); b) increasing with elevation, decreasing at highest elevations: precipitation in temperate mountains; c) peak at mid-elevation, decreasing towards mountain top: precipitation in tropical mountains; d) decreasing with elevation: temperature.

Climate change alters abiotic variables in mountain habitats. Abiotic variables discussed in this chapter can be grouped into the larger categories climate, hydrology and water chemistry, which will be also discussed in the following Chapter 2, when it comes to their relevance in host-pathogen relationships and expression of chytridiomycosis in mountain freshwaters.

Temperature changes (Pepin & Lundquist, 2008), changes in the cryosphere (snow and ice), (Stewart, 2009; Huss *et al.*, 2017; IPCC *et al.*, 2019), and changes in mountain hydrology (Viviroli & Weingartner, 2004; Immerzeel *et al.*, 2020) have already affected mountains globally, as well as their connected lowland ecosystems and human societies. Mountain freshwater ecosystems are recording these large-scale changes in abiotic variables connected to atmosphere and hydrology, and in addition are capturing related changes in erosive processes and biogeochemical cycles that in consequence control variables such as UV-radiation (hereafter: UVR) levels in water, water chemistry and aquatic pollutant levels (Sommaruga, 2001; Adrian *et al.*, 2009) (Figure 5). These changes in abiotic variables can be crucial for health and survival of mountain freshwater species and disease dynamics.

The intention of this chapter is to give an overview of the complexity of main abiotic variables in mountains and their alterations due to global change, as well as potential consequences for freshwater species. These will then be discussed in relation to chytridiomycosis in mountains in the following chapters.

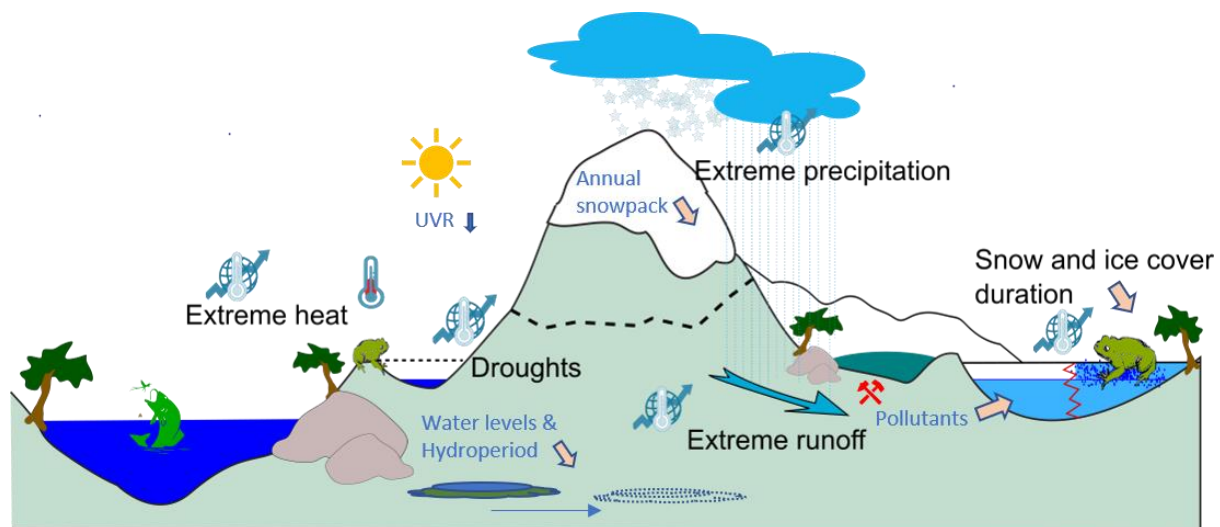


Figure 5: Schematic view of climate change effects on abiotic variables in mountains, which affect mountain freshwater ecosystems: Increasingly occurring are extreme heat, droughts, extreme precipitation, extreme runoff events and changes in snow and ice cover duration. Additional and related abiotic variables (blue): Changes in UVR, decreases in accumulated snowpack, lower water levels and shorter hydroperiod in freshwaters, and increased introduction of pollutants from catchment surfaces.

1.2 Temperature

1.2.1 Temperatures in mountains

Air temperature gradients at the land surface in mountains can be estimated to be linear with a standard decrease of 6.5 °C per km upslope (Körner, 2007). Measured lapse rates cover a range of values in different mountains: for example, -5.4 °C to -5.8 °C per km on a 30-year average in the European Alps (Rolland, 2003), or between -2.5 °C and -7.5 °C per km in the north American Cascade Mountains. Topography creates thermal meso- and microclimates by influencing insolation and air circulation (Dobrowski *et al.*, 2009). On small and medium scales, slope aspect, wind intensity and wind origin influence surface temperature patterns (Lundquist & Cayan, 2007). Evidence from soil and surface temperature data shows that landscape features such as small depressions, rocks and vegetation can create a heterogeneous thermal landscape (Scherrer and Körner, 2009). Topographic shading may control ecologically relevant abiotic variables such as water temperatures or lake ice cover duration in mountain freshwater ecosystems (Novikmec *et al.*, 2013). Cold air drainage and cold air pooling in valleys can significantly lower air temperatures at valley sites compared to the surrounding mountain habitats (Daly, Conklin, & Unsworth, 2010). Climatic complexity in mountains needs to be taken into account to improve predictions of future species distributions under climate change scenarios (Slavich *et al.*, 2014). Microclimates in and outside of mountain freshwaters could effectively shield small animals such as amphibians from climatic changes (Isaak *et al.*, 2016).

Water temperatures in mountains also generally decrease with increasing elevation. Water temperatures at a specific elevation depend on air temperature, topographic setting and local hydrology. Especially when factors such as lake freezing, meltwater and shading by steep topography become more important with increasing elevation, water temperatures are difficult to predict from air temperature gradients (Livingstone, Lotter, & Kettle, 2005). In steep terrain at high elevation, snow and topography become the dominant influence on water temperatures, which leads to a cooling effect on downstream freshwater ecosystems (Lisi *et al.*, 2015).

1.2.2 Changes in mountain temperatures

Long-term climate change such as global warming may lead to species migrations along environmental gradients, species invasions or negative impacts on species health (Walther *et*

al., 2002; Parmesan, 2006). There are clear trends in warming of mountain surface air temperatures (López-Moreno, Beniston, & García-Ruiz, 2008), and high-latitude mountains are projected to warm faster than temperate and tropical mountains (Nogués-Bravo *et al.*, 2007). The amount of warming depends on mountain region, elevation and season and may not be of the same magnitude when viewed as mean, maximum or minimum temperatures (Pepin & Lundquist, 2008). In the Alps, the mean annual temperature has already increased by 1.5 – 2 °C since 1970, and future projections predict an additional rise of 0.25 - 0.36 °C per decade within the next century (Einhorn *et al.*, 2015), while in the Pyrenees, an average rise of 0.24 °C per decade was found (OPCC_CTP, 2018). Warming has decreased the annual amount of snow with most impacts at mid- and lower elevations (Laternser & Schneebeli, 2003), and has caused shifts to earlier snow melt (Kapnick & Hall, 2012), earlier lake-ice melt (Franssen & Scherrer, 2008), and a globally accelerated deglaciation process (Zemp *et al.*, 2015). Climate extreme events are increasingly occurring globally (Easterling, 2000), and more frequent temperature extreme indices have been observed in mountains (Lin *et al.*, 2017; Amblar-Frances *et al.*, 2020), that have also been related to changes in mountain freshwater functioning (Parker, Vinebrooke, & Schindler, 2008).

1.3 Precipitation and Hydrology

1.3.1 Precipitation and hydrology in mountains

Their precipitation regimes define the role of mountains as global water towers to human populations (Viviroli & Weingartner, 2004). Precipitation gradients are created by orographic uplift of air masses, creating a windward-side maximum of precipitation with the mountain crest acting as a rain shadow, and descending dry air masses on the leeward side (Roe, 2005). Mountains in tropical regions show precipitation maxima at lower and mid-elevations while temperate mountains show increased precipitation towards the top (McCain & Grytnes, 2010). Seasonal dynamics in precipitation intensity cause a variety of precipitation regimes that are observably different between mountain ranges (Roe, 2005). On smaller spatial scales, precipitation gradients are due to dynamics in convection, turbulence and cloud microphysics (Körner, 2007). Topographic features such as valleys and smaller surface depressions are prone to accumulate moist air which will create a humid microclimate at those sites (Williams, McNamara, & Chandler, 2009).

Besides by climate, mountain hydrology is regulated by geology, soils and vegetation or human land use, which leads to a large variety of hydrological behaviours and stream flow

regimes in global catchments (Roa-García *et al.*, 2011; Dierauer, Whitfield, & Allen, 2018; Zuecco, Penna, & Borga, 2018). Rain and snow quantities and their variability and long-term change impact on soil moisture conditions and are important controls on water levels and the existence of shallow and temporary freshwater ecosystems (ponds and wetlands), which are important habitats for freshwater species (Brooks, 2009; Lee *et al.*, 2015).

Temporary freshwater habitat can exist due to extreme precipitation events, when runoff fills surface depressions with water, or due to seasonal hydrology, for example due to snow and snowmelt (Brooks, 2009). Temporary or seasonal freshwaters are a class of freshwaters which are characterized by hydrologic regimes and a certain hydroperiod, of which the length can vary between years. In this context, the term hydroperiod is used to refer to the time period in which a freshwater ecosystem carries water, and based on which different classes range from short- over intermediate to perennial and permanent freshwater habitats (Brooks, 2009; Kissel *et al.*, 2019).

1.3.2 Changes in mountain precipitation and hydrology

Change and variability in mountain hydrology crucially impact on the vulnerability of mountain ecosystems and their downstream human societies to climate change (Viviroli & Weingartner, 2004). While more extreme precipitation events can be expected in the future (Rajczak & Schär, 2017), climate change will most likely result in the disappearance of many intermediate and ephemeral habitats in mountains due to droughts, with potential severe consequences for freshwater organisms such as amphibians (Ryan *et al.*, 2014; Lee *et al.*, 2015). Mountains around the world are experiencing more drought periods, extreme precipitation events, and rapid glacial retreat (Gobiet *et al.*, 2014).

In Mediterranean climates, the rain-snow altitude threshold is shifting upwards due to global warming and increased number and duration of droughts (McCullough *et al.*, 2016). For the European Alps, climate projections predict more summer droughts and more heavy rainfall events (Rajczak & Schär, 2017). Subtropical mountains tend to experience more frequent summer droughts (McCullough *et al.*, 2016), while for example the Hindu Kush and the Himalaya, have been experiencing increased amounts of heavy rainfall events (Hartmann & Andresky, 2013). Mountains with snow regimes are most sensitive to current and predicted climatic changes (Viviroli & Weingartner, 2004), while sensitivity to climate change impacts on snow differs between global mountain ranges (López-Moreno *et al.*, 2017). Snow regulates ice cover dynamics in mountains (Preston *et al.*, 2016), and controls catchment

water levels, runoff and soil moisture (Huss *et al.*, 2017). Decreased annual snow amounts lead to earlier spring ice-out in mountain lakes and many of them are especially sensitive to the effects of rising air temperatures, because they are located at elevations and settings in which their ice-cover duration is mostly controlled by air temperatures (Thompson, Kamenik, & Schmidt, 2005). Less snow and retreating glaciers will continue to profoundly reshape mountain freshwater habitats and affect human economies and water supply to human societies (Pons *et al.*, 2015; Huss *et al.*, 2017).

1.4 Ultraviolet radiation (UVR)

1.4.1 UVR in mountains

Ultraviolet radiation (UVR) levels reaching aquatic ecosystems increase with elevation (Sommaruga, 2001). Especially effects of high levels of UVR in the UV-B spectrum (wavelength range 280-320 nm) have been studied concerning their effects on aquatic organisms at higher elevations (Sommaruga, 2001). Dissolved organic carbon (DOC) and phytoplankton may attenuate penetration of UV-B in water, shielding freshwater species from radiation (Sommaruga, 2001). In many high mountain freshwaters, there are limited catchment sources of DOC which results in clear water, facilitating penetration of radiation into the water column (Catalan & Donato Rodón 2016, Fujii *et al.* 2016). Variability in UV-B reaching mountain freshwaters depends on factors such as cloud cover and air pollution (Cabrera, Bozzo, & Fuenzalida, 1995; Blumthaler *et al.*, 1996; Sommaruga, 2001).

1.4.2 Changes in UVR

Long-term changes in UVR reaching terrestrial ecosystems are due to changes in atmospheric ozone levels as well as effects of climate warming. Atmospheric ozone layers have been recovering since emission regulations are in force according to the Montreal protocol (Barnes *et al.*, 2019), but there are reports of ongoing lower stratospheric ozone depletion (Ball *et al.*, 2018) and new ozone depleting agents are found in the atmosphere (Fang *et al.*, 2019). In the future, increased radiative stress to freshwater species due to earlier snow and ice cover melt or decreased stress due to higher DOC import from catchment reforestation are potential opposite effects of global warming in mountains (Sommaruga, 2001).

1.5 Water chemistry and pollution

1.5.1 Water chemistry and pollution in mountains

Chemical and physical weathering of rocks, productivity, vegetation and topography are main natural controls on element and nutrient input and cycling in freshwater ecosystems (Kamenik *et al.*, 2001) and can explain gradients and differences in water chemistry in and between mountains. Productivity in freshwaters and hydroclimate influence water acidity, measured as pH, on diurnal to seasonal scales (Maberly, 1996). Seasonal events such as snowmelt can change pH up to a full unit (Nodvin *et al.*, 1995), with early snowmelt water generally leading to an acidity pulse and late snowmelt to a basic pulse (Fay Baird, Buso, & Hornbeck, 1987). These acidic pulses may occur together with spikes of toxic element concentration in water (Havas & Rosseland, 1995). Acidification of mountain freshwater ecosystems originating from anthropogenic Sulphur and Nitrogen oxide emissions has been well-documented (Catalan *et al.*, 2013). Precipitation and the amount of atmospheric dust deposition are further controls on the pH of mountain freshwaters (Catalan *et al.*, 1992; Mosello *et al.*, 2002). Saline freshwater habitats, such as permanent or seasonal ponds or wetlands at high elevations occur mostly due to evaporation exceeding precipitation inputs in arid or semi-arid climates (Williams, 2002). Salinity fluctuations in water may play a role when hurricanes occur in wetlands of coastal mountains (Walls, Barichivich, & Brown, 2013).

The scavenging of atmospheric organic and inorganic pollutants is pronounced in high elevations and can take place as dry and wet deposition of aerosols to the ground surface (Skjelkvåle & Wright, 1998; Le Roux *et al.*, 2016). Main categories of atmospheric pollutants transferred to freshwater ecosystems in mountains are Nitrogen and Sulphur oxides, with their importance for acidification (Heard *et al.*, 2014a), trace elements (Camarero *et al.*, 2009), and synthetic pollutants such as pesticides, fungicides or microplastics (Reid *et al.*, 2019; Allen *et al.*, 2019; Brahney *et al.*, 2020). Volatile compounds are introduced into the atmosphere in warmer environments and progressive condensation takes place with decreasing temperature as air masses travel over mountain slopes (Blais *et al.*, 1998). As a consequence, organic and inorganic micropollutants can be introduced atmospherically to mountains (Camarero *et al.*, 2009; Zhang *et al.*, 2013). Transfer of inorganic and organic pollutants to mountain freshwaters can originate from legacy and recent local human activities. Fish stocking (Hansson *et al.*, 2017b), present and historic mining activities (De

Vleeschouwer *et al.*, 2014; Hansson *et al.*, 2017a) or forestry and agriculture (Alpers *et al.*, 2016) introduce pollutants or mobilize their catchment sources such as soils and sediments. Evidence for introduced potentially harmful trace elements (PHTE, for example: As, Hg, Pb, Se, Sb, Zn, Cu) from distant or local sources is found in mountain lake sediments, peatlands and in snow (Le Roux *et al.*, 2008; Bacardit & Camarero, 2010).

1.5.2 Changes in water chemistry and pollution in mountains

Climate change, with higher temperatures and longer and more frequent periods of drought, are likely to decrease water levels and persistence of saline lakes, and increase salinities due to higher evaporation (Williams, 2002). Higher rates of erosion and weathering as a result of more frequent events of heavy precipitation may also lead to a higher import of base cations and increase pH of mountain freshwater (Kopáček *et al.*, 2017). Many mountain freshwaters have recovered from acidification related to reduced deposition of acid pollutants (Catalan *et al.*, 2013), but others have not, which is explained by complex controls of catchment soil and rock characteristics and climate-induced weathering on water chemistry (Mosello *et al.*, 2002). Present-day acidification can play a role if mountains are located downwind of Nitrogen or Sulphur emission sources and buffering capacity of freshwater is low (Nodvin *et al.*, 1995). Extreme precipitation events or droughts will not only change water levels, they will also influence water chemistry due to their control on erosive processes (Walls *et al.*, 2013). Changes in water salinity and pH will thus depend on future dynamics in atmospheric Nitrogen and Sulphur emissions and climate change effects on mobilization and transfer of catchment-origin material to freshwater ecosystems. Glacier melt and snowmelt can mobilize legacy atmospheric pollution and catchment sources of pollutants (Meyer & Wania, 2008; Bogdal *et al.*, 2009). Increased pollutant introduction or increased mobilization of legacy pollutants may increase exposure of freshwater species to micropollutants, challenging health of mountain freshwater ecosystems (Schmeller *et al.*, 2018).

1.6 Impacts of changes in abiotic variables on mountain freshwaters and freshwater species

Climate change threatens health and survival of species globally by increasing threats posed by alien species invasions, disease or habitat loss (Parmesan, 2006). In mountain freshwater ecosystems, distinct effects of single or combined stressors related to climate change and pollution may lead to species mortalities, species extinctions and loss of ecosystem services to humans (Schmeller *et al.*, 2018; Le Roux *et al.*, 2020).

Mountain freshwater ecosystems are very exposed to dynamics in atmosphere and hydrology. Their catchments have a low buffering capacity for hydroclimatic events, as they are most often characterized by shallow soils and little vegetation cover (Skjelkvåle & Wright, 1998; Le Roux *et al.*, 2008). This inherent sensitivity and exposure to changes in atmosphere and hydrological changes makes for the role of mountain freshwaters as sentinels of climate change (Adrian *et al.*, 2009). Water levels, water temperature, ice cover dynamics and water chemistry parameters (e.g. DOC levels, pH) are examples of abiotic variables that are measurable indicators of climate change in freshwater ecosystems (Adrian *et al.*, 2009).

For freshwater species, changes in these abiotic variables can have severe consequences. A combination of changes in water temperature and ice cover duration, including changes in water chemistry, may alter plankton community composition and favour species that are more adapted to warming (Brown, Hannah, & Milner, 2007; Parker *et al.*, 2008). Changes in ice-over duration and water temperatures after ice-out in mountain freshwaters can profoundly change the composition of the microbiome (Jiang *et al.*, 2019). These alterations may also affect directly the development and health of freshwater species at higher trophic levels, such as amphibians (Clare *et al.*, 2016). Extreme hydroclimatic events such as droughts or floods can threaten survival of amphibians, as they are very exposed to hydrologic dynamics (Walls *et al.*, 2013). Increased drought due to lower annual precipitation and higher temperatures will lead to amphibians facing habitat loss due to shortening of pond and wetland hydroperiods or their dry-out (Ryan *et al.*, 2014; Lee *et al.*, 2015). Temporary and seasonal freshwaters are especially important for mountain freshwater species such as amphibians (Brooks, 2009; Lee *et al.*, 2015). Shallow and temporary freshwaters are habitats for early life stages of many amphibian species as they are used during breeding of eggs and during tadpole development (Griffiths, 1997) and they have gained much of their importance also for adult amphibians due to stocking of many permanent alpine ponds and lakes with predatory fish for sport fishing (Knapp & Matthews, 2000).

Thermal and hydric stress is predicted to become increasingly more threatening to amphibians that inhabit shallow freshwater habitats such as alpine ponds or wetlands (Lertzman-Lepofsky *et al.*, 2020). Despite all these stressors, some mountain freshwater species might effectively also be shielded by warming, as high terrain complexity in mountains is likely to create shaded microhabitats where lower temperatures persist (Isaak *et al.*, 2016).

As this chapter illustrated, the complexity of abiotic variables in mountains bears the potential to translate into complex responses of species to climatic and hydrologic change.

Chapter 1: Abiotic variables in mountains & their response to global change

Many of current and future changes in mountain freshwaters may either directly or indirectly challenge the health and survival of freshwater species. The occurrence of disease in mountain freshwater species should be seen in the context of these changes in their environment, which impact all three species categories of the disease pyramid – host, pathogen and microbiome (Bernardo-Cravo *et al.*, 2020). In the following Chapter 2, abiotic variables which can impact disease dynamics in amphibians in mountains under global change will be discussed in more detail.

Chapter 2: A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen

2.1 Introduction

Chytridiomycosis in amphibians globally has regularly been related to abiotic variables, most often to climatic variables. *Batrachochytrium dendrobatidis* (hereafter: *Bd*) occurrence and chytridiomycosis disease dynamics in mountain areas are subject of many previous studies. Yet, to date, the abiotic variables that are potentially impacting host-pathogen-environment interactions in mountains have not been systematically discussed. Mitigating ongoing amphibian declines in mountains is an interdisciplinary challenge, in which experts in the areas of climate, hydrology, biogeochemistry and ecology need to join their knowledge and further, be able to communicate it to conservation managers and the larger public.

In this chapter, abiotic variables that have the potential to impact chytridiomycosis disease (and potentially disease caused by other pathogens) in mountains are defined from reviewing existing literature on chytridiomycosis in amphibians. Based on knowledge gaps indicated in reviewed works and others that were derived from the lack of studies, directions for further interdisciplinary research are suggested. The term *disease expression* in context of *Bd* infections in amphibians describes how the outcome of disease is observed in infected individuals or populations, which can be controlled by many abiotic and biotic factors, such as climate, pathogen virulence, host immune response, host skin microbiome properties, etc. (Fisher & Garner, 2020; Bernardo-Cravo *et al.*, 2020).

This chapter (starting from following section 2.2) was submitted as a literature review article entitled *A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen* in the peer-review journal *STOTEN (Science of the Total Environment)*³. As a result from the reviewed literature, potential abiotic drivers in chytridiomycosis disease were classified into the categories climate, hydrology and water chemistry (Figure 6).

³ submitted to STOTEN on 21/07/2021 (and previously submitted to *Biological Reviews* on 20/01/2021, rejected by *Biological Reviews* on 24/05/2021).

Chapter 2: A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen

2.2 A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen (Submitted publication, currently under review in STOTEN⁴)

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2.2.1 Introduction

Wildlife diseases caused by fungal pathogens lead to an attrition of biodiversity, putting at risk ecosystem health and food security (Fisher *et al.*, 2012; Scheele *et al.*, 2019). Aquatic pathogens can affect entire freshwater ecosystems when they cause local extinctions of key biological elements such as amphibians (Whiles *et al.*, 2013; Hocking & Babbitt, 2014). Declines of such key species related to disease and environmental stress should be seen as warning system for global ecosystem change, degradation, and health repercussions for ecosystems, wildlife and the human population (Hopkins, 2007).

The chytridiomycete fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*), causing the disease chytridiomycosis, has been linked to the decline of a dramatically increasing number of amphibian species globally (Wake & Vredenburg, 2008; Scheele *et al.*, 2019; Lambert *et al.*, 2020). *Bd* has been described as a generalist pathogen and is showing extraordinary

⁴ First submitted to *Biological Reviews* 20/01/2021, Rejected after review 24/05/2021; Re-submitted in revised format to STOTEN on 21/07/2021, Currently (as of 12/10/2021) under review at STOTEN.

Chapter 2: A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen

virulence among amphibian species in a wide range of biogeographical and climatic contexts (Daszak *et al.*, 1999; Fisher, Garner, & Walker, 2009; Fisher & Garner, 2020). Increasingly globalized trade (Auliya *et al.*, 2016), and the resulting intended or unintended release of amphibian hosts into the wild, is facilitating invasion of highly virulent *Bd* lineages into naïve host populations (Garner *et al.*, 2006; Vredenburg *et al.*, 2013). The *Bd* global pandemic lineage (*Bd*-GPL) is the main driver of epizootic mass mortalities of amphibians and may go back to lineages from Asia. In Asia, coevolution of endemic *Bd* lineages with amphibian hosts may have occurred over millions of years, allowing Asian species to control *Bd* infections and maintain infection burdens at enzootic levels (O’Hanlon *et al.*, 2018). Although *Bd* has been reported to affect amphibians at low and high elevations, severe mass mortalities due to *Bd* have been observed mainly in mountain populations (Lips *et al.*, 2008; Vredenburg *et al.*, 2010a; Catenazzi *et al.*, 2011).

The disease expression caused by *Bd* or other pathogens is the result of complex biotic and abiotic interactions, recently conceptualized in a disease pyramid (Bernardo-Cravo *et al.*, 2020). Abiotic variables that impact disease outcome are environmental factors that affect the host, the pathogen, and host microbiome. Mountain freshwaters integrate catchment responses to both global changes and local stressors and can therefore be seen as sentinel ecosystems (Adrian *et al.*, 2009). Future distribution of *Bd* and other aquatic pathogens will depend crucially on pathogen, host and microbiome responses to resulting changes in abiotic variables (Bernardo-Cravo *et al.*, 2020)(see also Figure 8). Effective disease management for conservation of freshwater biodiversity relies on accurately predicting and communicating these results (Schmeller *et al.*, 2018).

Here we review abiotic variables that can affect chytridiomycosis disease dynamics in freshwater ecosystems, and discuss them for mountain freshwaters and in the context of global change. Based on the existing knowledge of their importance on infection dynamics, we defined climate, hydrology and water chemistry as abiotic variables. Our review seeks to facilitate interdisciplinary collaboration by proposing a framework for abiotic variables that control *Bd*, and focuses on mountain freshwater ecosystems where global change is expected to have the most extensive impacts on ecosystems and wildlife.

Chapter 2: A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen

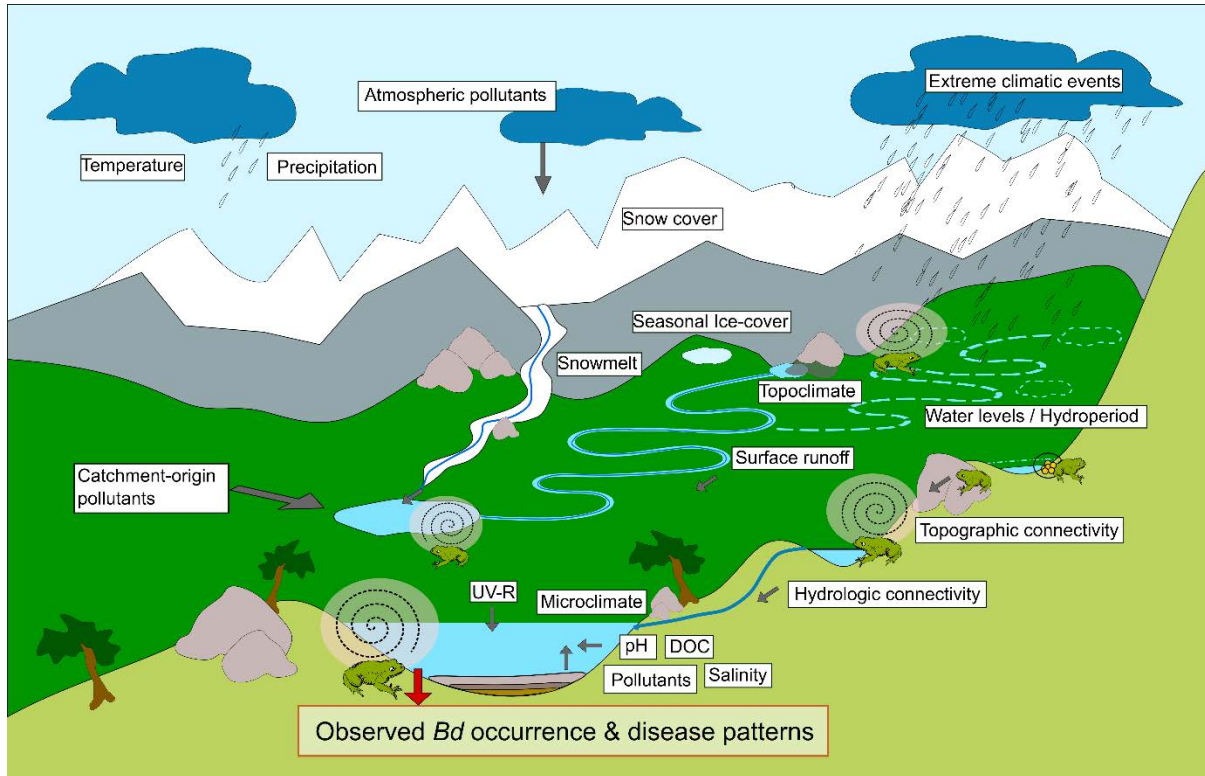


Figure 6: Abiotic variables related to mountain climate, hydrology and water chemistry which may drive chytridiomycosis disease in amphibian populations in mountains. The swirls represent observed *Bd* occurrence and disease patterns. (This figure was submitted as graphical abstract of submitted review article on which this Chapter is based).

2.2.2 Abiotic variables controlling *Bd*

2.2.2.1 Climate and Hydrology

Climate affects the physiology of all organisms in the disease pyramid: the pathogen, the host, the host microbiome and biotic factors known to interact with the pathogen, such as nano-, micro- and macro-zooplankton (Bernardo-Cravo *et al.*, 2020). Concerning impacts on hydroclimatic variables on *Bd*, the majority of research has been focusing on temperature, while fewer studies have been investigating the role of precipitation, UVR and hydrology.

Temperature

In culture, optimal growth of *Bd* cultures is between 17 °C and 25 °C, while temperatures below 17 °C slow down fungal growth rates (Piotrowski, Annis, & Longcore, 2004). Exposure to higher temperatures from 27 °C and above were shown to kill *Bd* within hours to days (Johnson *et al.*, 2003; Piotrowski *et al.*, 2004; Schmidt *et al.*, 2011), while *Bd* produced more zoospores at temperatures of 7 °C to 10 °C (Woodhams *et al.*, 2008). *Bd* exhibits a

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broad thermal tolerance with differences between *Bd* lineages, where lineages may show differences in fitness related to temperature (Voyles *et al.*, 2017). The general pattern observed in experimental and field studies shows that temperatures below its optimum growth range rarely limit *Bd*, whereas temperatures above the upper tolerance limit of around 26 °C can restrict or kill the fungus and thus may offer hosts refuge from infection. Complex controls of temperature on *Bd*, host and host microbiome may explain differences in disease dynamics observed in the laboratory and in the field and may also play a role in the different disease outcomes between mountain sites (Table 1, Figure 7 and Figure 8).

Temperatures in the *Bd* optimal range and wet climate were early on related to outbreaks of chytridiomycosis in mountains of Australia (Berger *et al.*, 2004). While the role of climate as single driver of *Bd*-related amphibian epidemics has since been ruled out, climatic variation, temperature extremes and large-scale climatic events such as El Niño are potentially negatively affecting immune defences of amphibians towards infection in favour of the pathogen (Rohr & Raffel, 2010; Raffel *et al.*, 2015). Climate change may be a driver of *Bd* epidemics in mountains, if the altered climatic conditions enlarge the available habitat for the pathogen and affect host immune reactions (Catenazzi, Lehr, & Vredenburg, 2014). Global and regional species distribution models predict current and future suitable habitats for *Bd* by taking into account its climatic niche, habitat variables such as vegetation and the potential of new introductions (Ron, 2005; Xie, Olson, & Blaustein, 2016). Simulations of future distributions of *Bd* and its sister species *Batrachochytrium salamandrivorans* (*Bsal*) suggest expansions to higher latitudes and elevations, especially on the northern hemisphere (Beukema *et al.*, 2018; Xie *et al.*, 2016). Warming can benefit *Bd* by providing optimal growth temperatures across larger areas and access to more amphibian hosts (Adams *et al.*, 2017).

Because amphibians are ectotherms, their physiology (e.g., immunity and growth) is directly linked to environmental temperature. Host resistance to infection is temperature-dependant, and thus host populations can find refuge from disease if temperatures are limiting *Bd* survival or growth (Brannelly *et al.*, 2021). A so-called “behavioural fever” is a defence mechanism for amphibians to fight pathogen infection by raising body temperatures through choosing warm microhabitat (Richards-Zawacki, 2010). The amphibian strategy to seek warm microhabitat may reduce *Bd* infection load (Forrest & Schlaepfer, 2011; Daversa *et al.*, 2018), especially when body temperatures are above 25 °C (Rowley and Alford, 2013).

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Increasing amounts of short-term exceedance of daily maximum temperatures above *Bd* tolerance limits or warm microclimates may also decrease infection loads of hosts (Becker *et al.*, 2012; Sonn, Utz, & Richards-Zawacki, 2019). These thermal refugia in mountain freshwaters that exist due to topographic heterogeneity may shield freshwater species from rapid warming (Isaak *et al.*, 2016). Thus, amphibian exposure to climatic stress with potential impacts on disease dynamics has to be evaluated taking into account macroclimatic trends and microclimate responses to climate change.

More severe infection outcomes might be a consequence of thermal stress lowering amphibian immune responses (Cohen *et al.*, 2017; McMillan *et al.*, 2019). Rising temperatures in mountain freshwaters related to climate warming may impact health and survival of amphibians, especially of early life stages that are restricted to aquatic habitat and have low heat tolerance (Enriquez-Urzelai *et al.*, 2019; Fernández-Loras *et al.*, 2019). While high winter temperatures may favour amphibian resistance to *Bd* (McCaffery & Maxell, 2010), low temperatures in more severe winters can lead to low immune reactivity which may favour severe outbreaks of chytridiomycosis (Rollins-Smith *et al.*, 2011). Temperature effects on the expression of chytridiomycosis (prevalence, infection burden) will crucially depend on how the amphibian host species' thermal optimum relates to experienced temperature change and sudden temperature shifts, as those are likely to favour *Bd* over host adaptability, which is also termed "thermal mismatch hypothesis" (Cohen *et al.*, 2017).

These direct impacts of temperature on the host-pathogen relationship are complemented by indirect effects. Such an indirect effect is e.g. driven by zoospore predation by zooplankton that may reduce infection pressure on amphibians (Schmeller *et al.*, 2014). Water temperature being a major factor for determining the growth and species composition of micropredatory zooplankton communities (Schmeller *et al.*, 2018), changes in water temperature have indirect effects on host-pathogen interactions. With changes in temperature regimes, the differences in plankton egg hatching may profoundly differ between years. As there are differences in zooplankton ability to consume *Bd* zoospores (Schmeller *et al.* 2014), such temperature regimes may constitute or not if a zooplankton community is able to protect amphibian tadpoles from *Bd* infection. Hence, between years and breeding seasons, amphibian hosts may find very different conditions under which they breed and thus face very different *Bd* infection pressures.

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In addition, it has been stated that the protective amphibian skin microbiome is also impacted by temperature (Catenazzi *et al.*, 2018). Temperature fluctuations may create opportunities for bacterial replacement on the amphibian skin, which attenuate pathogen transmission and improve host persistence against pathogen infection (Longo & Zamudio, 2017). More research is needed on the ecology of the amphibian skin microbiome, but seasons and temperature appear to be important determinants of the composition of amphibian skin microbiome determining disease outcome (Bernardo-Cravo *et al.* 2020).

Precipitation

It is known that *Bd* is adversely affected and potentially is killed from desiccation (Johnson *et al.*, 2003; Piotrowski *et al.*, 2004). Precipitation regime and vegetation density were shown to be a significant predictor of global pathogen distribution (Liu, Rohr, & Li, 2012) and humid climate has been related to more severe chytridiomycosis (Puschendorf *et al.*, 2011)(Table 1). Amphibians in continuous contact with water are more likely to develop lethal chytridiomycosis (Bustamante, Livo, & Carey, 2010; Murphy, St-Hilaire, & Corn, 2011), while dry environments lead to lower mortality (Raffel *et al.*, 2015; Scheele *et al.*, 2015). Precipitation deficits in combination with thermal stress may lower the amphibian capacity of an immune response under climate change (Lertzman-Lepofsky *et al.*, 2020) and thus lower their resistance to infection. This may explain the severe disease outcomes that have led to mass mortalities in some mountain settings (Lampo *et al.*, 2006) (Table 1).

Hydrology

Global warming impacts on precipitation, ice and snow regimes, water levels and hydroperiod of freshwater habitat (Adam, Hamlet, & Lettenmaier, 2009; Bavay *et al.*, 2009; Stewart, 2009; Preston *et al.*, 2016), which can be important variables in freshwater pathogen and host distribution, health and life cycles. *Bd* new habitat colonization and inter-annual chytridiomycosis dynamics have been related to glacier melt (Seimon *et al.*, 2017) and changes in lake ice phenology (Clare *et al.*, 2016) (Table 1).

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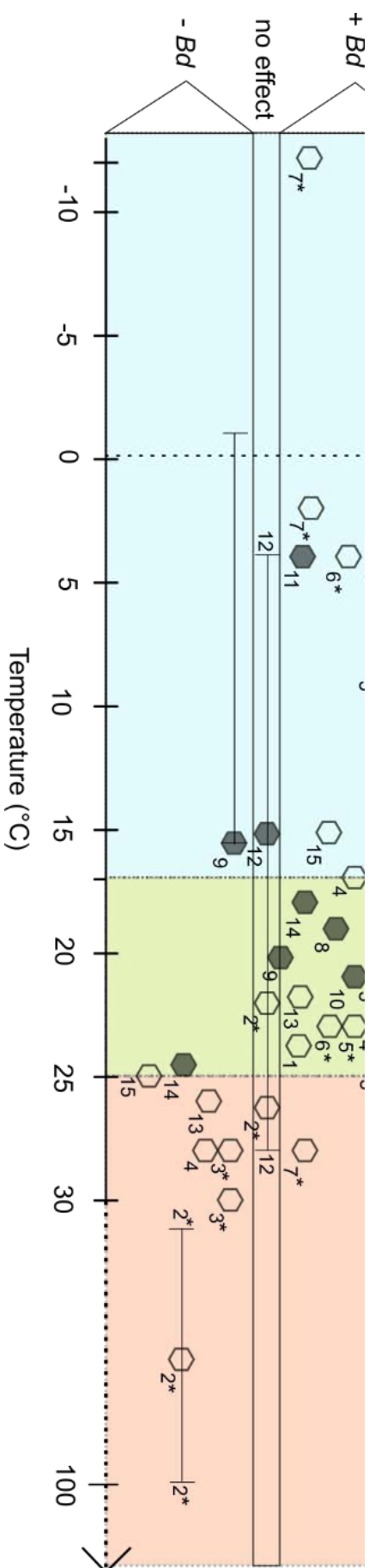


Figure 7: Temperature effects on *Batrachochytrium dendrobatidis* (*Bd*) from selected studies (studies represented by hexagons). Temperature effect on *Bd* infection intensity / *Bd* zoospore count, divided into favouring *Bd* (+ *Bd*, rectangle upper half) limiting/eliminating *Bd* (- *Bd*,

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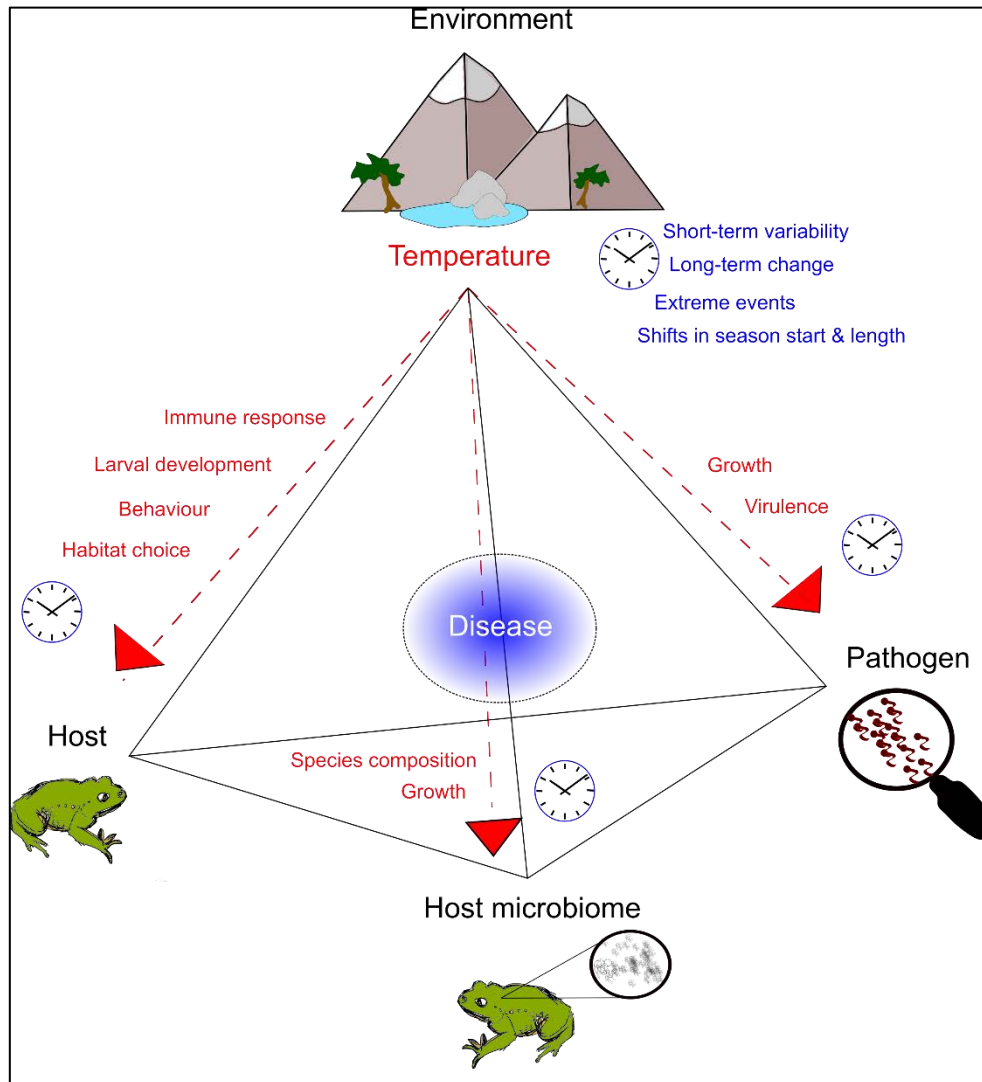


Figure 8: The disease pyramid (modified, after Bernardo-Cravo *et al.*, 2020), illustrating the role of temperature as abiotic variable impacting all corners of the disease pyramid: host, pathogen, host microbiome, and their habitat as part of the environment. Temperature regulates growth, immune system and behaviour of the host, growth and virulence of the pathogen as well as growth and species composition of the host microbiome (dashed axes). We stress the importance of temporal variability of abiotic variables such as temperature on disease dynamics (represented by a clock: short-term variations and long-term change, extreme events, seasonal changes).

Transport by water in streams is a potential directional and long-distance dispersal mechanism for microorganisms (Hohl, Václavík, & Meentemeyer, 2014). Streams may transport pathogens over greater distances downstream in a catchment (Meentemeyer, Haas, & Václavík, 2012; Rinaldo, Gatto, & Rodriguez-Iturbe, 2018). The mechanisms of abiotic

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dispersal of *Bd* in complex landscapes such as mountains are still largely unknown, but dispersal over smaller distances by rain, water movements in lakes or via downstream transport has been suggested (Sapsford, Alford, & Schwarzkopf, 2013; Hagman & Alford, 2015; Kolby *et al.*, 2015; Courtois *et al.*, 2017) (Table 1). *Bd* dispersal and host reinfections may further be facilitated by short distances between freshwater habitats (Padgett-Flohr & Hopkins, 2010) (Table 1).

Freshwater ecosystems with seasonal regimes can be a pathogen-free refuge for amphibians if *Bd* has not been able to colonize them or to survive their dry-out (Scheele *et al.*, 2015). But seasonal and extreme climatic events may put amphibians inhabiting mountain freshwaters at higher risk, when they change the availability of breeding habitats and impact on the density of tadpoles due to changes in the volume of water bodies (Scheele *et al.*, 2015; McMillan *et al.*, 2019). *Bd* may further profit from drought through higher presence of reservoir species, hosts weakened by hydric stress (Walls *et al.*, 2013; Catenazzi, von May, & Vredenburg, 2013; Adams *et al.*, 2017) or crowding of hosts causing higher pathogen transmission rates (Adams *et al.*, 2017; Courtois *et al.*, 2017). Changes in snow amount and snow cover duration are potential additional negative effects on amphibian health when they lead to shifts in breeding phenology, with earlier breeding start due to earlier snowmelt (Corn, 2003). Climate warming will continue to have far-reaching consequences for the mountain hydro- and cryosphere and potentially influence disease dynamics in mountain freshwater ecosystems.

UVR

High UVR levels in mountain freshwaters may induce *Bd* mortality (Hite *et al.*, 2016) and radiation in the UV-B spectrum was found to lower *Bd* infection levels in hosts (Ortiz-Santaliestra *et al.*, 2011) (Table 1). However, sublethal effects of UV-B may also hamper immune responses of amphibians to pathogen infection (Cramp & Franklin, 2018). Negative effects of UVR on amphibians have mostly been studied for radiation in the UV-B spectrum and can depend on sensitivity of amphibian species to radiation (Blaustein *et al.*, 1994; Blaustein & Belden, 2003) and on the presence of cofactors such as pathogens, contaminants or low temperatures that characterize their mountain habitats (Kiesecker & Blaustein, 1995; Blaustein & Kiesecker, 2002; Davidson, Shaffer, & Jennings, 2002; Morison *et al.*, 2020).

It has been shown that UV-B changes the aquatic microscopic species composition due to different radiation sensitivity of species (Sommaruga, 2001). Hence, UV-B radiation intensity

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may lower the presence, abundance, and diversity of zooplankton micropredators of *Bd*, increasing the infection pressure on amphibians (Hite *et al.*, 2016). Currently, we know little about the effect of UV-B on the amphibian skin microbiome as a first immune response capacity of amphibians against infections. We can therefore only suggest that earlier findings (Ortiz-Santaliestra *et al.*, 2011) may be linked to UV-B impacts on the amphibian skin microbiome.

The threat of damaging UV-B for biota, including mountain amphibians threatened by disease, is likely to persist in the future. Besides the potential damage to atmospheric ozone by new ozone depleting agents (Fang *et al.*, 2019), a larger threat of UV-B exposure to mountain freshwater organisms can be a change in freshwater ice phenology, leading to earlier annual exposures to high radiation levels in spring (Williamson *et al.*, 2014). Especially in freshwater habitats that offer only limited protection from UV-B radiation, for example due to shallow water levels or low DOC in water, freshwater organisms may face radiative stress (Kiesecker, Blaustein, & Belden, 2001; Sommaruga, 2001). On the other hand, upwards shifts of the treeline (Gottfried *et al.*, 2012), glacier melt (Sommaruga, 2015) or extreme precipitation events (Sadro & Melack, 2012; Williamson *et al.*, 2014) may potentially increase future import of DOC in water, which would lead to lower UV-B levels in water.

2.2.2.2 *Water chemistry and pollution*

Water chemistry, including pH, salinity and the presence of environmental pollutants in water may impact chytridiomycosis outcomes through their effects on pathogen, host or the aquatic microbiome. Water chemistry and pollution impacts on *Bd* and chytridiomycosis occurrence in a mountain context are still largely lacking evidence (but see Chestnut *et al.*, 2014, Table 1).

Acidity and salinity

Water acidity and salinity levels in water affect both *Bd* and amphibian hosts. *Bd* has been suggested to be limited in its habitat by pH 6 - 7 (Piotrowski *et al.*, 2004; Johnson & Speare, 2005). So far, only a few studies have investigated the effect of salinity on pathogen infections, including *Bd*. It is known that elevated salinity of more than 2 ppt in water can hamper *Bd* growth by disturbing osmotic balance (Stockwell *et al.*, 2015), reduce *Bd* infection loads in saline ponds and wetlands at low elevation study sites (Heard *et al.*, 2014b; Stockwell *et al.*, 2015), or block *Bd* transmission altogether (Clulow *et al.*, 2018).

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Amphibians can adapt to environments with low pH (Räsänen, Laurila, & Merilä, 2003), but low water pH (5.5) may also disrupt their immune system response which can make them more vulnerable to infection (Vatnick *et al.*, 2006). Nevertheless, low pH in water may lead to increased micropollutant concentrations with potential lethal to sublethal effects (e.g. stress, malformations, slow growth) on amphibians (Freda, 1991; Havas & Rosseland, 1995). Aquatic zooplankton community composition is as well influenced by acidity (Hořická *et al.*, 2006) and salinity (Gutierrez *et al.*, 2018). Thus, impacts of pH on the pathogen-host system are possibly indirect via the effects of those variables on host susceptibility and planktonic predators of *Bd* zoospores.

Many mountain freshwaters have recovered from acidification related to reduced deposition of acid pollutants (Catalan *et al.*, 2013). Present-day acidification can play a role if mountains are located downwind of Nitrogen or Sulphur emission sources buffering capacity of freshwater is low (Nodvin *et al.*, 1995). Higher rates of erosion and weathering as a result of increased events of heavy precipitation may on the other hand lead to a higher import of base cations to freshwaters, thus to potential increase in pH (Kopáček *et al.*, 2017). Salinity fluctuations in water may play a role when hurricanes occur in wetlands of coastal mountains (Walls *et al.*, 2013). Due to direct and indirect effects of acidity and salinity changes on the physiology of *Bd*, and on physiology and community composition of amphibians and microbiome, those abiotic variables should be assessed in chytridiomycosis surveys.

Pollutants

Organic micropollutants, including many pesticides, have been shown to be detrimental to *Bd* and amphibian health. Fungicides and different commonly used pesticides lead to *Bd* mortality and were shown to inhibit *Bd* zoospore production (Johnson *et al.*, 2003). Fungicides and pesticides show adverse effects on amphibian health but due to potential effects on hosts and *Bd* their effect on infection dynamics can be equivocal (Battaglin *et al.*, 2016). Exposure to pesticides at the tadpole stage can have a long-term negative impact on the amphibian immune system (Rohr *et al.*, 2014). Differential responses of disease dynamics to pesticides have been related to the importance of changes in sensitivity of amphibians during their lifecycle (Rumschlag & Rohr, 2018). Exposure timing and type of pesticide may affect tadpole survival chances in case of simultaneous pathogen infection (Hanlon & Parris, 2014).

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While pesticides in water might be limiting *Bd*, they may favour *Bd* infections of tadpoles and mortality due to *Bd* infection in metamorphic or adult amphibians at a later, terrestrial life stage (Rohr *et al.*, 2017).

Microplastic pollution in water was found to enhance *Bd* infection in exposed amphibians, which may be caused by a synergy between amphibian stress response to these pollutants and the immune system (Bosch *et al.*, 2021).

The impact of presence of inorganic compounds on *Bd* growth and survival may be depending on their concentration in water. For example, high concentrations of copper due to mining or agriculture were hypothesized to locally limit *Bd* growth (Boisvert & Davidson, 2011). Inorganic micropollutants such as potentially harmful trace elements may accumulate in amphibian tissue and lead to poor amphibian health and higher amphibian mortality (Unrine *et al.*, 2004, 2007). As such, mercury (Hg) bioaccumulation was found in amphibian populations close to local pollution hotspots originating from historical mining spots (Hothem, Jennings, & Crayon, 2010) or industrial sites (Bergeron *et al.*, 2010).

Catchment sources of pollutants may be increasingly mobilized by extreme climate, such as longer hot and dry periods and heavy rain (Zaharescu *et al.*, 2016). Remote sources deliver pollutants to mountain freshwater ecosystems. For example, pesticides originating from downwind agriculture have been found in mountain amphibians and have been suspected to play a role in amphibian declines (Fellers *et al.*, 2004; Davidson & Knapp, 2007). In addition, emerging pollutants are currently being introduced into mountains with their effects on freshwater organisms still widely unknown (Brahney *et al.*, 2020; Catalan *et al.*, 1992; Reid *et al.*, 2019).

2.2.3 Future research directions

The usage of more uniform metrics and spatiotemporal data resolutions should be considered for future studies of climate impacts on chytridiomycosis dynamics, as they allow for a higher comparability between studies and places when investigating disease expression under climate change. Different abiotic variables and metrics with different spatiotemporal resolutions used in studies (see also references in Table 1) may each relate to different responses of freshwater ecosystems, pathogens and hosts.

Widely available are climatic data of spatial resolution in the km-range, and with annual or monthly temporal resolution (Muths *et al.*, 2008; Rohr & Raffel, 2010; James *et al.*, 2015;

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Sette, Vredenburg, & Zink, 2015). Compared to other vertebrates, the importance of microclimates (cm-scale) increases significantly for ectotherms such as amphibians (Enriquez-Urzelai *et al.*, 2019), which may be one explanation for heterogeneous responses of disease dynamics to climate change (Slavich *et al.*, 2014). Despite the importance of micro- and topo-climate, regional climate variables (>100 m spatial resolution) are essential as they allow for long-term studies of climatic variables in order to approximate climate effects on *Bd* and its host.

We should take advantage of high-resolution data and models to assess climate and hydrology at spatiotemporal scales relevant for amphibian host, host microbiome and pathogen. For disease dynamics, climate extremes and sudden temperature shifts may be more important than long-term mean temperature changes, as they generally favour pathogens over hosts according to the thermal mismatch hypothesis (Cohen *et al.*, 2017). Data with fine temporal resolution of at least daily resolution are needed to describe these climatic variations sufficiently well. The availability of small and inexpensive data loggers has made it more feasible to track microclimatic variables such as air and water temperature and its short-term variability (Bramer *et al.*, 2018). Recent microclimate models can even take into account amphibian heat- or moisture budget and allow to model water temperatures as mechanistic variable (Catenazzi *et al.*, 2014; Enriquez-Urzelai *et al.*, 2019).

Temperature changes should be studied together with changes in moisture conditions, as the interaction of both variables defines amphibian behaviour and physiological vulnerability to warming (Lertzman-Lepofsky *et al.*, 2020) and will impact their response capacities to disease. Hydrological changes relevant to *Bd* and host, such as changing soil moisture conditions, accumulation of surface water, snow melt and runoff dynamics need to be tracked at high, at least daily, temporal resolution. We need to include hydroperiod and water level changes of mountain freshwaters in sampling for *Bd* due to their importance for disease dynamics in mountain amphibians. Here, high-resolution remote sensing may allow to study changes in catchment surface water levels, and for example monitor hydroperiods of ephemeral or permanent wetlands, but also changes in the volumes of water bodies (Raffel *et al.*, 2015; Maclean *et al.*, 2017; Lertzman-Lepofsky *et al.*, 2020). Metrics such as the topographic wetness index and the topographic position index can help to indicate water saturated areas or wetlands in topographic depressions, facilitating mapping of intermediate or ephemeral ponds (Riley *et al.*, 2017). Mountain freshwater ecosystems should further be

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evaluated in terms of their hydrological and landscape connectivity, in order to identify freshwater sites at risk for infection spill-over (Sapsford *et al.*, 2013; Hagman & Alford, 2015; Scheele *et al.*, 2015). Similar hydrological functioning of different mountain catchments, for example due to climate, geological and topographical setting may help to extrapolate freshwater habitat characteristics from one mountain setting to another (Zaharescu *et al.*, 2016b). Further, hydrologic models, remote sensing and new ground-based monitoring techniques may also allow transfer of parameters and conceptual models of hydrologic functioning between different catchments (Hrachowitz *et al.*, 2013). Increasing our knowledge on functioning and changes of hydrology will enable further understanding of chytridiomycosis dynamics in mountain freshwater ecosystems.

A better knowledge of the complex interactions between UVR (UVB) and the host-pathogen system in mountain freshwaters is crucial. More work on post-hatching effects of UVR on hosts (Vredenburg *et al.*, 2010b) and on indirect effects on host infection dynamics has been recommended (Hite *et al.*, 2016). We need more understanding of the immunosuppressive effects of UVR in amphibians and on the amount of radiation necessary to produce adverse effects (Cramp & Franklin, 2018). Further, DOC concentrations and changes in water levels or hydroperiod of shallow freshwater habitat should be included in sampling, as both variables regulate UVR exposure to *Bd* and amphibians in mountains as a potential cofactor in disease vulnerability and disease outcomes in mountains.

The interactions between water chemistry and disease in amphibians are still poorly understood and we therefore call for more research attention and more studies to assess those stressors in mountain amphibian populations. Changes in weathering and element cycles, persisting accumulation of atmospheric pollution and mobilization of legacy pollution are threats to freshwater ecosystems that are likely to exacerbate stress to mountain amphibian populations in the future. We need to identify the types of pollutants present in mountain freshwaters and the local pollution fingerprint (Schmeller *et al.*, 2018). New dynamics of pollutant transfer to freshwater ecosystems (Bogdal *et al.*, 2010; Hansson *et al.*, 2017b; Le Roux *et al.*, 2020) and new types of pollutants (Reid *et al.*, 2019; Allen *et al.*, 2019) call for experimental and field studies in mountain ecosystems that evaluate local pollution threats as additional stressors to amphibians facing disease. Finally, timing of changes in water chemistry is crucial for amphibians as their life stages show different sensitivities towards changes in water chemistry (Hanlon & Parris, 2014; Rumschlag & Rohr, 2018). We therefore

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need more frequent and increased sampling efforts to collect water samples during periods when amphibians are most vulnerable and when the aquatic environment is most dynamic, for example during spring snowmelt (McClain *et al.*, 2003) to better understand how anthropogenic pollution may drive disease.

2.2.4 Conclusion

It is vital that we acknowledge the role of amphibian health as an early warning system for current degradations in freshwater and environmental health. Amphibian mortalities due to chytridiomycosis disease are evidence of profound changes in mountain aquatic ecosystems. Mountains are complex and dynamic environments, which translates into differences of observed disease between global mountain ranges. Climate, hydrology and water chemistry can either directly impact *Bd* distribution, growth and virulence, or act as co-factors impacting amphibian health and exposure to potential infection. Our review reveals that interdisciplinary research is critical to advance disease ecology and conservation efforts for amphibians in mountains, which extends to freshwater health at low elevations. Global climatic changes with more frequently expected climate extremes and the response of microclimates, hydrologic processes, water chemistry and pollutant levels in freshwater ecosystems represent important leverages in disease dynamics. While mountains can offer climatic refuges for freshwater organisms in microhabitats due to topographic heterogeneity (Isaak *et al.*, 2016), amphibians are likely to be negatively affected by climate warming at the same time as pathogens may experience advantages over hosts (Cohen *et al.*, 2017). The usage of data with high spatiotemporal resolution will benefit future epidemiology models and will lead to better predictions of environmental controls on wildlife disease dynamics. In order to achieve effective and preventive conservation management, *Bd* occurrence and disease dynamics should be studied in different mountain settings, using the same abiotic metrics with comparable spatiotemporal data resolution. Interdisciplinary formulated, spatially transferable models for landscape epidemiology will facilitate risk assessment and conservation efforts in the perspective of present and future environmental change.

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Table 1: Mountain field studies relating abiotic variables of categories climate, hydrology, UVR and water chemistry to observed *Bd* distribution and/or infection severity or outcomes.

Abiotic Category	Abiotic Variable	Study location / mountain range	Variable range / conditions in experiment / environment	Effect on <i>Bd</i>	Study on <i>Bd</i> only / <i>Bd</i> & host (species)	Reference
Climate	Temperature (Water)	Great Dividing Range & coast, Eastern Australia	<i>Bd</i> infected hosts, Laboratory infection experiments at 17 °C; 23 °C; 27 °C	Chytridiomycosis surveys in the field show higher amphibian mortalities in winter	<i>Bd</i> & host (34 amphibian species in field surveys and <i>Mixophyes fasciolatus</i> in infection experiment)	Berger et al., 2004
Climate	Temperature (Air)	Central & South American tropical mountain sites	Field surveys, daily minimum and maximum temperatures converge towards 17 – 25 °C (<i>Bd</i> optimum growth)	Chytrid thermal optimum hypothesis: observed daytime cooling and nighttime warming suggest <i>Bd</i> epidemics related to recent shifts of daily temperature range towards <i>Bd</i> optimum	<i>Bd</i> & host (<i>Incilius periglenes</i>)	Pounds et al., 2006
Climate	Temperature (Air)	Rocky Mountains, USA	Field surveys, daily maximum temperature < 17 – 25 °C (<i>Bd</i> optimum growth)	Lower <i>Bd</i> infection intensities observed at higher altitudes explained by low maximum temperatures	<i>Bd</i> & host (<i>Anaxyrus boreas boreas</i>)	Muths et al., 2008
Climate	Temperature (Air)	Peñalara Natural Park, Guadarrama Mountains, Spain	Field surveys of mountain ponds, Increasing number of days with maximum temperature > 21 – 27 °C	Increased <i>Bd</i> infection intensity explained by warmer temperatures related to recent regional warming	<i>Bd</i> & host (<i>Alytes obstetricans</i>)	Bosch et al., 2007
Climate	Temperature (Air)	Guadarrama Mountains & Pyrenees, Spain	Field surveys of mountain ponds, average / minimum temperature warmest quarter & minimum temperature coldest month	No evidence for temperature control on <i>Bd</i> prevalence; weak relationship of infected sites and temperatures < <i>Bd</i> optimum	<i>Bd</i> & host (<i>Alytes obstetricans</i>)	Walker et al., 2010
Climate	Temperature (Water)	Sierra Nevada, California, USA	Field surveys, Mountain lakes, temperature range of 4 – 28 °C; metrics: average annual temperatures and number of	No <i>Bd</i> limitation by lower water temperatures with increasing altitude	<i>Bd</i> & host (<i>Rana sierrae</i>)	Knapp et al., 2011

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			days between 15 – 25 °C (for 0 – 100 d/year)			
Climate	Temperature (Water)	Adirondack Mountains, USA	Field surveys, Mountain Ponds, metrics: average daily temperature & average daily minimum and maximum temperature, average daily maximum temperature frequently above > 25 °C	Highest <i>Bd</i> infection intensities observed at closed canopy sites with lower water temperature	<i>Bd</i> & host (<i>Craugastor fitzingeri</i> , <i>Litoria lesueuri</i>)	Becker et al., 2012
Climate	Precipitation (drought)	Andes, Venezuela	Survey of museum specimen for signs of infection, Analysis of precipitation records revealed deficit of annual accumulated rainfall	Suggested climatic forcing of chytridiomycosis: Severe drought event coincides with epidemics due to <i>Bd</i> infection	<i>Bd</i> & host (<i>Atelopus carbonerensis</i> , <i>Atelopus mucubajiensis</i> , <i>Atelopus soriano</i> , <i>Mannophryne cordilleriana</i>)	Lampo et al., 2006
Climate	Precipitation (Humid climate vs. dry climate)	Queensland, Australia	Field surveys, mountain river, lack of canopy cover, low annual precipitation, dry conditions at dry sclerophyll forest site vs wet conditions in rainforest	<i>Bd</i> suggested to be limited by drier and warmer microclimatic conditions and favored by wet conditions	<i>Bd</i> & host (<i>Litoria lorica</i> , <i>Litoria nannotis</i>);	Puschendorf et al., 2011
Hydrology	Lake ice regime	Pyrenees, France	Field survey, timing of lake thaw from water temperature data, mountain lake	Early timings of lake spring thaw followed by high prevalence of infection	<i>Bd</i> & host (<i>Alytes obstetricans</i> , <i>Bufo spinosus</i> , <i>Rana temporaria</i>)	Clare et al., 2016
Hydrology	Habitat availability	Cordillera Vilcanota, Andes, Peru	Field survey, deglaciation ponds	Recently developed deglaciation ponds allowing <i>Bd</i> and host to extend habitat range to higher altitudes	<i>Bd</i> & host (<i>Pleurodema marmorata</i> , <i>Bufo spinulosus</i> , <i>Telmatobius marmoratus</i> , <i>Rhinella spinulosa</i>)	Seimon et al., 2017
Hydrology	<i>Bd</i> dispersal via water (Hydrological connectivity)	Cusuco National Park, Honduras	Field survey, <i>Bd</i> in rainwater samples	Short-distance dispersal of <i>Bd</i> zoospores by rainwater as possible spreading mechanism	<i>Bd</i>	Kolby et al., 2015
Hydrology	<i>Bd</i> dispersal via water (Hydrological connectivity)	Queensland, Australia	Field experiment & survey, rainforest streams,	Transport of <i>Bd</i> zoospores in streams as downstream transmission mechanism	<i>Bd</i> & host (<i>Litoria rheocola</i>); <i>Bd</i> & host (<i>Litoria serrata</i>);	Sapsford, Alford, & Schwarzkopf, 2013; Hagman & Alford, 2015

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Hydrology	<i>Bd</i> dispersal via water (Hydrological connectivity)	Pyrenées, France	Field experiment & survey, Co-housing of infected & non-infected hosts, mountain lake	<i>Bd</i> zoospore dispersal in water as a means of passive transmission of the pathogen	<i>Bd</i> & host (<i>Alytes obstetricans</i>)	Courtois et al. 2017
Hydrology	Habitat density / habitat connectivity	Sierra Nevada, USA	Field survey, distance between ponds	Perennial ponds and ephemeral ponds in spatial proximity (1000 – 1500 m) are <i>Bd</i> hotspots in the landscape	<i>Bd</i> & host (<i>Anaxyrus boreas halophilus</i> , <i>Ambystoma Californiense</i> , <i>Pseudacris regilla</i> , <i>Taricha torosa torosa</i> , <i>Rana draytonii</i> , <i>Rana catesbeiana</i>)	Padgett-Flohr and Hopkins, 2010
UVR	UV-B (280-315 nm)	Peñalara Natural Park, Guadarrama Mountains, Spain	Field experiment & survey, full or no exposure to UV-B, mountain pond	<i>Bd</i> prevalence lower in hosts exposed than those not exposed to UV-B	<i>Bd</i> & host (<i>Bufo Bufo</i>)	Ortiz-Santaliestra et al., 2011
UVR	UVR (250–399 nm)	Peñalara Natural Park, Guadarrama Mountains, Spain	Field experiment & survey, full or no exposure to UVR, mountain pond	UVR exposure reduced <i>Bd</i> survival by 50 %; suggested further controls on UVR and thus on disease are hydroperiod, pond depth, dissolved organic carbon (DOC)	<i>Bd</i> & host (<i>Salamandra salamandra</i> , <i>Alytes Obstetricans</i>)	Hite et al., 2016
Water chemistry	pH	Western US field sites (100 – 2500 m elevation)	Field survey, pH 6.5 – 10.4	Negligible effect of pH range on detection probability of free <i>Bd</i> zoospores	<i>Bd</i>	Chestnut et al., 2014

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3.1 Study area & sampling sites

As a main objective, the P³ (People, Pollution, Pathogens, see theoretical background in Schmeller *et al.*, 2018) studies biotic and abiotic variables that may inform on the health of mountain freshwater ecosystems and this, including important environmental gradients that exist in mountains. The P³ study sites in the Pyrenees are 25 small high-elevation lakes (including one stream/wetland site). The Pyrenees extend over roughly 500 km as a natural topographic barrier between France and Spain, with the Atlantic Ocean to the West and the Mediterranean Sea to the East. The highest elevation in the Pyrenees, peak Aneto, reaches 3404 m elevation asl (above sea level).

The study sites are situated along 5 elevation gradients (hereafter= EG), and cover a longitudinal gradient (Figure 9, Figure 10, Table 2), and maps in Suppl. Figure 41 -Figure 45). With the exception of two sites, all sites are situated on the northern, French side of the mountain chain.

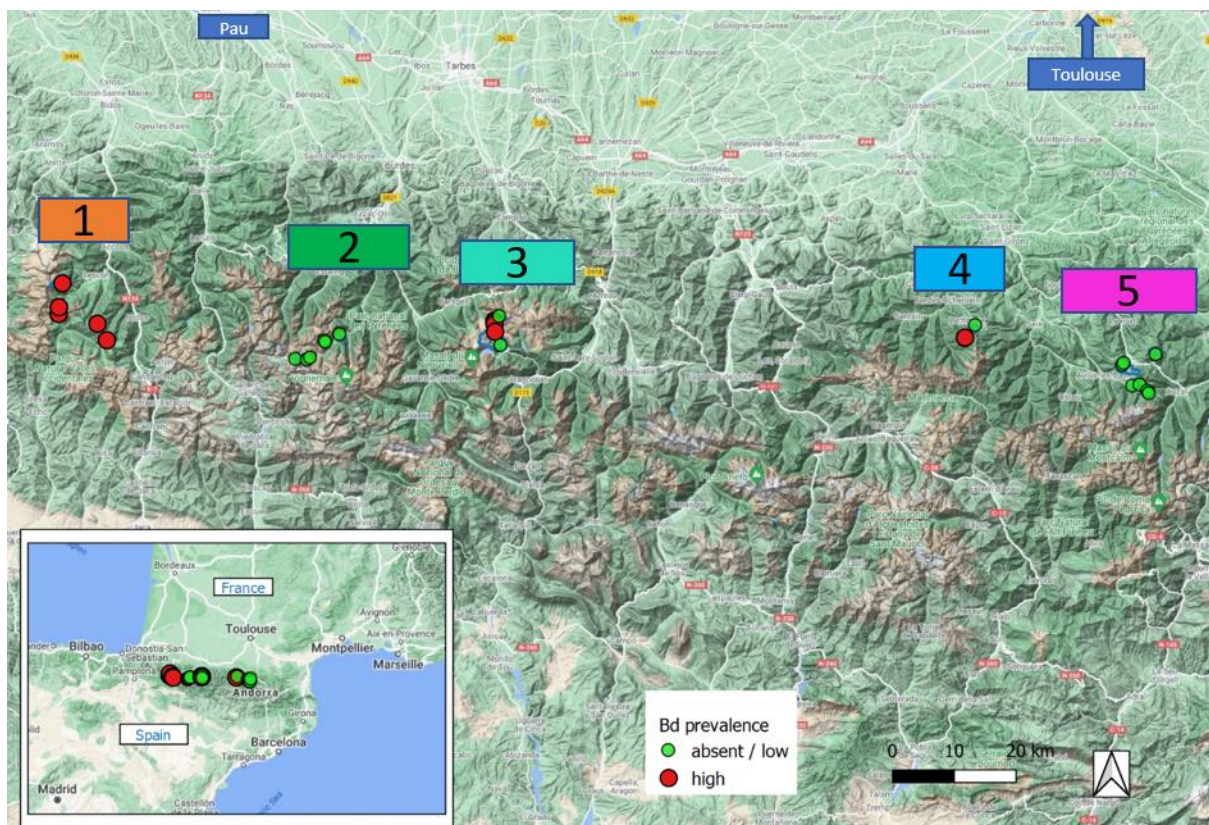


Figure 9: Study sites in the Pyrenees: Overview map (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid). The 25 sites are situated along a latitudinal gradient and separated

into 5 elevational gradients (EG), from EG 1= Western Pyrenees to EG5 = Central-Eastern Pyrenees. Red site= high *Bd* prevalence, Green site = low *Bd* prevalence or no *Bd* observed.

Of the 25 study sites, high prevalence of *Batrachochytrium dendrobatidis* (hereafter: *Bd*) and related amphibian mortalities has been observed at currently 8 sites (Figure 10), belonging to the EG 1, 3 and 4.

The sampling sites distinguish themselves by their climate and their ecotone, defined by the local catchment geomorphology, vegetation cover and geology. Some study sites are surrounded by pasture and have visible aquatic vegetation/macrophytes, other, higher-elevation sites are surrounded mainly by bare rock surfaces (Figure 11). A majority of the sites are in close proximity to hiking routes, which are popular among tourists in the snow-free season and which also connect many of the sites along each EG.

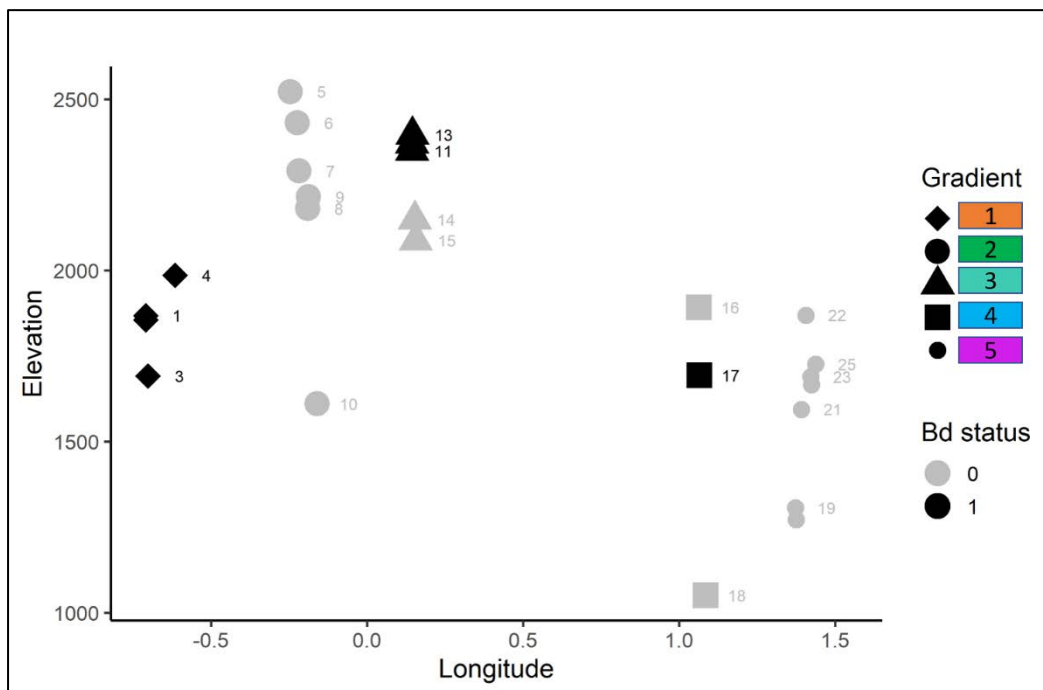


Figure 10: Elevation of sampling sites along longitude . Symbol labels represent lake site / catchment number from west to east in the Pyrenees (see also Table 1 / Maps). Black symbols: *Bd* present, grey symbols: *Bd* absent. 5 different symbols represent each of the 5 elevation gradients.

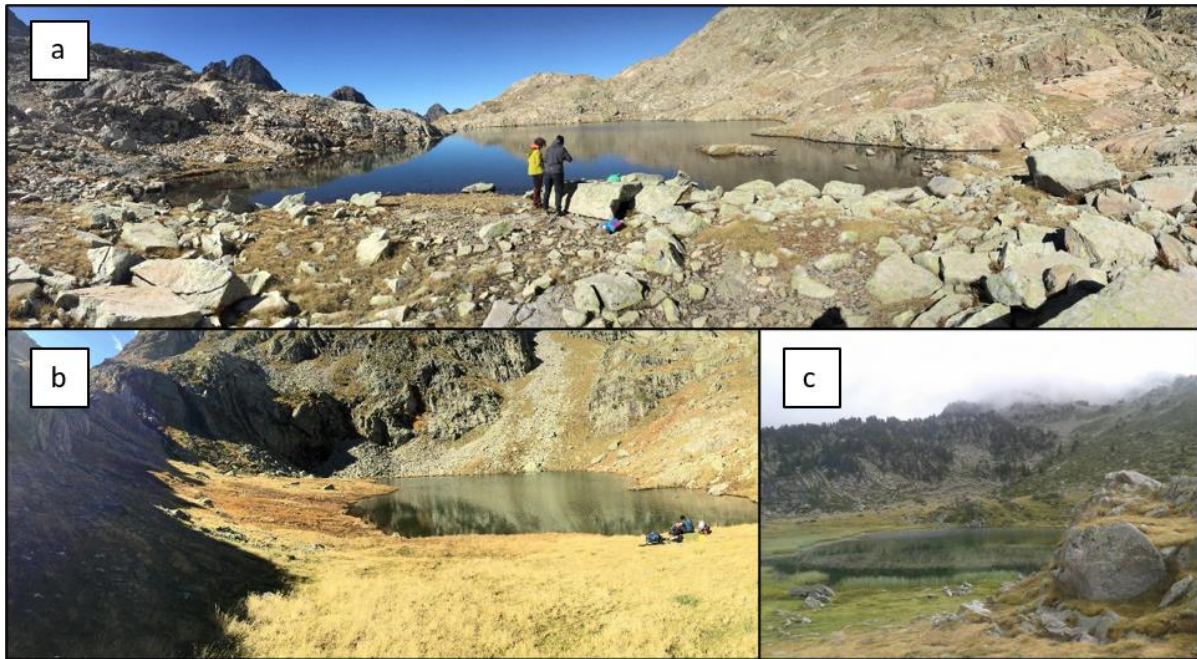


Figure 11 a-c: Diversity of lake ecotones which are the result of different elevation, longitude geology and climate. a- Site 5 (Ibon de la Faja 2), b- Site 7 (Petit lac de la Fache), c- Site 14 (Lac de Coueyla Gran). (Picture credit photos a-b: Judit Laufer, c- own photo)

Table 2: Sampling sites and catchment specifications : Geographic information and physical data of the lakes / lake catchments. Catchment number and elevation gradient number, as it was defined for this study. Lake names in *italic* = given name, in most cases due to lack of official name of the lake or site.

Lake name	Short name or Abbreviation (As used in this study)	Catchment no.	Gradient / cluster no.	Latitude	Longitude	Elevation (m asl)	Lake surface area (ha)	Catchment surface area (ha)	Catchment slope (%)	<i>Bd</i> status (+ = <i>Bd</i> present)
Ibón de Acherito	Acherito	1	1	42.9	-0.7	1868	6.2	49.7	47.3	+
Lac d'Ansabère	Ansabere	2	1	42.9	-0.7	1855	0.2	13.7	43	+
Lac de Lhurs	Lhurs	3	1	42.9	-0.7	1691	3.2	256.9	59.9	+
Lac d'Arlet	Arlet	4	1	42.8	-0.6	1986	3.2	34.1	41.9	+
Ibón de la Faja 2	Fache Spain	5	2	42.8	-0.3	2522	0.6	94.6	55.8	-
Lac de la Fache	Fache Grand	6	2	42.8	-0.2	2431	0.8	6.4	29.2	-
Petit lac de la Fache	Fache Petit	7	2	42.8	-0.2	2290	0.3	53.1	52	-
Embarrat stream	Embarrat	8	2	42.8	-0.2	2180	0.01	36.1	61.6	-
Embarrat - ponds (Vallon)	Vallon	9	2	42.8	-0.2	2215	0.1	22.6	48.4	-
Lac du Paradis	Paradis	10	2	42.9	-0.2	1610	0.4	29.5	54	-
Lacs de Madaméte	Madamete 1&2	11	3	42.9	0.1	2298	2.6	94.4	33.2	+
Laquet de Madaméte (<i>Madamete Pecheur</i>)	Madamete Pecheur	12	3	42.9	0.1	2371	0.4	50.3	40	+
Gourg de Rabas	Gourg de Rabas	13	3	42.9	0.2	2396	1.3	32.7	48	+
Lac de Coueyla Gran	Coueyla	14	3	42.9	0.2	2149	0.5	49.6	42.1	-
Les Laquettes	Laquettes	15	3	42.8	0.2	2087	3.3	874.2	46.2	-
Étang de Bellonguère	Belonguere	16	4	42.8	1.1	1892	0.1	57.3	50.7	-
Étang d'Ayes	Ayes	17	4	42.8	1.1	1693	1.8	146.2	55.9	+

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Étang de Bethmale	Bethmale	18	4	42.9	1.1	1051	2.9	122.5	65.2	-
L'Estagnon	Estagnon	19	5	42.8	1.4	1306	0.2	370.5	39.9	-
Étang de Lers	Lers	20	5	42.8	1.4	1272	7.4	446	41	-
Étang de Labant	Labant	21	5	42.8	1.4	1594	0.5	57.4	52.8	-
Étang d'Alate	Alate	22	5	42.8	1.4	1869	2.0	10.9	41.5	-
Mare Triton	Mare Triton	23	5	42.8	1.4	1689	0.0	29.8	53.5	-
Étang Mort	Etang Mort	24	5	42.8	1.4	1666	0.8	10.1	27.1	-
Étang d'Arbu	Arbu	25	5	42.8	1.4	1726	4.4	178.5	42.4	-

3.2 Data and data analysis

3.2.1 Data

Bd prevalence data

Prevalence is a measure of *Bd*-infection status in a sampled amphibian population and describes the fraction of *Bd* positive individuals in a sample. *Bd* infection prevalence data for the years 2008 to 2014 at lake Arlet were obtained from Figure 1d) of the Clare *et al.*, 2016 paper. Further data on *Bd* prevalence for the study sites is based on field work between the years 2016 and 2018, when sites were sampled during three field work campaigns (late June/early July, August, and late September/early October). At each sampling site, tadpoles, if present, were caught and swabbed (sampling of the amphibian mouth part, using a sterile cotton swab) in order to later assess *Bd* prevalence (zoospore count/infection intensity) in the lab, using qPCR (quantitative polymerase chain reaction) on DNA extracted from the swabs (Boyle *et al.*, 2007).

Topographic data

Topographic data used for the definition of the catchments for the 25 sites were obtained from a digital elevation model (DEM), which is based on the Shuttle Radar Topography Mission (SRTM) data product of 1-arc second resolution (approximately 30 m resolution at the ground surface), provided by USGS (Earth Explorer website). Catchments for the sites were then calculated based on this DEM and using QGIS, and these were used for extracting climate and hydrology variables from the gridded data (Suppl. Figure 47).

Climatic variables

Daily resolution climate data were obtained from ECA&D (<https://www.ecad.eu>), which provides the e-obs dataset (Version used here: 19.0e), consisting of daily gridded climatic data at a resolution of 0.1 degrees, corresponding to around 10 km. Daily maximum and minimum temperature and precipitation data from this dataset were used to calculate annual climate extreme indices for the study catchments, for the years 1950-2018, using the Climdex index definition. The Climdex climate extreme indices are a standardised set of indices recommended by the ETCCDI (Expert Team on Climate Change Detection and Indices).

For this thesis, the gridded e-obs dataset was chosen for several reasons. E-obs is available open source, and at the needed daily resolution, covering a time period back until 1950 and updated annually. Further, for practical reasons and needs of further data-users, e-obs was chosen due to availability of data for the lake site sampling years 2016-2018. As a gridded

dataset, e-obs covers the entire range of the Pyrenees, and is also available for all Europe. E-obs data offered thus a good compromise of spatiotemporal resolution, availability for sampling years and accessibility, making this study framework reproducible.

Hydrologic variables

6 annual-resolution hydrologic variables with a gridded 5 km spatial resolution were obtained as model output variables from two hydrologic models, for the time period 1960-2014. The mesoscale hydrologic model simulates catchment hydrology at a grid-scale (Suppl.

Figure 46 for schematic illustration) (Samaniego, Kumar, & Attinger, 2010; Kumar, Samaniego, & Attinger, 2013; Thober *et al.*, 2019). Further hydrologic variables stem from the noahmp multi-parameterization land surface model (Niu *et al.*, 2011), which takes into account key land-atmosphere interaction processes and is able to simulate for example canopy interception. Two hydrologic variables obtained from the mhm model and 4 variables from the noahmp model were used in this study (Table 3). The variables runoff and soil moisture both models provide. Daily-resolution snow cover data for lake Arlet was obtained from the MODIS (Moderate Resolution Imaging Spectroradiometer) satellite mission-derived snow product dataset, for which data for the Pyrenees are provided open-access for the years 2000-2015 (Data source: Gascoin, 2016).

Table 3: Units and description of annual-resolution hydrological variables (annual mean values) obtained from the mhm and the noahmp model.

Model	Variable	Unit	Description
mhm	Q (routed runoff)	m ³ /s	Water (from rainfall) that is routed as surface runoff through conceptual model storages to represent behaviour of overland and channel flow
	Soil Moisture	-	Fraction of saturation, all soil layers (no unit)
Noah-mp	Evapotranspiration	mm/h	Evapotranspiration
	Q (routed runoff)	m ³ /s	Water (from rainfall) that is routed as surface runoff through conceptual model storages to represent behaviour of overland and channel flow
	Q (runoff)	mm/h	Mean annual total runoff = baseflow + surface flow (Groundwater runoff and surface runoff combined)

Model	Variable	Unit	Description
	Soil Moisture	-	Fraction of saturation, all soil layers (no unit)

Table 4: Description and units of climdex climate extreme indices

Index (abbreviation)	Index name	Description	Unit
Annual temperature indices – Heat and cold			
FD	Number of frost days	Annual count of days when $TN < 0\text{ }^{\circ}\text{C}$	days
SU	Number of summer days	Annual count of days when $TX > 25\text{ }^{\circ}\text{C}$	days
ID	Number of icing days	Annual count of days when $TX < 0\text{ }^{\circ}\text{C}$	days
GSL	Growing season length	Annual count of days between first span of at least consecutive 6 days with daily mean $T > 5\text{ }^{\circ}\text{C}$ and of at least 6 consecutive days with daily mean $T < 5\text{ }^{\circ}\text{C}$	days
WSDI	Warm spell duration index	Annual count of days with at least 6 consecutive days when $TX > 90^{\text{th}}$ percentile	days
CSDI	Cold spell duration index	Annual count of days with at least 6 consecutive days when $TN < 10^{\text{th}}$ percentile	days
Annual precipitation indices – Wet and dry			
SDII	Simple precipitation intensity index	mean RR in wet days (days with $> 1\text{mm}$ of precipitation)	mm
R10mm	Number of wet days	Annual count of days when $PRCP \geq 10\text{ mm}$	days
R20mm	Number of very wet days	Annual count of days when $PRCP \geq 20\text{ mm}$	days

Chapter 3: Methods

Index (abbreviation)	Index name	Description	Unit
CDD	Maximum dry spell length	Maximum number of consecutive days with RR ≤ 1 mm	days
CWD	Maximum wet spell length	Maximum number of consecutive days with RR ≥ 1 mm	days
R95pTOT	Very wet day precipitation	Contribution to total precipitation from very wet days	mm
R99pTOT	Extremely wet day precipitation	Contribution to total precipitation from extremely wet days	mm
PRCPTOT	Total wet day precipitation	Annual total precipitation on wet days	mm

3.2.2 Data analysis

All data extraction, formatting, visualization and statistical analysis was performed using R and RStudio and the tidyverse package (Version 1.3.0).

Climate extremes and hydrology at the study sites (Chapter 5)

The 14 annual climate extreme indices (Table 4) were calculated based on the extracted e-obs daily climate data for the 25 study site catchments (see climate data grid and catchment example in Suppl. Figure 47), in R studio, using the R package *climdex.pcic* (Version 1.1-11) (see R scripts in Appendix C.1 and C.2). The six hydrology variables (Table 3) were extracted for the 25 study site catchments in R studio, analogous to the climate extreme indices. Temporal evolution of extreme climate and hydrology variables for all sites were visualized for the years 1950-2018 (climate), and 1960-2014 (hydrology), using a heatmap visualization. This type of visualization was chosen to allow for the plotting of a long time series, in addition to showing spatial and elevational differences in climate and hydrology along the sites in the same plot⁵. All visualizations were performed in R studio and using the *ggplot2* package (Version 3.3.3).

Spring start & infection risk (Chapter 6)

Spring start timing was calculated from daily average air temperature data (e-obs daily data) (see R script in Appendix C.3). Spring start from air temperature data was manually calibrated for lake Arlet and the years 2008 to 2014, with the intention to find a suitable air temperature threshold, which defines the start of the growing season (for freshwater organisms in general and the amphibian active season), and which corresponds to observed spring start from water temperature measurements (=lake ice-out) at lake Arlet, in this case the start of the annual period with a mean daily water temperature above 1 °C, as retrieved from Clare *et al.*, 2016. This threshold was determined via testing several temperature thresholds and a duration above this threshold of least 30 days (Spring start date = date, following which ≥ 30 days of $\geq X$ °C), and the comparison with observed spring start dates. The calibration results are shown for temperatures of 3, 4 and 5 °C (Chapter 6, Table 5). Using a 5 °C threshold failed to reproduce the observed early spring start of 2011 (DOY=120) (Chapter 6, Table 5, Figure 29), but the thresholds 4 °C and 3 °C both gave better results in reproducing this early spring event.

⁵ For further examples of heatmap plots, see for example their use in visualizing spatial and temporal patterns in COVID-19 infections in France: <https://germain-forestier.info/covid/>

In addition, data on snow cover days for the coordinates of lake Arlet were extracted from the MODIS snow cover dataset and used to calculate the start of the annual snow-free period at the lake site, in order to compare this variable to observed and calculated spring start dates. Start of the snow-free period, was calculated as start of the annual period with at least 5 days of no snow captured by MODIS. Due to the observed correspondence with MODIS snow cover data (Chapter 6, Table 5, Figure 29), the decision was taken to use the 4 °C threshold, as it is more ecologically relevant for defining start of the growing season in spring than 3 °C. Spring start date (Julian Day = DOY (Day of year), abbreviation used hereafter) from daily average air temperature data was then calculated for all study sites and for the years 1950-2018, using the e-obs daily average air temperature data: *Spring start DOY = DOY following which ≥ 30 days of ≥ 4 °C.*

The spring start from air temperature, as well as the start of the snow-free season from MODIS data were calculated using the *exceedance* function of the heatwaveR package (Version 0.4.5).

In order to extrapolate risk for high infection intensities due to early spring start from lake Arlet to the other sampling sites, the spring start timing (DOY calculated from air temperature, Air T ≥ 4 °C for ≥ 30 days) was related to observed *Bd* prevalence between 2008 and 2014 in the amphibian species *Alytes obstetricans* (hereafter: *A. obstetricans*), *Rana temporaria* (hereafter: *R. temporaria*) and *Bufo spinosa* (hereafter: *B. spinosa*) (Figure 12) (Clare *et al.*, 2016). A linear regression was used to fit the data as it was the best mathematical fit to the available observed prevalence sample points. The linear equations obtained from this regression were then used to extrapolate infection risk per species for all other study sites for a) 2008-2014 and b) 1950-2018. Infection risk was visualized as heatmap plots, using the calculated infection risk and in order to be able to visualize temporal trends in infection risk and longitudinal, as well as elevational differences in potential for infection (Chapter 6, section 6.2.2).

Spring temperatures (Chapter 6)

Based on the observed relationship between different temperature thresholds used and calculated spring start during calibration, and due to the known importance of temperatures around 4 °C to 5 °C for defining the start of growing and activity season for freshwater species, three temperature metrics were calculated, based on e-obs daily average temperature data and taking into account the annual period of 60 days after observed and simulated spring

start (See R scripts in Appendix C.4). This was done for lake Arlet 2008-2014 (Chapter 6, Table 6) and for all study sites for the years 1950-2018 (Chapter 6, Figure 35 to Figure 34). The temperature metrics are defined as follows:

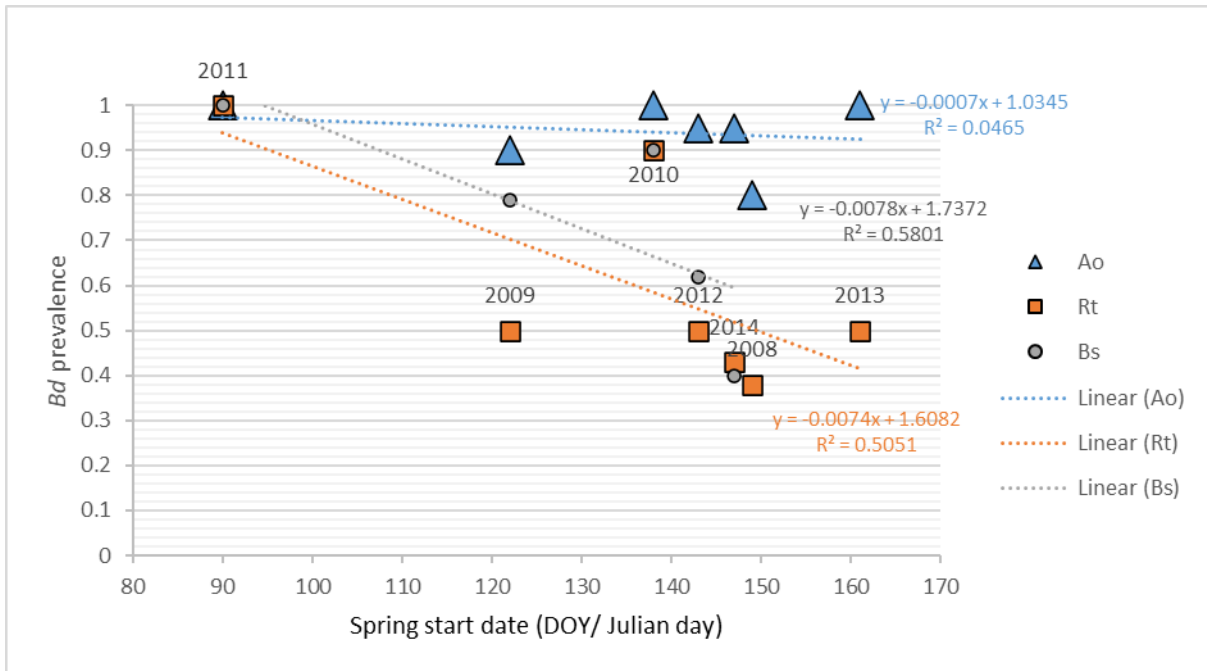


Figure 12: Linear regression plot between *Bd* prevalence Clare *et al.*, 2016 and annual spring start (calculated from air temperature, Spring start = DOY following which ≥ 30 days of ≥ 4 °C) for lake Arlet, for the three different species *A. obsetricans* (Ao), *R. temporaria* (Rt) and *B. spinosa* (Bs) (blue, orange and grey dotted lines and equations for the three species). Some years present overlay of data points, e.g. 2010 for *B. spinosa* and *R. temporaria*.

- 1) number of days with temperatures between 4 °C and 5 °C,
- 2) number of days with temperatures below 4 °C and
- 3) average temperature.

These temperature metrics were correlated with annual calculated spring start timing for all sites for the years 1950-2018, in order to test their relationship with infection risk (spring start timing as proxy for infection prevalence) (Chapter 6, Figure 60). For average temperature, the correlation to spring start was strongest, and the time series of this temperature metric for all years 1950-2018 was plotted for all study sites in order to visualize the temporal trend in this metric (Chapter 6, Figure 59).

Climate and hydrology variables related to infection risk (Chapter 6)

In order to find potential climatic and hydrological predictors of disease risk, all annual climate extreme indices and hydrology variables were correlated to observed spring start

Chapter 3: Methods

timing at lake Arlet for the years 2008-2014 (observed from water temperature, Clare *et al.*, 2016) (Chapter 6, Table 7). Spring start timing is considered as proxy for infection risk, due to the correlation found between spring start (lake ice-out timing) and annual *Bd* prevalence in *R. temporaria* and *B. spinosa* at lake Arlet (Clare *et al.*, 2016). The significance of the correlation between climate extreme and hydrology variables and spring start was tested using the Kendall rank correlation test, at a significance level of 5 % ($p < 0.05$). Among the climate extreme indices, PRCPTOT and CWD and among the hydrology variables, routed runoff (mhm model) and mean annual runoff, routed runoff and soil moisture (noahmp model) were significantly related to annual observed spring start (DOY) (Chapter 6, Table 7).

Chapter 4: Pyrenees under global change: Changes in climate and hydrology & responses of freshwater species

4.1 Introduction

High altitude freshwaters in the Pyrenees are composed of a multitude of lakes, ponds, wetlands and streams. Amphibians inhabiting these high elevation freshwater ecosystems have adapted their life-cycles to extreme climatic conditions and seasons (Vences *et al.*, 2000). Climate change is one of the major environmental stressors that are threatening health and survival of Pyrenean amphibians (Dalibard *et al.*, 2020). Climate change effects on abiotic variables in freshwaters and catchment hydrology have also the potential to increase loss of species due to disease, by complex alterations in biotic and abiotic variables affecting host-pathogen relationships (Chapter 2).

Most annual precipitation is stored in Pyrenean snow cover, which exists at least during 50 percent of the year at elevations above 1600 m, between the months December and April (Gascoin *et al.*, 2015). The spring snow melt releases this important water storage for downstream human demands. The major rivers Ebro, Garonne and Adour originate in the Pyrenees and supply large regions on both the Spanish and French downstream regions with water for industry, agriculture and households. This water tower function is seriously threatened by current and expected future climatic changes, which will most likely reduce water availability (López-Moreno *et al.*, 2008).

This fourth chapter is giving an overview of major ongoing and expected future changes in climate and hydrology in the Pyrenees (Section 4.2). Further, examples of observed effects of changes in climate and hydrology on freshwater species in the Pyrenees are discussed. This includes the occurrence of chytridiomycosis disease in the Pyrenees, which has been related to global change in this mountain range (Section 4.3).

4.2 Changes in climate and hydrology in the Pyrenees

The Pyrenees, like other global mountain ranges, are already showing responses of climate and hydrology to anthropogenic climate change. Climate change and its consequences for the Pyrenees has been studied for several decades now, but only recently, efforts by the Pyrenean climate change observatory (hereafter: OPCC) allowed to summarize climate change indicators and effects of climate change on natural ecosystems, human populations and economy (OPCC_CTP, 2018). The OPCC reports results of research from several associated transnational research groups which have been focusing their monitoring and analysis on

climatic changes or impacts on vegetation and hydrology in the Pyrenees (OPCC_CTP, 2018).

4.2.1 Changes in temperature and precipitation

A significant trend of rising temperatures is evident based on the analysis of long-term temperature data (Figure 13, OPCC_CTP, 2018). On average, for the period of 1959 – 2015, the rise in temperature amounts to an increase of 0.28 °C annual average temperature per decade (OPCC_CTP, 2018). On a seasonal scale, spring, summer and autumn temperatures are also rising significantly (Serrano-Notivoli *et al.*, 2019), with the strongest increase in summer temperatures of on average +0.4 °C per decade, followed by spring temperatures rising with +0.2 °C per decade over the last five decades (OPCC_CTP, 2018). Further rises in Pyrenean temperatures are predicted, with annual maximum temperatures rising between 1 and 2.7 °C and minimum temperatures between 0.9 and 2.2 °C until 2030 (2 - 4 °C and 1.7 – 3.3 °C until 2050, respectively) for the climate change scenario RCP 8.5 (OPCC_CTP, 2018). In addition, heat waves are predicted to increase in intensity, frequency, duration and spatial extent in the Iberian peninsula (Lorenzo, Díaz-Poso, & Royé, 2021). Recently provided high-resolution climatic data for the Pyrenees have permitted to further analyse future climatic trends at the scale of mountain massifs in the Pyrenees (Amblar-Frances *et al.*, 2020). Overall, results indicate as well a rise in both maximum and minimum temperature most pronounced towards the 2090 horizon for all scenarios, with stronger warming in the summer months and on southern slopes (Amblar-Frances *et al.*, 2020). More frequent soil moisture deficits can be expected when combining results of climate extremes due to decrease in precipitation and increase in evapotranspiration (Amblar-Frances *et al.*, 2020).

Precipitation patterns show great inter-annual and spatial variation in the Pyrenees, and a weak, but statistically significant trend of decreasing annual precipitation since 1950, with -2.5 % annual precipitation per decade (Figure 14) (OPCC_CTP, 2018). Simulations of future precipitation trends and variability are predicting a similar trend, in combination with an increase in extreme precipitation events, more drought periods and a significant decrease in annual precipitation falling as snow (OPCC_CTP, 2018). Considerable uncertainties exist in predictions of future temperatures and especially of future precipitation and precipitation extremes and depend on the model and the emission scenario used for the predictions (Amblar-Frances *et al.*, 2020).

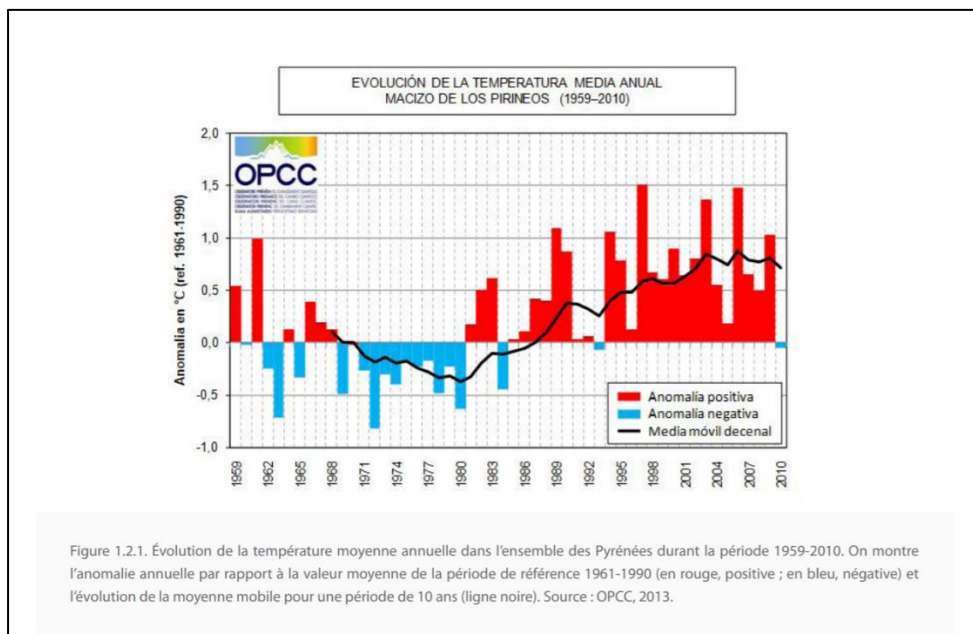


Figure 13: Mean annual temperature anomalies between 1959 and 2010 , relative to the mean of the 1961-1990 reference period, as published in the 2018 OPCC report on climate change in the Pyrenees (OPCC_CTP, 2018).

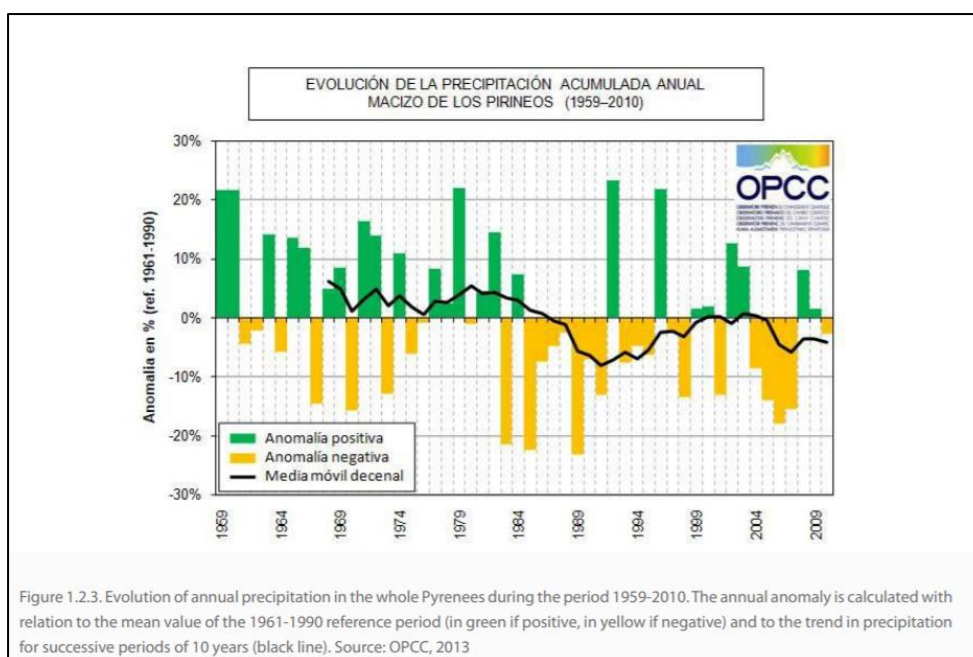


Figure 14: Mean annual precipitation anomalies between 1959 and 2010 , relative to the mean of the 1961-1990 reference period, as published in the 2018 OPCC report on climate change in the Pyrenees (OPCC_CTP, 2018).

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Strong gradients and variability can be observed in the annual Pyrenean snowpack (Alonso-González *et al.*, 2020). Snow cover and snow depth show significant decreases since mid-20th century, which can be explained by the trend of rising air temperatures (López-Moreno *et al.*, 2020). The western Pyrenees show the strongest past declines in annual snow and stronger declines were found at 2100 m than at 1500 m elevation, which has been related to lower intensity of winter warming but higher spring warming (López-Moreno *et al.*, 2020) (Figure 15). Compared with other Mediterranean mountain ranges, the Pyrenees show a high sensitivity of their snowpack to climate changes (López-Moreno *et al.*, 2017). For the future, further significant decreases in future snowpack due to continued global warming are expected, which could lead to a loss of 50 % of snow in the central Pyrenees by 2050 (OPCC_CTP, 2018).

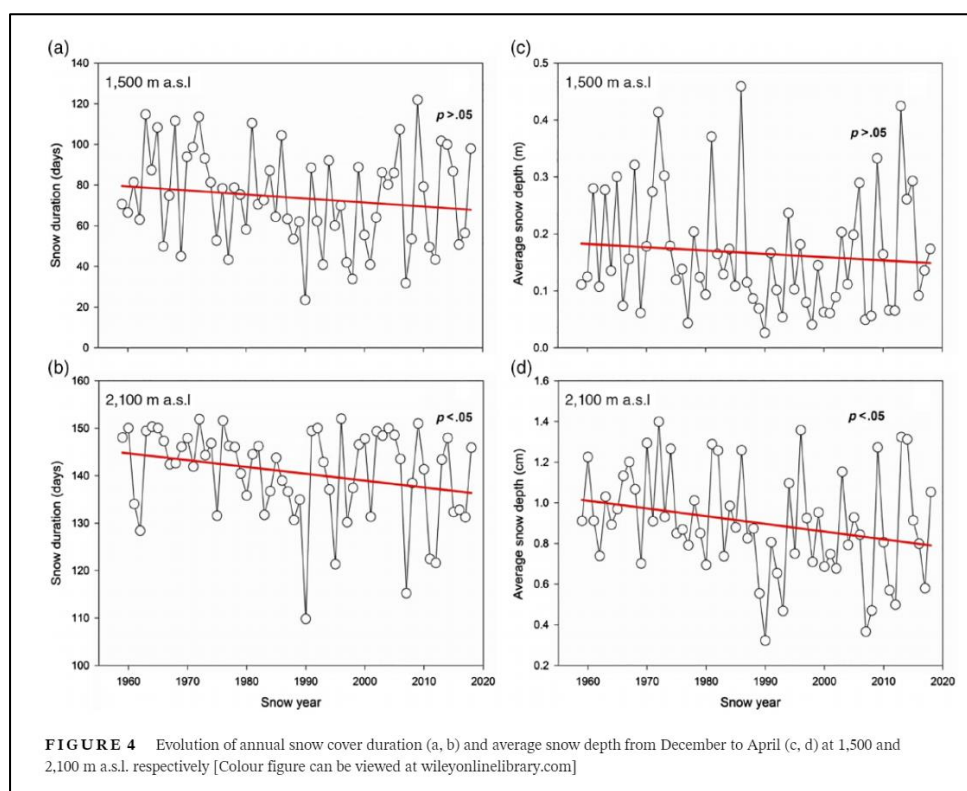


Figure 15: Trends in annual snow cover duration (days) and average snow (m) depth between December and April, at 1500 m (a-b) and 2100 m (c-d) elevation in the Pyrenees, Figure as published in López-Moreno *et al.*, 2020, Fig.4.

4.2.2 Changes in hydrology: runoff, ice cover, water temperatures

A combination of reforestation (and consequent water higher plant uptake and evapotranspiration) and changes in climate, especially the significant negative trend in annual

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snow accumulation, have been leading to overall reductions in runoff in Pyrenean rivers (López-Moreno *et al.*, 2008). Rivers in the Pyrenees are tending towards earlier high flow regimes due to earlier snow melt, and lower flow in summer, which affects stream ecosystems and human societies depending on this water supply during the summer months (García-Ruiz *et al.*, 2011). According to climate change scenarios, future runoff is predicted to decrease further in the Pyrenees due to lower winter precipitation amounts and higher temperatures, with the eastern part of the mountain range predicted to be more strongly affected (López-Moreno *et al.*, 2008; OPCC_CTP, 2018). Hydrological drought, as evidenced by reduced river runoff, is tightly related to meteorological (climatic) drought as well as to human management and has the potential of profoundly impacting on multiple socio-economic sectors in the Pyrenees (Vicente-Serrano *et al.*, 2021).

One important sentinel variable of change, lake ice cover duration (Adrian *et al.*, 2009), is greatly influenced by snow accumulation (Preston *et al.*, 2016). In the Pyrenees, the inter-annual variation in lake ice cover duration has found to be controlled by large-scale atmospheric dynamics, namely the NAO (North Atlantic Oscillation), through its control on annual snow accumulation (Sánchez-López *et al.*, 2015). There is little doubt that on a long term, ice cover duration of Pyrenean lakes will follow trends found for other mountains, with advances of ice break-up dates in spring due to reductions in snow cover and spring warming at all elevations (Franssen & Scherrer, 2008; Preston *et al.*, 2016).

Finally, water temperatures of freshwaters in the Pyrenees are expected to rise as a result of rising air temperatures and loss of snow and ice in their catchment (Thompson, Ventura, & Camarero, 2009). Already, ecological effects of rising Pyrenean stream water temperatures and advancement of lake ice break-up in spring have been recorded (Brown *et al.*, 2007).

4.3 Freshwater ecosystem responses to change in the Pyrenees

4.3.1 Freshwater species responses to change in the Pyrenees: amphibians as example

Pyrenean freshwater ecosystems have been showing responses to climate change, which are evidenced by altered hydrology, thermal and ice regimes, changes which affect freshwater species in the Pyrenees.

Among amphibian species, the example of the endemic Pyrenean brook newt (*Calotriton asper*) can be named, who is facing considerable range contractions through the loss of suitable bioclimatic habitat (de Pous *et al.*, 2016). In a recent review, other main threats for

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this newt species, besides climate change, were defined: disease, species introductions, water quality, logging industry, hydraulic activity and recreational aquatic activities (Dalibard *et al.*, 2020). Most of these named threats represent stressors also for other amphibian species in the Pyrenees. Many of these stressors are reinforced by climate change or act in synergy with each other, as discussed in Chapter 2 for interactions between disease and UVR, water chemistry and hydroclimatic variables. The following section and chapters focus on hydroclimatic variables and their potential role in disease expression in freshwaters of the Pyrenees. The occurrence of disease is seen as expression of underlying change in local abiotic variables which are likely to link to global change in this mountain range.

While climate change impacts on Pyrenean freshwater species need to be further documented and understood, examples from other mountain ranges with comparable climatic context allow to extrapolate challenges for local species. For example, the warming- or drought-induced loss of wetlands, small and temporary ponds, such as snowmelt ponds, or their limitation to higher altitudes, are profoundly changing amphibian habitat at high elevations (Ryan *et al.*, 2014; Lee *et al.*, 2015; McCullough *et al.*, 2016). Small seasonal freshwaters also exist in the Pyrenees and are used as breeding habitats for example by the common frog (*Rana temporaria*) in the Pyrenees (Oromi *et al.*, 2015).

In order to understand disease occurrence and dynamics (e.g., why fungal pathogens may be able to establish in new habitat), abiotic changes in Pyrenean freshwaters need to be mapped. Increasing our knowledge on the environmental impacts on disease dynamics will also help us to identify alterations in these ecosystems that concern the larger mountain ecosystems, with importance for the health of other freshwater species and humans (Schmeller *et al.*, 2018).

4.3.2 Chytridiomycosis in the Pyrenees: Potential indicator of change in local freshwater ecosystems

This section is a shortened and modified version of a published section⁶ entitled *Modifications in biological composition of aquatic ecosystems in the Pyrenees*, as part of

⁶ Chapter 2, *Water cycle and water resources*, Section 2.6.5 (Author: M. Haver), pp. 74-76 (in the french report version), ISBN: 978-84-09-06268-3, see full report online: <https://www.opcc-ctp.org/fr>

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Chapter 2 in the OPCC-report on climate change in the Pyrenees (OPCC_CTP, 2018), see Abstract of Chapter 2 in Appendix A.1.

Batrachochytrium dendrobatidis (hereafter: *Bd*) was first reported in central Spain in 1997 (Bosch, Martínez-Solano, & García-París, 2001). In the Pyrenees, epizootic spread of *Bd*, causing mass mortalities of the amphibian species *A. obstetricans* (indicator species for *Bd* in the Pyrenees), was first observed in 2002 in the area of the Parc National des Pyrénées (Walker *et al.*, 2010). Since, epizootic infections were mostly observed in this hot spot in the western Pyrenees and in another area in the eastern part of the National Park (Figure 16). Intriguingly, recent results show signs of *Bd* infection in preserved amphibian individuals from museum collections, in samples that date back to the early 20th century (H. Butler, personal communication, June 2021).

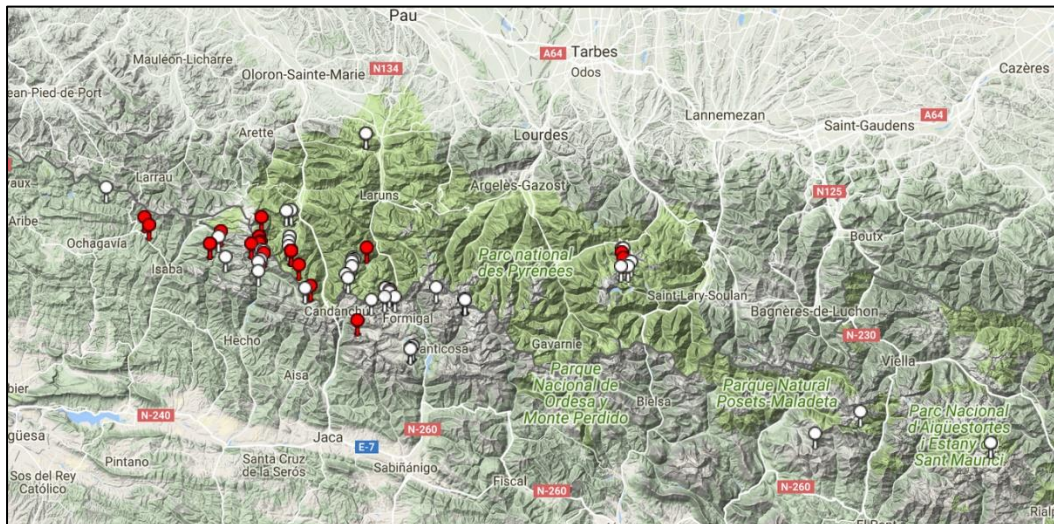


Figure 16: *Bd*-prevalence data for the central Pyrenees, screenshot retrieved in 2017 from www.bd-maps.net⁷; red points=*Bd*-positive, white points=*Bd*-negative.

Bd strains in the Pyrenees belong to the global panzootic lineage (GPL) of the pathogen and low genetic variety of local strains support the hypothesis that the pathogen was introduced into the Pyrenees from a single introduction event (as opposed to having evolved for a long time in the area, as endemic pathogen) (Walker *et al.*, 2010; Bates *et al.*, 2018). Yet, the non-infected state is seen as the norm in amphibians across the Iberian Peninsula (Walker *et al.*,

⁷ Bd-maps.net is offline, but this database in addition to new and future data are intended to be regularly updated via a new online platform (<https://amphibiandisease.org/>), offering open-access data on *Bd* and chytridiomycosis occurrence (Koo *et al.*, 2021).

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2010). In some sampled locations *Bd* is prevalent, but has not been causing any mass mortalities in amphibian populations— thus, it shows enzootic persistence (Walker *et al.*, 2010; Bates *et al.*, 2018).

The driving variables behind the current and past pattern of *Bd* prevalence and chytridiomycosis occurrence in Pyrenean amphibians are a current area of study. Previous studies have explored potential abiotic and biotic drivers of high infection prevalence, such as climate (Walker *et al.*, 2010), lake ice phenology (Clare *et al.*, 2016), zooplankton diversity (Schmeller *et al.*, 2014) or host skin microbiome communities (Bates *et al.*, 2018). Due to the complex network of species-environment interactions implicated in disease expression, it is most likely a combination of these and other variables, which are considerably tied to a local environmental context, including the ability of local hosts to fight infection (Walker *et al.*, 2010).

For conservation efforts directed at endemic Pyrenean species and for the surveillance of ecosystem health it is important to understand which environmental conditions govern pathogen spread. Temperature and water are among the most crucial factors controlling pathogen growth and survival (Walker *et al.*, 2010; Clare *et al.*, 2016). *Bd* optimal growth in culture has been observed to be between 17 and 25 °C, and the pathogen does not tolerate desiccation (Piotrowski *et al.*, 2004). Temperatures above this range represent a physiological limitation for the pathogen. Low temperatures, on the other hand, have been connected to trigger disease outbreaks, which can be explained by a changing strategy of *Bd*, which produces more zoospores in a colder environment (Woodhams *et al.*, 2008). In addition, moist environments generally favour fungal growth and increase infection rates. Higher infection rates have been related to humid climate (Berger *et al.*, 2004; Murray *et al.*, 2011), while amphibians inhabiting seasonally drying ponds were shown to be less afflicted by disease (Scheele *et al.*, 2015).

Changes in climate and hydrology together with land-use and land cover changes alter mountain habitats. Those alterations are likely to define *Bd* occurrence patterns in the Pyrenees. To explore the pathogen abiotic habitat, data on climate and hydrology as well as physicochemical habitat properties have to be analysed. This was done in the frame of the P³ project - People, Pollution and Pathogens (Schmeller *et al.*, 2018), which aimed at investigating the health of freshwater ecosystems in the Pyrenees and three other mountain ranges (USA, China, Oman).

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Within the research project, water quality, tadpole skin microbiome, microbiome in water and sediment of mountain lakes along elevation gradients were sampled and analysed. Further, climate and hydrological dynamics in the P³ Pyrenean freshwater sites were studied, using spatially distributed climate data and simulated hydrology. As hydrological data are sparse in high mountain environments as the Pyrenees, hydrologic modelling is a helpful tool to explore water balance and hydrologic processes on a regional scale. The P³ results for climate and hydrology are presented in the following thesis Chapters 5 and 6.

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Chapter 5: Changes in extreme climate & hydrology in Pyrenean freshwater ecosystems

5.1 Introduction

Climate change, more frequent occurrence of climate extremes and changes in hydrology have been affecting freshwater ecosystems in the Pyrenees (Chapter 4).

The 25 Pyrenean freshwater study sites that are subject of this analysis are situated along a longitudinal gradient, and along 5 elevation gradients. Thus, inherent differences in their general climatic conditions and responses to climatic and hydrologic changes can be expected. The gradients covered by the sites also most likely result in differing importance of abiotic stressors for amphibians at the sites, and different opportunities for infection success of the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter: *Bd*). Further, differences in past conditions may explain differences in disease dynamics presently observed. In other words, past climate, together with current climate as well as local geology, soil and vegetation are responsible for each site offering a unique “template” for freshwater organisms (Zaharescu *et al.*, 2016a, 2016b), which might as a consequence impact observed disease expression (Bernardo-Cravo *et al.*, 2020).

Pathogens such as *Bd* are able to adapt their growth faster than their hosts to altered conditions, such as extreme temperature fluctuations, and this mechanism may fuel infections in a changing climate with increased occurrence of climate extremes (Woodhams *et al.*, 2008; Raffel *et al.*, 2013). Amphibians are more susceptible to pathogen infection at low temperatures or due to climate shifts away from their optimum range, as their immune response is temperature-dependent (Rollins-Smith *et al.*, 2011; Raffel *et al.*, 2013)(see also: Chapter 2). Changes in hydrology can impact both pathogen and amphibians (Chapter 2), as they rely on water in their environment and do not tolerance extended conditions of drought (Johnson *et al.*, 2003; Lertzman-Lepofsky *et al.*, 2020).

When trying to understand current patterns of disease at a larger spatial scale, such as the Pyrenees, it is therefore crucial to map temporal changes in climate, climate extremes and changes in hydrology at high temporal resolution and along longitudinal and elevational gradients, and study these together with the occurrence of pathogen infections. A first step in this direction is presented in this Chapter, via analysis of temporal evolution of 14 daily-resolution extreme climate indices (Climdex extreme indices: Table 4) and 6 annual-resolution hydrology variables (mhm and noahmp-model variables: Table 3) at the Pyrenean study sites, by year, for the period 1950-2018 for climate and 1960-2014 for hydrology.

5.2 Results

5.2.1 Climate extreme indices - Temperature

Several of the analysed temperature extreme indices indicate warming in the Pyrenees in recent decades, but differences can be seen along longitude (= elevation gradient, EG hereafter) and elevation of sites. Both annual summer days (SU) (Figure 17) and warm spell duration (WSDI) (Figure 18) are increasing in the last two decades in the eastern Pyrenees (EG 4 & 5). Differences in length of growing season (GSL) (Suppl. Figure 48) are due to the elevation and longitude of the sites, with higher sites of EG 2 and 3 having shorter GSL, and eastern sites longer GSL. All sites show a slightly increasing trend of GSL since 1980 (Suppl. Figure 49).

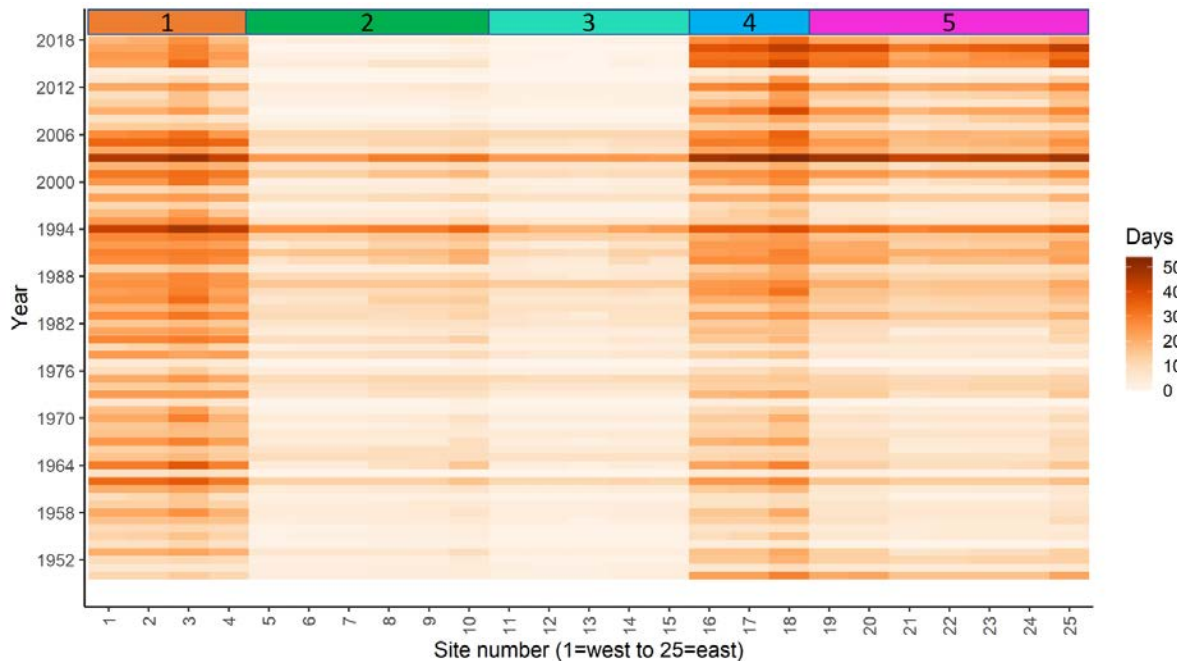


Figure 17: Annual count of summer days (SU), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

Annual frost days (FD) (Figure 19) and icing days (ID) (Suppl. Figure 50) show a decreasing trend since around 1980, for all sites and EGs, while elevational differences also are clearly visible. Annual cold spell duration (CSDI) is also decreasing towards the present, after a cold period around 1960 seen for all EGs, and another cold period in the western sites between 1970 and 1980 (Figure 20). In the recent decades, some of the most eastern show several years of longer cold spells around 2012.

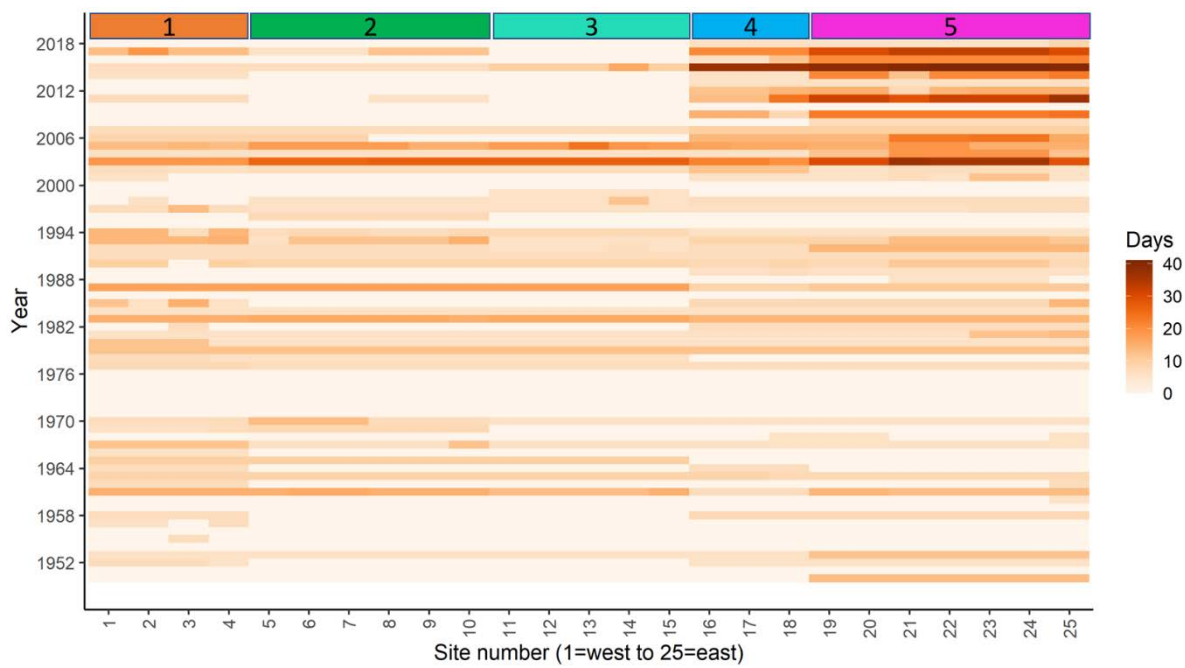


Figure 18: Annual count of warm spell duration (WSDI), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

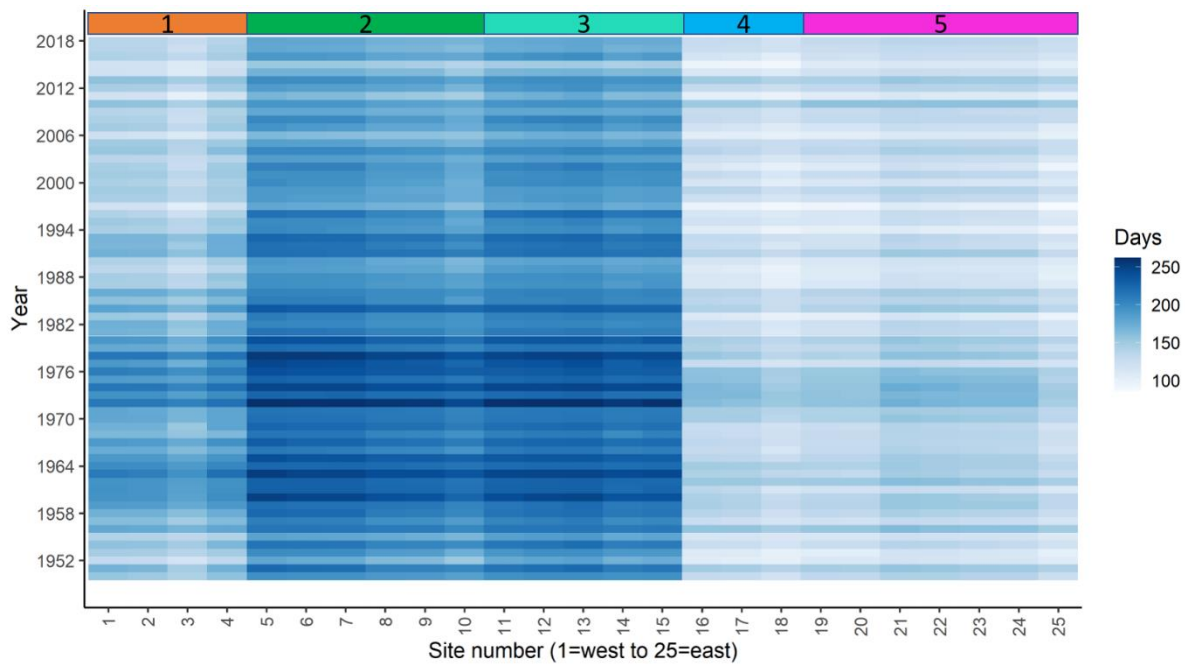


Figure 19: Annual count of frost days (FD), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

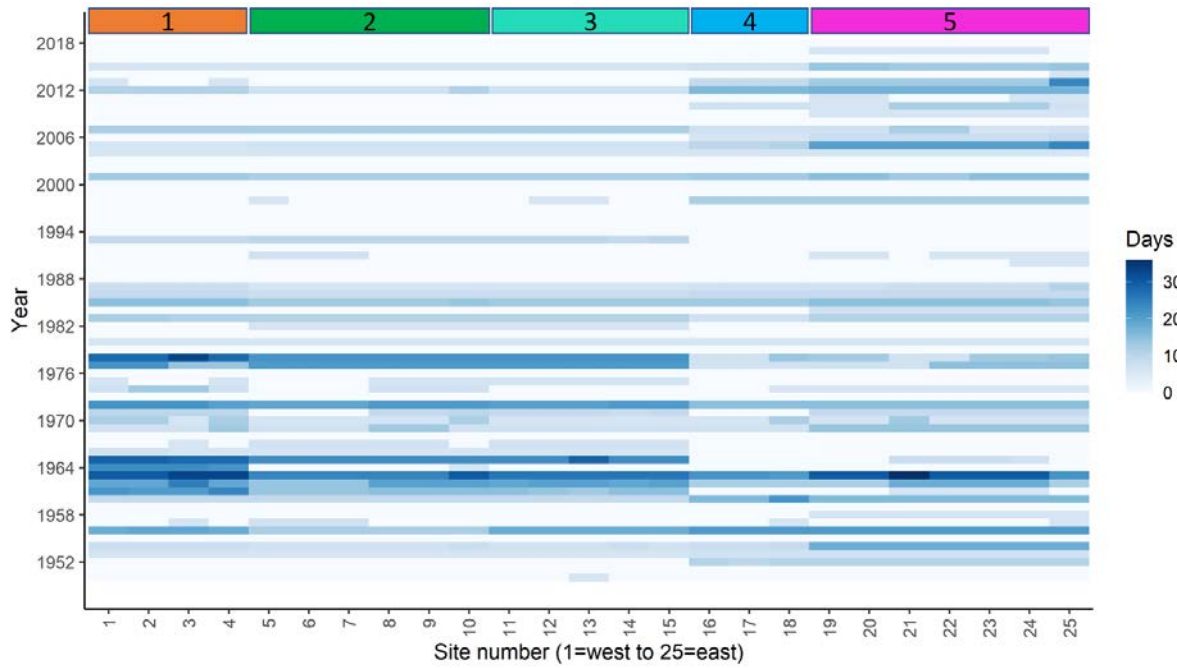


Figure 20: Annual cold spell duration index (CSDI), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

5.2.2 Climate extreme indices - Precipitation

The precipitation extreme indices, in accordance with previous results for climate change in the Pyrenees, show less clear trends attributable to climate change (OPCC_CTP, 2018; Amblar-Frances *et al.*, 2020). Nevertheless, some indices show trends for certain periods or successive years with higher or lower precipitation extremes. The standard precipitation index (SDII) shows a decreasing trend in the last two decades, thus indicating that overall, mean precipitation in wet days is decreasing (Figure 21). This is the case for all EGs since around the year 2000 and most evident for sites at higher elevations belonging to EGs 2 and 3. Annual counts of days with extreme precipitation (R10mm and R20mm) show a decreasing trend at the eastern sites (EGs 4 and 5), thus drier condition in the last two decades and generally less extreme precipitation days in the period 2000-2006 for all EGs (Figure 22, Suppl. Figure 51).

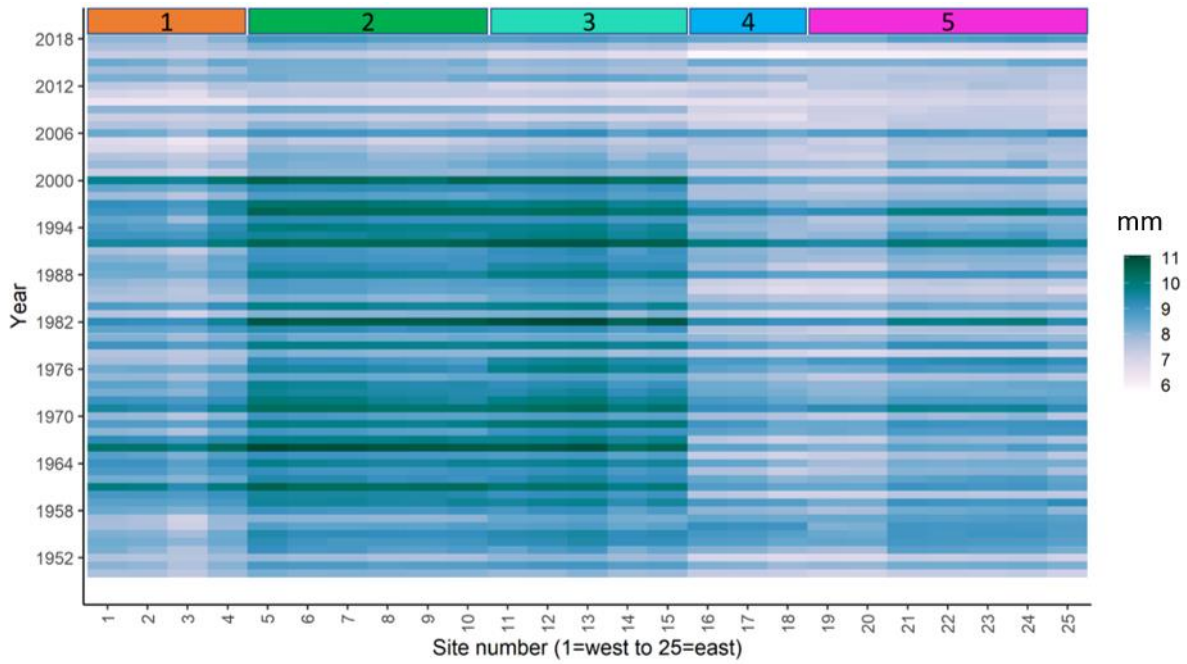


Figure 21: Annual precipitation intensity index (SDII), in mm, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

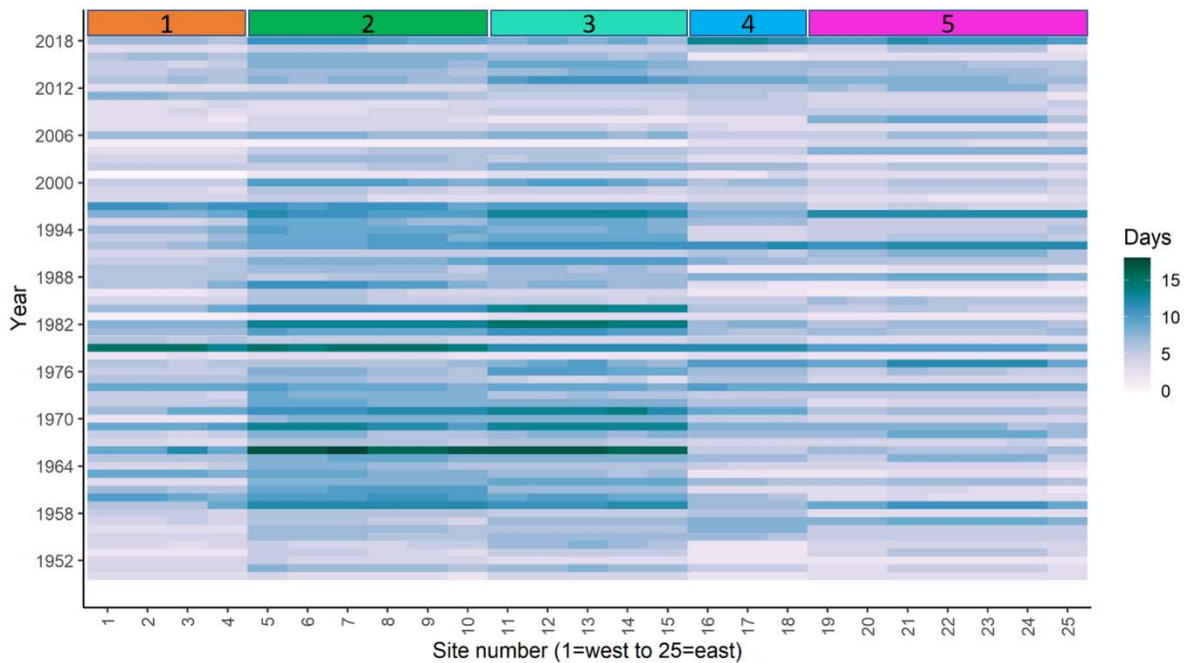


Figure 22: Annual count of very wet days (R20mm), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

While for annual dry spell duration (CDD), there is no visible trend that can be related to climate change in the Pyrenees, the year 2003 sticks out as exceptionally dry year, at least at EGs 2 and 3, thus the higher sites (Figure 23). Longer wet spells (CWD) are visible for the

western sites (EG 1 to 3) in the recent 5 to 10 years of the time series, while the eastern sites show no such trend for the same period (Figure 24, Suppl. Figure 54).

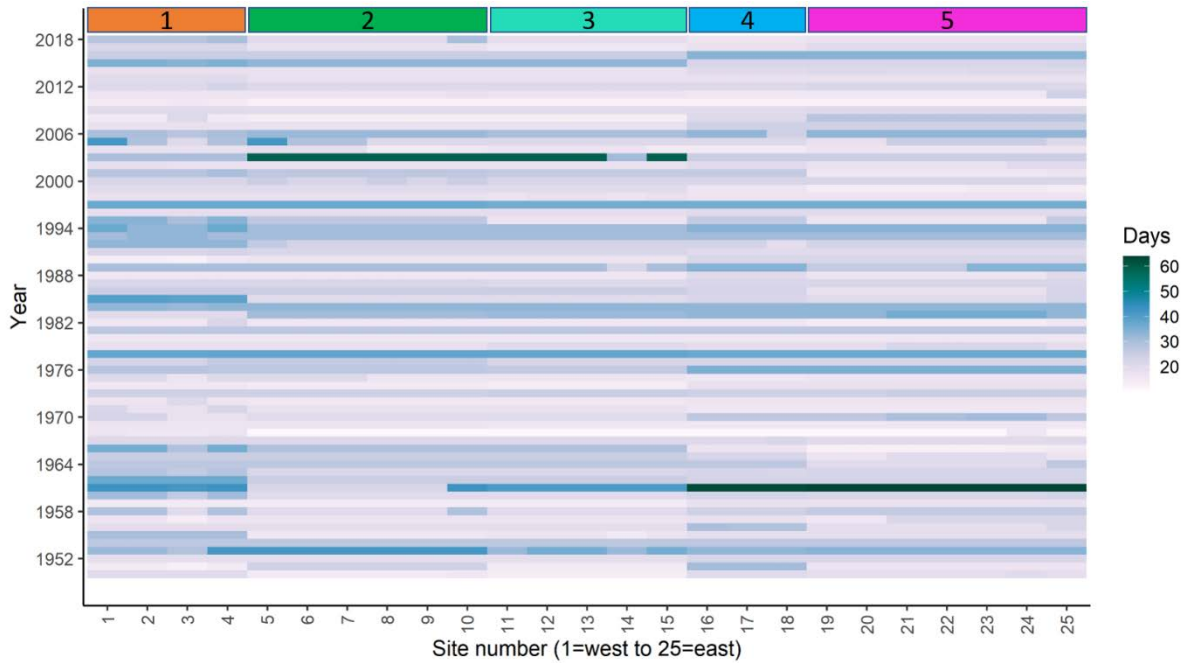


Figure 23: Annual maximum dry spell duration (CDD), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

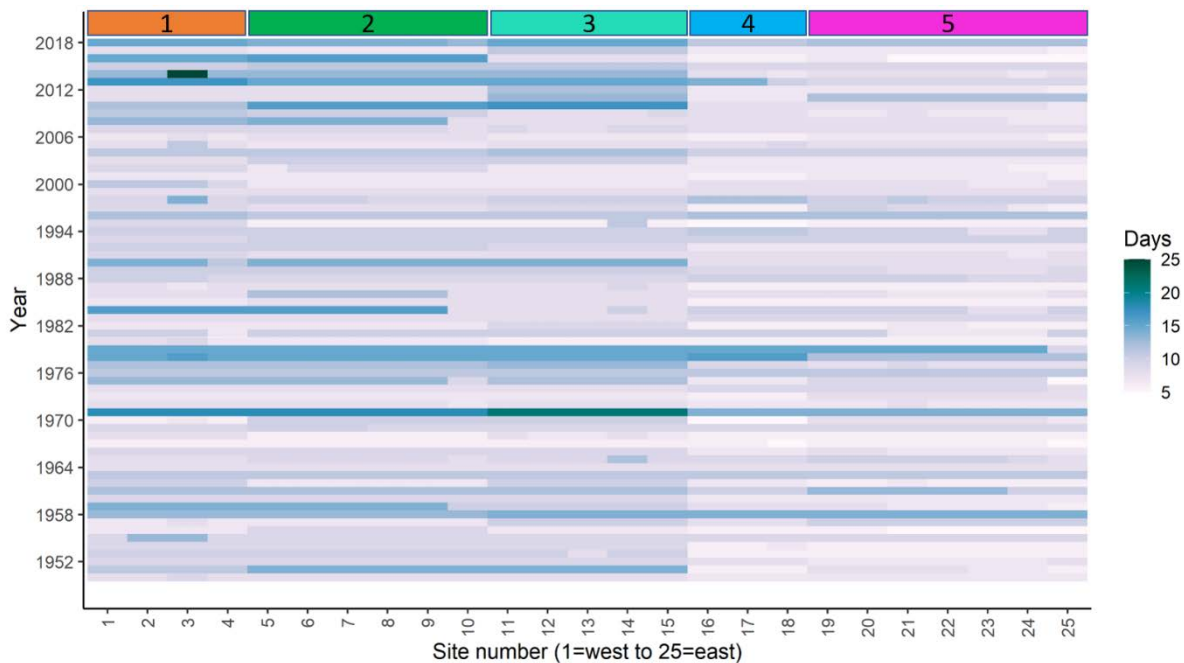


Figure 24: Annual maximum wet spell duration (CWD), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

Less precipitation was recorded on days classified as very wet (R95PTOT) since around 2000, a picture which is most pronounced at western sites (EGs 1 to 3), while eastern sites (EGs 4 to 5) show more very wet day precipitation in the most recent years (Suppl. Figure 52). Extremely wet day precipitation (R99PTOT) is higher for all EGs around 1990 and most pronounced in the western Pyrenees (Suppl. Figure 53).

Finally, the results for total annual wet day precipitation (PRCPTOT) show a decrease in precipitation after 1980 for, in general, all EGs (Figure 25, Suppl. Figure 55). Among the recent years the years 2013 and 2014 stick out as being wetter years across all sites.

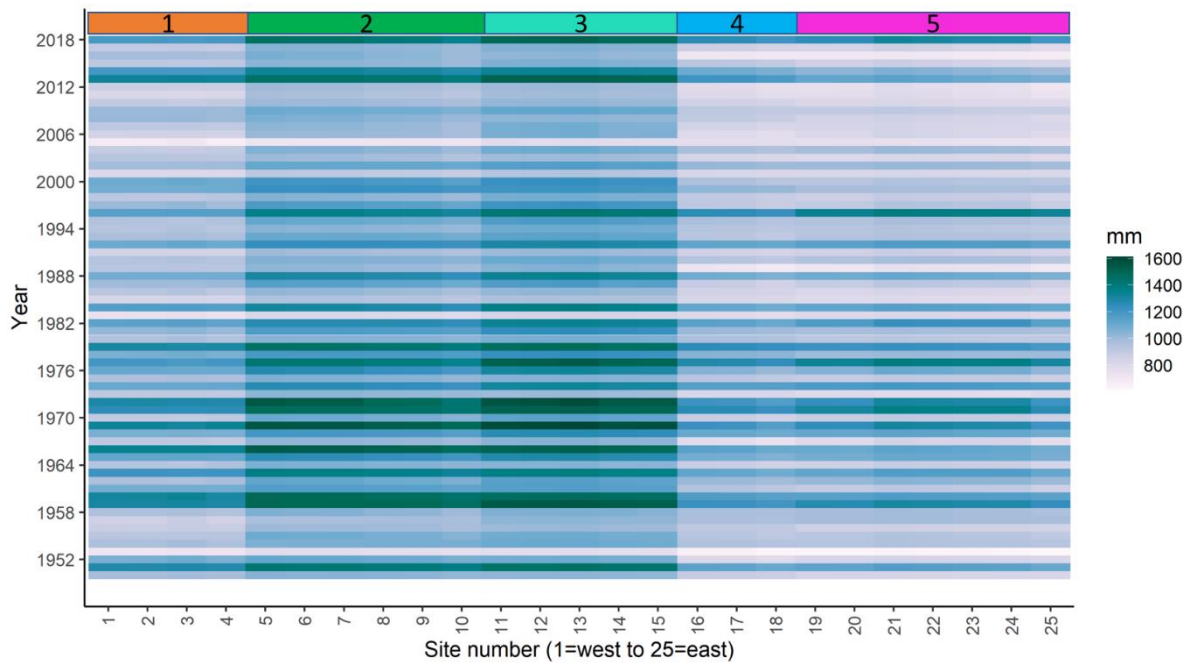


Figure 25: Annual total precipitation on wet days (PRCPTOT), in mm, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

5.2.2 Hydrology

Annual simulated hydrology variables show further signatures of climate change in the catchments of the study sites.

Mean annual runoff (Q, noahmp model) shows a long-term decreasing trend for all EGs, thus across the mountain range, and generally higher runoff for higher elevation sites (EG 2 and 3) (Figure 26, Suppl. Figure 56).

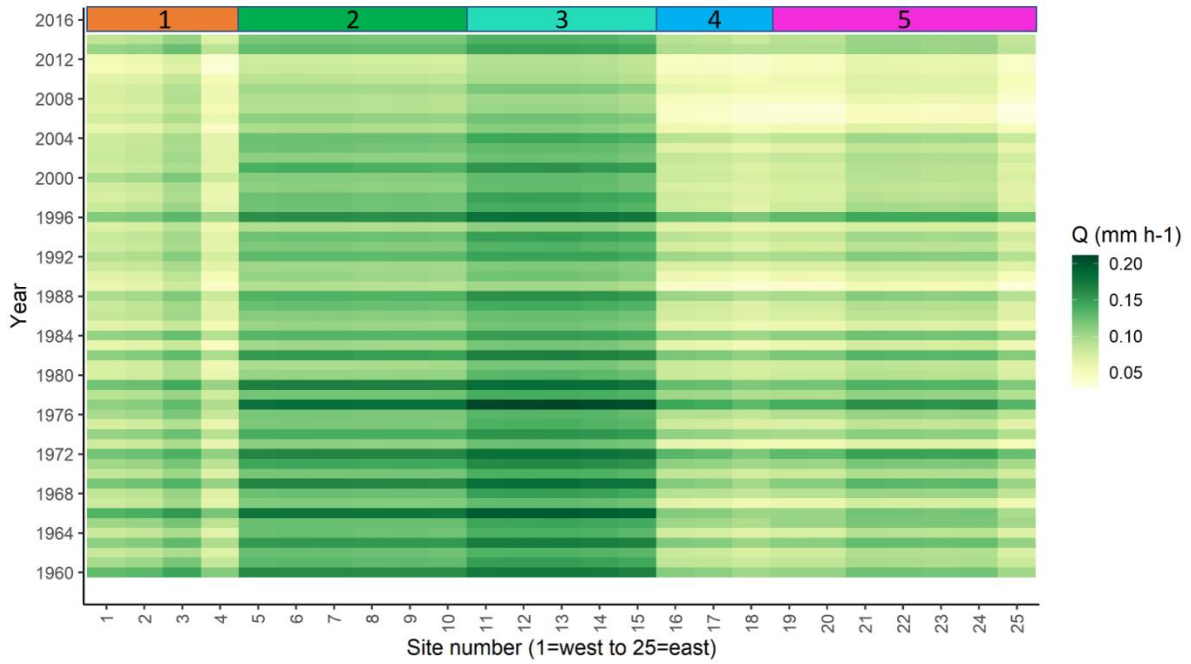


Figure 26: Mean annual runoff (Q) (noahmp model), in mm per hour, for the years 1960-2014, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

Soil moisture saturation levels are generally higher at higher elevations EG 2 and 3 (Figure 27, Suppl. Figure 57 and Suppl. Figure 58). The eastern sites (EG 4 to 5) have generally lower simulated soil moisture values and also show a recent period of drier conditions between 2003 and 2012 before returning to wetter conditions in 2013 and 2014.

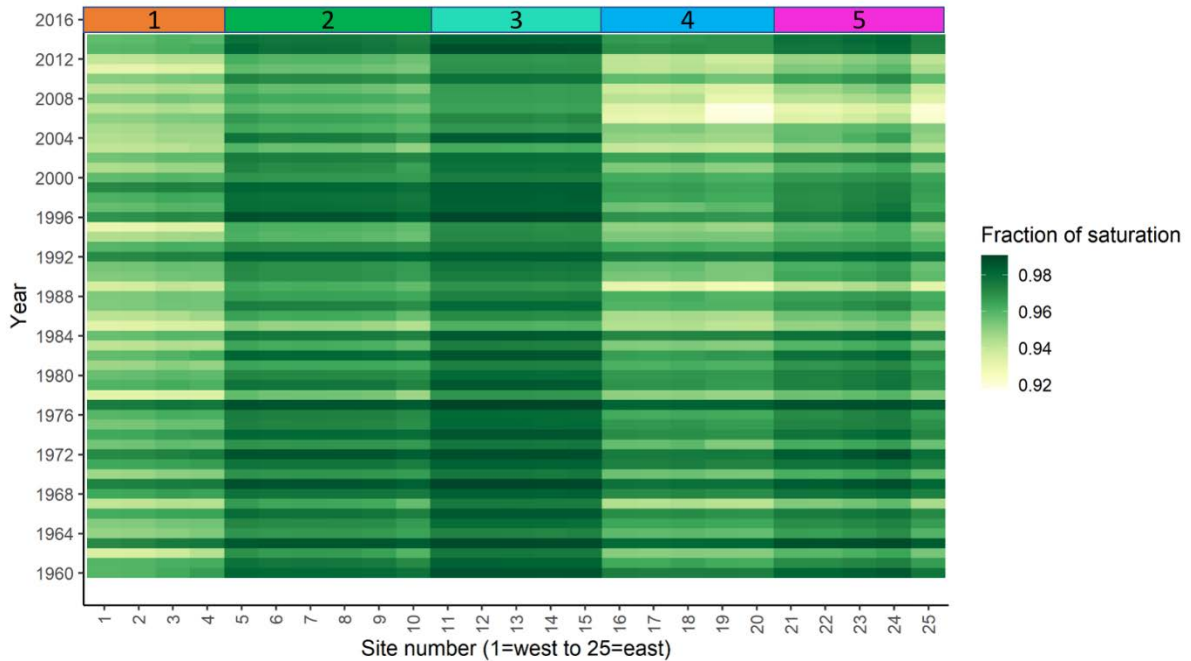


Figure 27: Annual soil moisture (mhm model), in fraction of saturation, for the years 1960-2014, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

5.3 Discussion

Climate change in the Pyrenees is evidenced by both temporal trends in climate extremes and in hydrologic variables. The effects of increasing global warming are clearly affecting climate and hydrology at all study sites, independent of longitude or elevation. However, differences in sensitivity exist between sites due to geographic and elevation gradients in climate change effects (López-Moreno, Goyette, & Beniston, 2009; López-Moreno *et al.*, 2017; OPCC_CTP, 2018). Results for precipitation extreme indices reflect the expected pattern of lower temperature (e.g., SU, Figure 17) and higher precipitation (e.g., SDII, Figure 21) at higher elevations (EG 2 and 3).

The Pyrenees show a large climatic diversity due to these longitudinal, latitudinal and elevational gradients and local atmospheric circulation patterns, for example resulting in distinguishable precipitation regions (Lemus-Canovas *et al.*, 2019). The sites in the Western Pyrenees are characterized by higher annual precipitation, with strong influence of the Atlantic Ocean (EG 1). Further towards the east, the Mediterranean climate influence increases, creating a generally warmer climate, with less amounts of wet extremes (EG 4 and 5)(e.g., SU and CWD, Figure 17 and Figure 24).

A general climate warming trend is found for the study sites, evidenced by the majority of temperature extreme indices, and which is most pronounced in the eastern Pyrenees (evidenced by SU, WSDI, GSL, FD, ID, CSDI). For example, sites situated in the eastern Pyrenees show a clear trend of increasing hot days and longer warm periods (SU, WSDI)(Figure 17 and Figure 18). These results are in accordance with other findings of climate change-related recent warming in the Pyrenees (OPCC_CTP, 2018)(Chapter 4).

A range of precipitation indices (SDII, R10mm, R20mm and PRCPTOT) show a decrease in annual extreme precipitation and total precipitation towards the present (Figure 21, Suppl. Figure 51, Figure 22, Figure 25, Suppl. Figure 55), thus a signal of increased meteorological drought. Such decreases in annual precipitation are also reported by OPCC, for the spatial scale of the entire Pyrenees, with a decrease in annual precipitation of 2.5% per decade in the last 50 years (OPCC_CTP, 2018). However, the largest decreases are due to decreases in winter precipitation (thus, including snow) and trends in precipitation, both positive and

negative, show large variations between different regions in the Pyrenees (OPCC_CTP, 2018).

Dry and wet periods, as well as single wet or dry years might be important drivers of change in freshwater ecosystems, that act on a shorter time scale than effects due to global warming. Study sites in the western Pyrenees for example experienced several recent periods of successive dry or wet years (CDD, CWD, Figure 23 and Figure 24). Such variability in dry and wet extremes are climatic factors important for inter-annual changes in availability of temporary freshwater habitat, which is of great importance for Pyrenean amphibian species (Oromi *et al.*, 2015).

The results for changes in hydrologic variables in the study sites evidence of climate change impacts on Pyrenean hydrology towards increases of hydrological drought (Vicente-Serrano *et al.*, 2021). This is indicated by a long-term decrease in mean annual runoff for all sites (Figure 26, Suppl. Figure 56) and decreases in annual soil moisture levels (Figure 27, Suppl. Figure 57 and Suppl. Figure 58). These alterations in water balance in the Pyrenees can be related to both warming and decreases in snow cover which is indicated by the results of climate extreme indices presented here and recent results for past snow cover trends the mountain area (López-Moreno *et al.*, 2020). Most affected by decreases in soil moisture are the sites in the eastern Pyrenees, which generally also experience warmer Mediterranean climate (Figure 27, Suppl. Figure 57 and Suppl. Figure 58). Rising temperature as driver in hydrological drought in the eastern sites is also evidenced by the climate extreme indices, namely SU and WSDI in the same area (Figure 17 and Figure 18). Drought is effectively expected to increase in frequency and is likely to be pronounced in the Mediterranean part of the mountain range (OPCC_CTP, 2018; Amblar-Frances *et al.*, 2020) (Chapter 4).

For Pyrenean amphibians, as for those in other mountain regions, warming and drought may cause thermal and hydric stress and potential habitat loss due to disappearance of ponds and wetlands (Ryan *et al.*, 2014; Lee *et al.*, 2015; Wilkins *et al.*, 2019; Carlson *et al.*, 2020). Potentially, these stressors will be more severe in the eastern Pyrenees, which are drier and are predicted to be more affected by near-future heat and drought. Decreasing snow amounts affect the entire Pyrenees and are expected to be even more severe in the future (OPCC_CTP, 2018). Reduced snow cover and thus earlier snow melt might expose amphibians to negative effects of spring frost (Bison *et al.*, 2021) or lead to earlier lake ice-out, the latter having been correlated to increased infections in the Pyrenees (Clare *et al.*, 2016) (Chapter 6).

Apart from climate-change related temporal trends, the results show longitudinal differences (thus, differences between the 5 EGs) in runoff and soil moisture (Figure 26 and Figure 27), potentially reflecting impacts of regional climate on hydrology as well as the climatic gradient from the Atlantic to the Mediterranean. Such differences in local hydrology may shape important abiotic variables in the freshwater habitat of amphibians and their importance could be tested in further studies, for example using hydrological models and climate data (temperature, precipitation and snow) of higher spatial resolution, if available.

5.4 Conclusion

The potential of increased climatic and hydrologic stress to Pyrenean amphibians towards the present is visible across the elevational and longitudinal gradient covered by the 25 freshwater study sites. These recent alterations in climate and hydrology in the Pyrenees signify changes in abiotic variables impacting local freshwater ecosystems which are likely to impact the risk for disease due to pathogen infections in amphibians.

Main significant changes for freshwater species in the study sites in the last decades involve increases in temperature extremes and in meteorological and hydrological drought, indicated by the results for annual climate extreme indices and hydrology.

Temperature extreme indices evidence of effects of recent global warming on surface temperatures in the Pyrenees, with most pronounced signals of warming from around 1980 in sites at lower- to mid-elevation and stronger warming in the eastern range. Decreases in annual precipitation extremes are indicated for the entire mountain range from the 1950s towards present, while the eastern range is more affected by meteorological drought. The results for hydrology variables show a signal of increasing hydrological drought, via decreases in runoff and soil moisture across the mountain range.

Main limitations of this analysis are due to the relatively coarse spatiotemporal resolution of climate data, which cannot resolve fine-spatial scale climate which creates some uncertainty of the actual climate experienced by the species at the study sites. Further, the results give no information on the timing of extreme events in each year, which can be an important factor defining their impact on mountain species. Nevertheless, this chapter allows to point out the general picture of stressors related to climate change and hydrology, which is in accordance with latest reports on climate change in the Pyrenees. Future studies are now needed to study climate-change response in freshwater ecosystems in higher spatiotemporal resolution while taking elevational and longitudinal gradients into account.

Chapter 6: Climate and hydrology impacts on *Bd* prevalence in the Pyrenees

6.1 Introduction

There is cumulating evidence of climate change impacts on climatic and hydrologic variables in the Pyrenees (Chapter 4 and 5), which lead to impacts on species health and altered habitat conditions experienced by freshwater species (Schmeller *et al.*, 2018).

The spatial pattern of epizootic versus enzootic *Bd* presence in freshwaters in the Pyrenees (Bates *et al.*, 2018) raises the important question of which drivers are most important in defining disease in the Pyrenees. For climate variables (average and minimum temperature of the warmest quarter, and minimum temperature of the coldest month) only a weak signal was found for their effect on conditional prevalence of infection in the Pyrenees (Walker *et al.*, 2010). In a more recent study, inter-annual differences in lake ice-out timing, marking the start of the growing season, also referred to as spring start, were found to control infection intensities in a multi-host community at lake Arlet, in the *Bd* hotspot of the western Pyrenees (Clare *et al.*, 2016). Here, early spring start was related to higher infections. The importance of spring start timing in a lake for infection prevalence is an important finding of a mountain-specific, and climate-change sensitive variable, as advances of the growing season start are likely under continued climate warming.

In high mountain freshwaters, amphibians have adapted to winters with extended ice and snow cover in their habitat. Advances of spring start, thus snow and ice melt timing impact the whole ecosystem. For example, breeding phenology of some amphibian species was recently found to depend on the timing of snowmelt, with earlier snowmelt additionally increasing the risk of frost damage, due to amphibians being more exposed to air temperatures once the insulating snow cover disappeared (Bison *et al.*, 2021). Further impacts of earlier growing season, for example during years with lower snowpack, can be higher water temperatures (Sadro *et al.*, 2019), which can define annual microorganism growth and thus community composition (Jiang *et al.*, 2019).

In this chapter, observed disease dynamics between 2008 and 2014 at lake Arlet (Clare *et al.*, 2016) serve as starting point for studying infection risk and potential climate and hydrological drivers of infection in the 25 study sites. Three amphibian species were sampled for *Bd* at lake Arlet, *A. obstetricans*, *R. temporaria* and *B. spinosa*. While *A. obstetricans* carries high infection levels in overwintering larvae over several years, infection levels in the other two species were found to fluctuate within the seven-year period (Clare *et al.*, 2016).

Here, *Bd* prevalence for *R. temporaria* and *B. spinosa*, as observed at lake Arlet, is translated into infection risk, in order to extrapolate infection risk to all study sites and the past. Further, spring start, early growing season temperature conditions, as well as annual extreme climate and hydrology are discussed as potential variables in defining disease risk in mountain freshwaters.

This study aims at helping to identify essential climate and hydrology variables for mountain biodiversity conservation. These need to capture current and expected shifts in abiotic variables at a suitable time scale. Abiotic variables and their fluctuations and long-term changes, with importance for freshwater species (which potentially lead to the definition of essential mountain biodiversity variables), may define *Bd* colonization success and, perhaps more importantly, infection outcomes after colonization (Fisher & Garner, 2020). Differences in such variables, and their temporal trends, along the mountain chain may go a step further towards explaining observed disease dynamics.

6.2 Results

6.2.1 Spring start & infection risk

6.2.1.1 Spring start at lake Arlet & simulated spring start for the 25 study sites

Between the years 2008-2014, ice-out at Arlet, here referred to as spring start at the lake, was exceptionally early in 2011, the year when the highest *Bd* prevalence was observed (Figure 28), (Clare *et al.*, 2016).

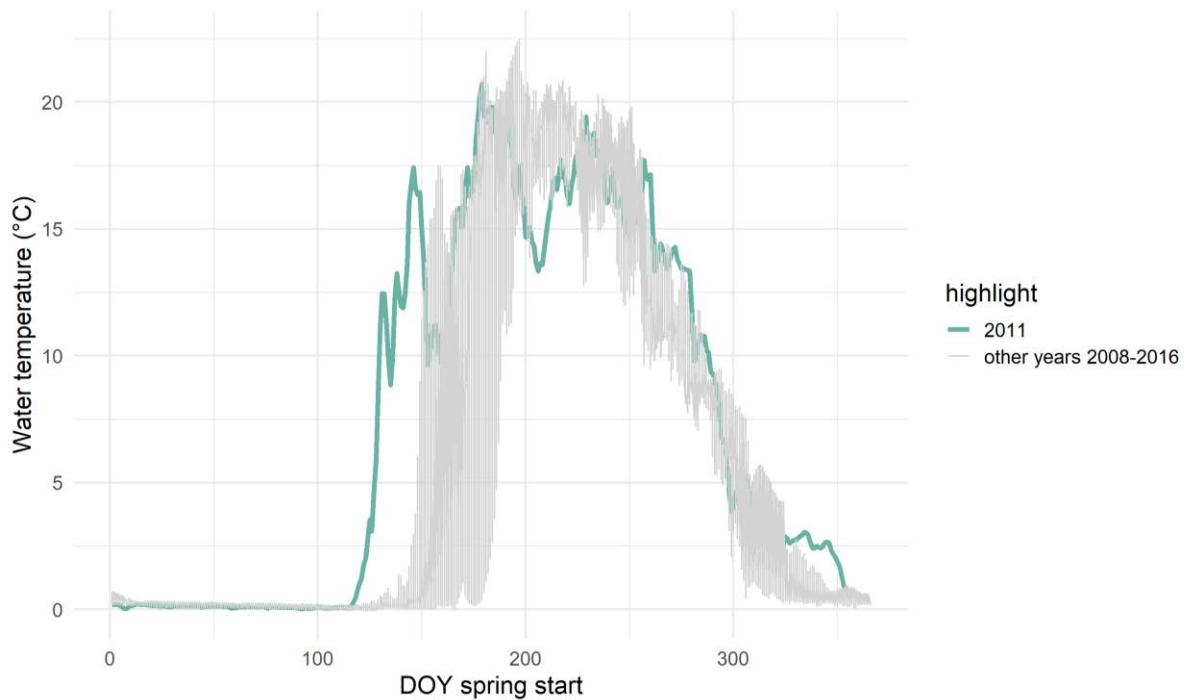


Figure 28: Spring start timing (Julian day =DOY of ice-out), as determined from water temperature versus water temperature time series for lake Arlet between 2008-2014 – analogue to Figure 1 in Clare et al 2016 – here highlighted is the early ice-out of the year 2011.

Early 2011 spring start was observed by water temperature loggers (Clare *et al.*, 2016), and reflected by air temperature data used in this analysis, when using a 4 °C or 3 °C air temperature threshold to simulate spring start timing (Table 5, Figure 29). The simulated spring start at lake Arlet (DOY spring start = date after which air T \geq 4 °C for at least 30 days) is earliest in 2011 (DOY= 90) followed by the year 2009 (DOY =122). The year 2013 shows a late spring start (DOY 161), being the latest spring during this period (2008-2014). MODIS data confirm an exceptionally short snow cover duration at lake Arlet in the year 2011 (DOY=100). In general, the MODIS snow cover data show that snow disappears at lake Arlet before the spring start observed from water temperature logger data, except for the year 2010 (Figure 29). In 2010, water temperature was not measured and spring start timing was thus interpolated from other years (Clare *et al.*, 2016).

The years with the shortest snow cover duration within the 7-year period are 2008 and 2011. For 2008, the observed ice-out timing (and the calculated/ simulated date using the 4 °C threshold) is relatively late after snow cover disappearance (lag = 39 days) compared to 2011 (lag = 20 days), indicating relatively cold spring in 2008, delaying the ice-out once the snow has melted. The year 2013 shows the longest lasting snow cover (until DOY = 157), and is a

year with record snow amounts in the Pyrenees (Anel *et al.*, 2014). The late spring start observed from water temperature data (DOY = 181) can be explained by this long lasting and deep snow cover, which takes longer time to melt, thus also delaying the onset of lake ice melt.

Table 5: Spring start observed at lake Arlet, Clare *et al.* 2016 (DOY water), start of the snow-free period, based on MODIS data (DOY MODIS) and spring start simulated from average daily air temperature, using 3 thresholds ≥ 3 °C, ≥ 4 °C and ≥ 5 °C. Marked in red are the values for the year 2011, due to their importance for choosing a threshold for further analysis. Asterix(*): The DOY water for 2010 was estimated based on biological data (Clare *et al.*, 2016).

	Observed		Simulated		
Air T threshold			3 °C	4 °C	5 °C
Year	DOY water	DOY MODIS	DOY air	DOY air	DOY air
2008	162	123	122	149	160
2009	150	132	120	122	136
2010	137*	140	137	138	138
2011	120	100	89	90	159
2012	145	130	143	143	144
2013	181	157	151	161	161
2014	160	150	145	147	149

Simulated spring start for the years 2008 to 2014 for all study sites shows the general picture of the exceptionally early spring 2011 being limited to the lower elevation sites (EG 1, 4 and 5) (Figure 30a). The eastern sites (EG 4 and 5) show a generally earlier spring than the western sites. In the high snowfall year 2013, a late spring occurs across all study sites. Reanalysis data of snow cover variability reflect largely the picture of simulated spring start, showing 2013, followed by 2014, 2009 and 2008 have a longer snow cover duration than 2011 and 2012 (López-Moreno *et al.*, 2020).

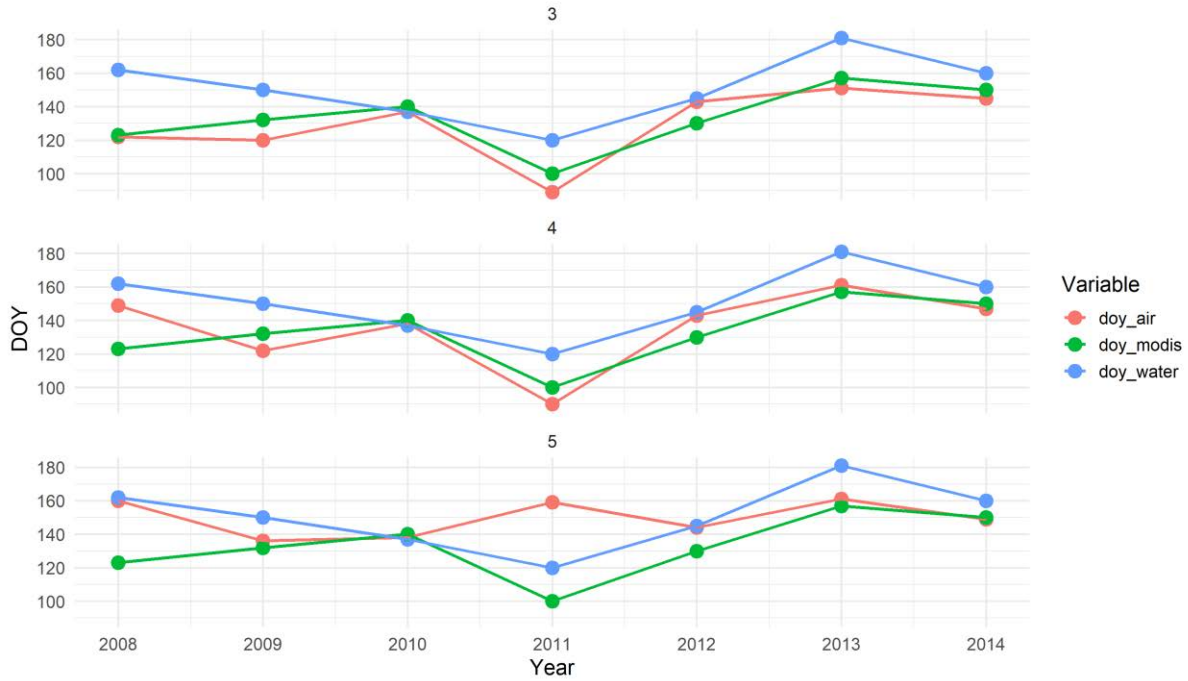


Figure 29: Comparison of simulated spring start 2008-2014 for lake Arlet, using the average daily air temperature thresholds ≥ 3 °C, ≥ 4 °C and ≥ 5 °C (series in red, “doy_air”), with the annual start of the snow-free season based on MODIS satellite data (series in green, “doy_modis”) and observed spring start based on water temperature data by Clare *et al.* 2016 (series in blue, “doy_water”, where 2010 is estimated, not observed).

The temporal evolution of spring start for all sites for the years 1950- 2018 (Figure 30b) shows a trend towards earlier spring start from around 1985, but this trend is only clear for the lower elevation sites (EG 1, 4 and 5), and more pronounced in the east. The higher elevations (EG 2 and 3) show periods with earlier or later spring start, for example a later spring in the 1970s and 1990s and earlier spring between the years 2000 and 2005.

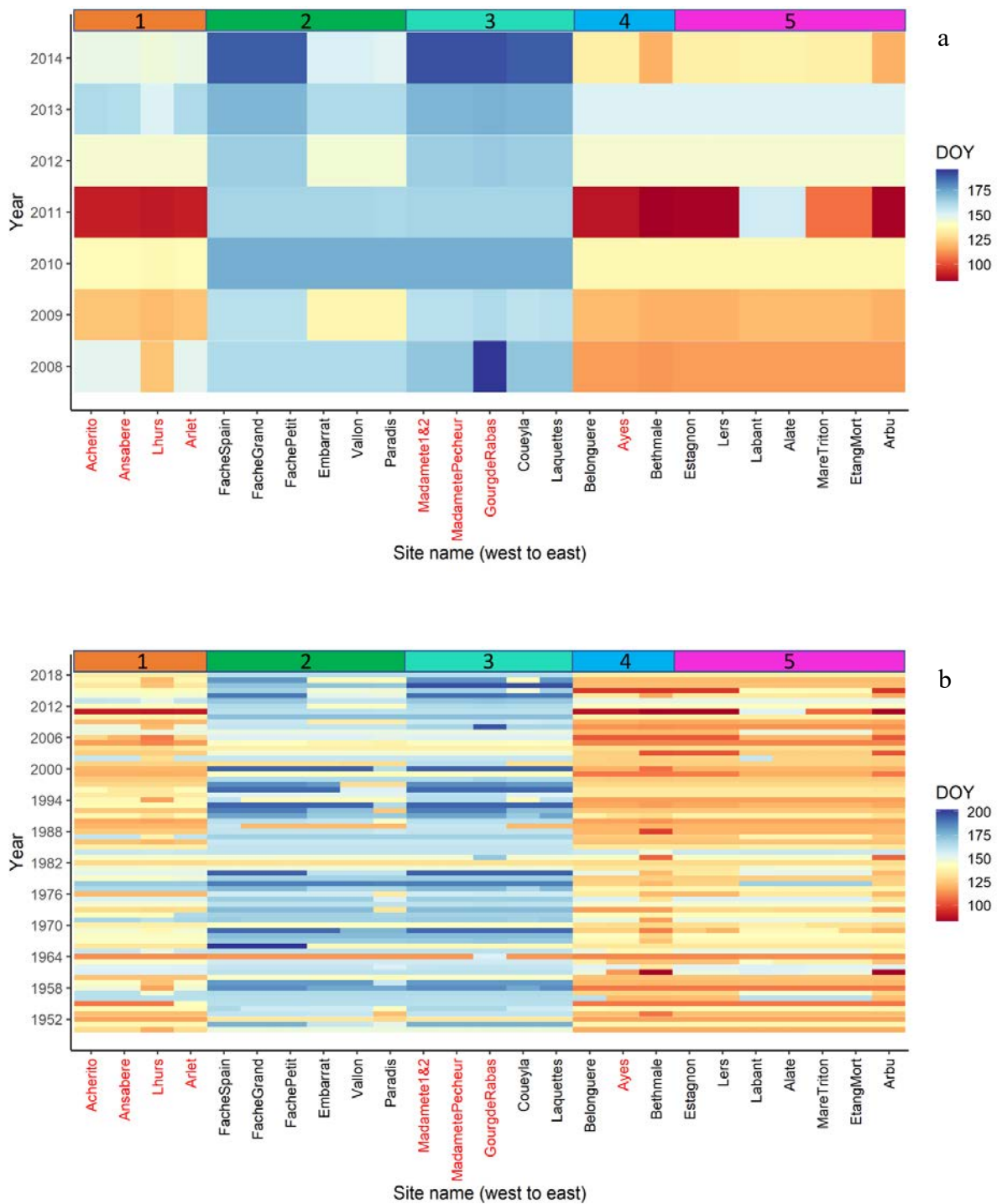


Figure 30 a-b: Time series of annual spring start for all sites from 2008-2014 visualized as heatmap for a) 2008-2014 and b) 1950-2018, respectively, Sites with high *Bd*-prevalence are marked in red.

6.2.1.2 Infection risk based on annual spring start timing

Differences in *Bd*-prevalence were found in the study system between the three amphibians species (Clare *et al.*, 2016). Here, infection risk was simulated based on the simulated spring-

start timing using daily average air temperature (section 6.2.1.1). Thus, the results generally reflect air temperature dynamics in the early spring period at the study, but with the additional information of risk for *Bd* prevalence in the local amphibian species *B. spinosa* and *R. temporaria*. No infection risk map is included for *A. obstetricans*. *A. obstetricans* is serving as inter-annual pathogen reservoir species where prevalence is constantly high with only minimal inter-annual differences in *Bd* prevalence, which show a similar pattern of increase and following decrease in prevalence as found and the other two species, *R. temporaria* and *B. spinosa*. The latter show strong inter-annual variation in prevalence, which was found to correspond to spring start evolution between 2008 to 2014 (Clare *et al.*, 2016, and Chapter 4, Figure 12).

Higher spring-start related infection risk is simulated for amphibians at lower elevation sites from around 1985 (EG 1, 4 and 5), and higher risk at eastern than at western low elevation sites (Figure 31 and Figure 32). In general, infection risk is higher when spring start is early which reflects the spatiotemporal pattern for spring start seen in Figure 30, and is as expected due to the here used definition of infection risk based on spring start timing. In other words, the gradients in temperature effects on spring start timing that exist due to elevation translate into gradients in infection risk. Lower air temperatures delay spring start at higher elevations (EG 2 and 3), and show generally lower infection risk for amphibians (Figure 31 and Figure 32).

A clear trend in increasing infection risk for amphibians is shown for lower elevation sites (EG 1, 4 and 5) due to advancing spring start timing, with a stronger trend for sites in the eastern Pyrenees (Figure 31 and Figure 32). Very early spring start years, with infection risk similar to 2011 at lake Arlet, are increasingly occurring, especially in the eastern sites (EG 4 and 5). The higher elevation sites show only sporadic signals of single or successive years with increased infection risk 1950-2018.

Lastly, the risk maps show strong inter-annual differences in risk between the study sites due to variability in spring start. For example, after the high-risk year of 2011 follow several years of lower risk due to later spring start for the lower elevation sites (Figure 31 and Figure 32). A tendency towards again later spring start in the recent years leads to a lower simulated infection risk observed across many of the sites after 2012.

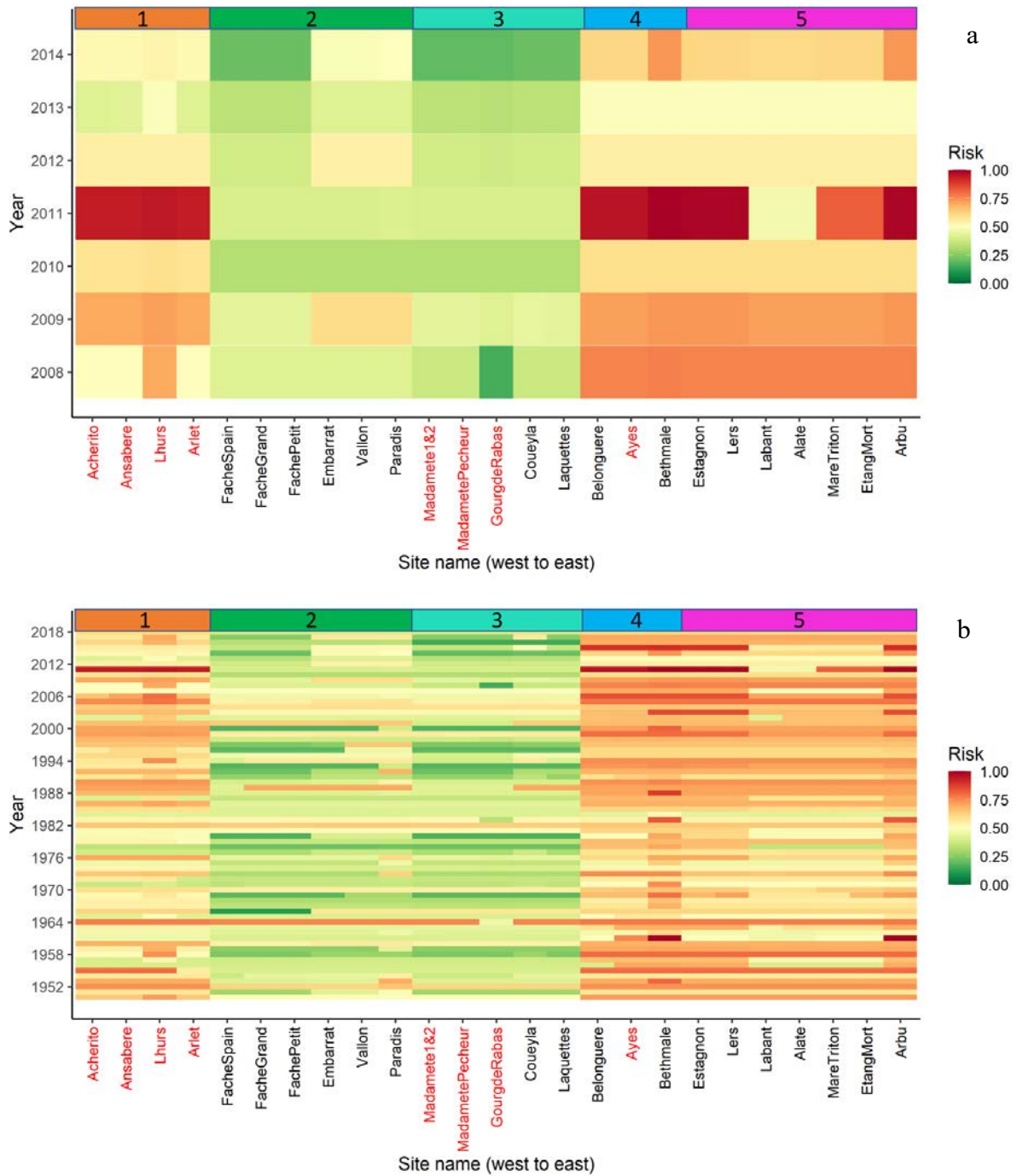


Figure 31 a-b: Annual infection risk for *R. temporaria* (*Rt*) at the study sites, a) 2008-2014 and b) 1950-2018 (analogue to *Bd* prevalence, high risk= high *Bd* prevalence). Sites with high *Bd*-prevalence are marked in red.

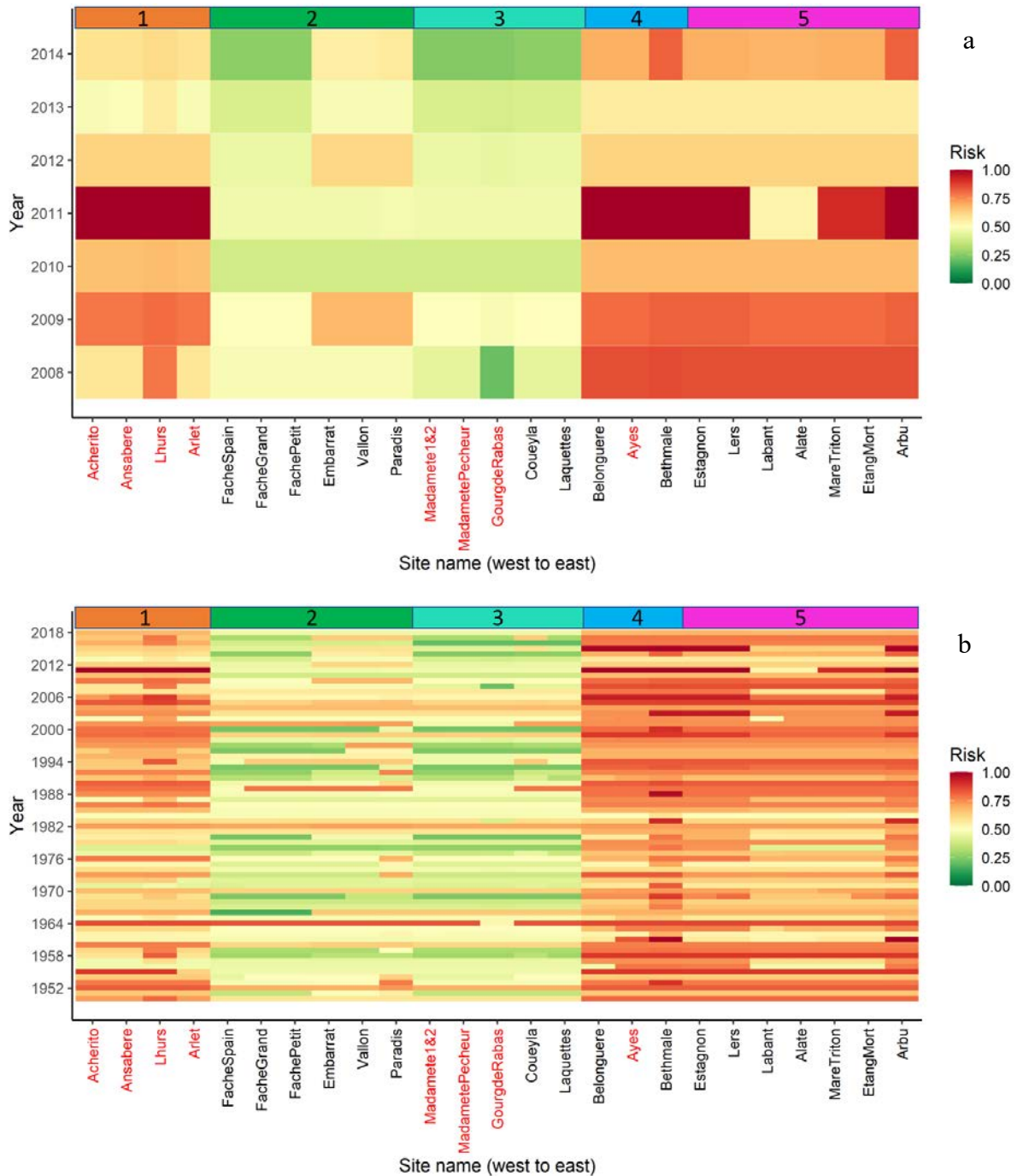


Figure 32 a-b: Annual infection risk for *B. spinosa* (*Bs*) at the study sites. a) 2008-2014 and b) 1950-2018 (risk is analogue to *Bd* prevalence, high risk= high *Bd* prevalence). Sites with high *Bd*-prevalence are marked in red.

6.2.2 Climate & hydrology as variables in infection risk

6.2.2.1 Spring temperatures

Post-spring-start daily average temperatures within the 2 months (60 days), thus, in the annual early growing season after the simulated spring start date at lake Arlet (2008-2014)

were used to identify the annual number of days within critical temperature range 4 °C – 5 °C and below this range (Table 6, Figure 33, Figure 35a-Figure 34a).

In the 2 months after the 2011 spring start, in total 3 days with daily average air temperatures between 4 °C and 5 °C occur and temperatures dropped below 4 °C for one day (at DOY = 135) (Table 6, Figure 33 & Figure 35a & Figure 34a).

The average temperature in this early growing season in 2011 was with 9 °C the lowest in the 7-year period (Table 6). For the 3 temperature metrics calculated, the year 2011 stands out with the coldest spring. The annual post spring start average temperatures at lake Arlet reflect differences in infection prevalence between the years. In the years 2008 to 2011, during which high or rising *Bd* infection prevalence were observed, average spring temperatures are lower (9-12 °C) than in the later, decreasing *Bd* prevalence years 2012 to 2014 (12, 13 and 14 °C) (Table 6, Figure 33, Figure 35a).

Early growing season average temperatures show great inter-annual variability, but also an overall upwards trend across all sites and elevations towards the present (Figure 34b, Suppl. Figure 59). Nevertheless, the timing of occurrences of cold early growing season differs between elevations. For example, the higher elevation sites (EG 2 & 3) show consecutive years of colder spring between 2007 and 2014, while this is not shown for sites at lower elevation (EG 1, 4 & 5). A cold early growing season similar to the year 2011 at lake Arlet occurs also at EG 4 and 5 in the same year (Figure 34a), and within the past 68 years, similarly cold or colder early growing season average temperatures did occur independent of elevation (Figure 34b).

The time series of temperature metrics calculated for the entire year range, 1950-2018 shows that days with temperature between 4 °C and 5 °C in early spring frequently occur, across all sites and elevations, with a weak signal of more days in that range at higher elevations, but no clear overall temporal trend (Figure 35b).

Days of temperature drops below 4 °C occur more often at higher elevation, which can be expected, as temperatures are generally lower there (Figure 36b). In the recent two decades, higher elevation sites (EG 2 & 3) show fewer occurrence of this metric, while the lower elevation sites show no temporal trend but rather single years of spring temperature drops below 4 °C.

Finally, early spring start is generally followed by lower average temperatures (Suppl. Figure 60a), but this correlation is considerably stronger at sites below 2000 m elevation (EG 1, 4

and 5), than above (EG 2 & 3). However, early spring start does not mean that there will be a higher number of days between 4 °C and 5 °C or below 4 °C in the 2 months afterwards (Suppl. Figure 60b-c).

Table 6: Annual number of days with daily average temperatures between 4 and 5 °C, below 4 °C and average temperature within the post spring start period (DOY + 60 days) at Lake Arlet, for the years 2008-2014. Shown are also observed and simulated spring start dates (DOY spring start = first date after which daily average temperature ≥ 4 °C for ≥ 30 days).

Year	Spring start (DOY) observed (Clare et al. 2016)	Spring start (DOY simulated) (Air T ≥ 4 °C for ≥ 30 days)	Number of Days ≥ 4 °C and ≤ 5 °C	Number of Days < 4 °C	T Average (°C) (+ 60days)
2008	162	149	1	0	12
2009	150	122	1	0	12
2010	137	138	1	0	12
2011	120	90	3	1	9
2012	145	143	1	0	13
2013	181	161	0	0	14
2014	160	147	1	0	12

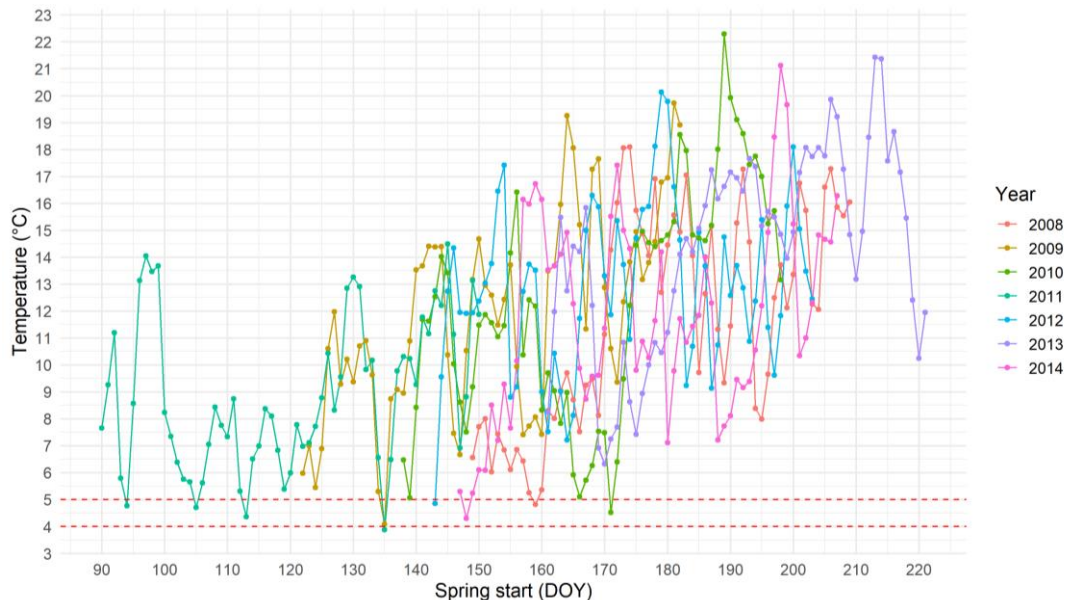


Figure 33: Post spring start temperature time series overlay for lake Arlet and the years 2008-2014, spring start (DOY spring start = first date after which daily average temperature ≥ 4 °C for ≥ 30 days). The dashed red horizontal lines mark the range between 4 and 5 °C.

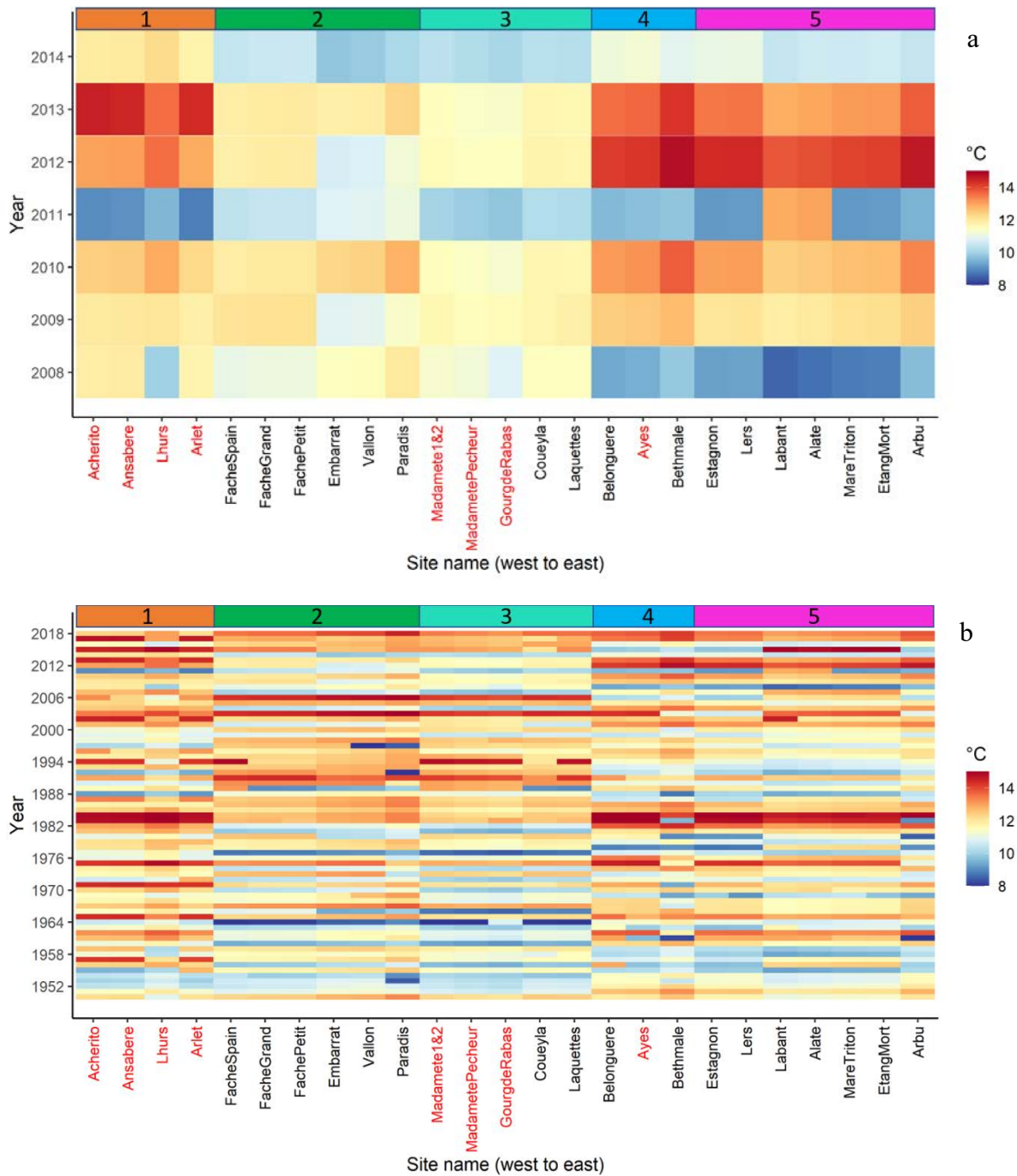


Figure 34 a-b: Heatmap time series for post spring start (DOY simulated spring start +60 days) average temperature (°C), all sites, a) 2008-2014, b) 1950-2018. Sites with high *Bd*-prevalence are marked in red. Elevation gradient number indicated in bars above the heatmap.

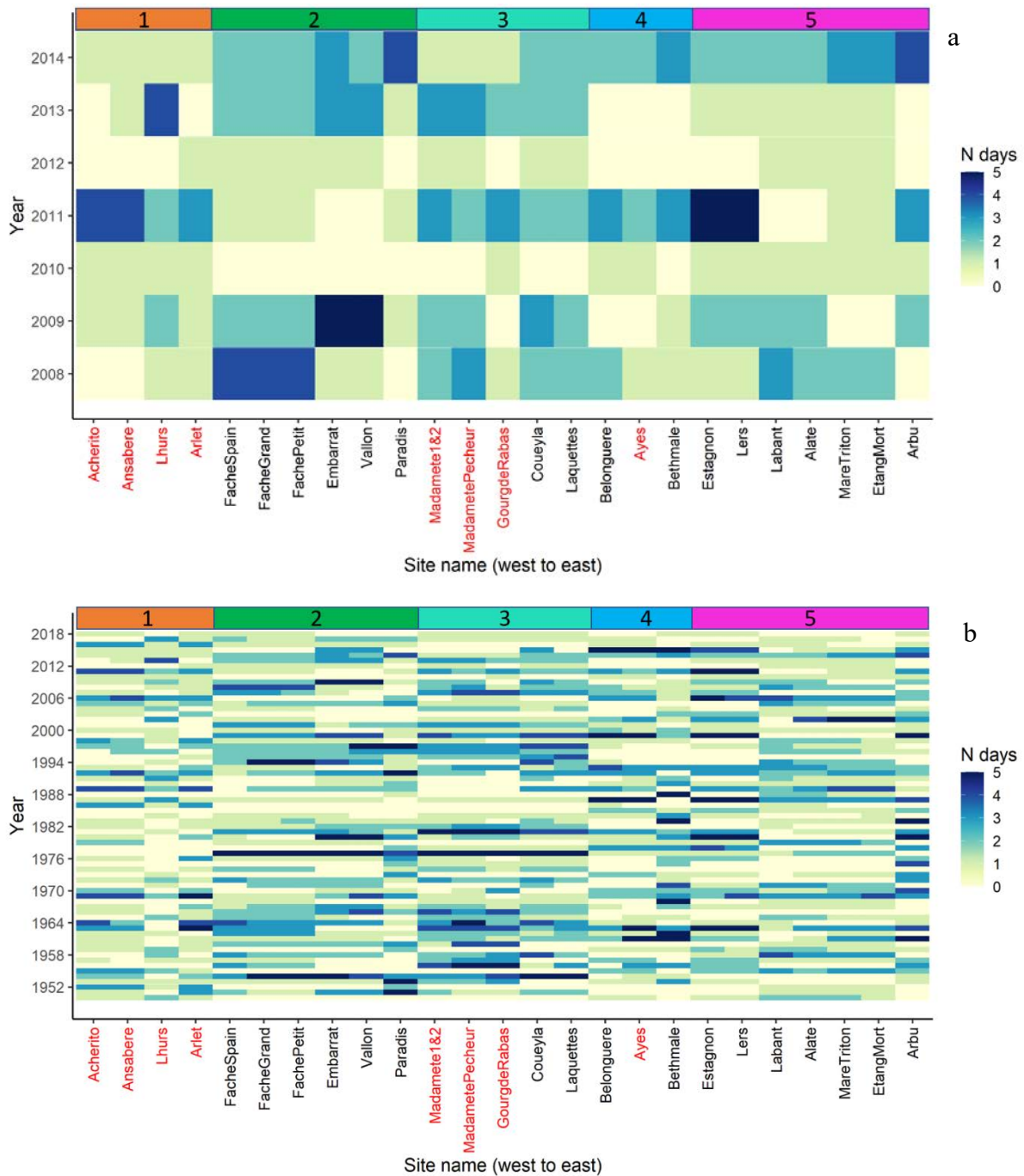


Figure 35 a-b: Heatmap time series for post spring start (DOY simulated spring start +60 days) number of days between 4 °C and 5 °C, all sites, a) 2008-2014, b) 1950-2018. Sites with high *Bd*-prevalence are marked in red. Elevation gradient number indicated in bars above the heatmap.

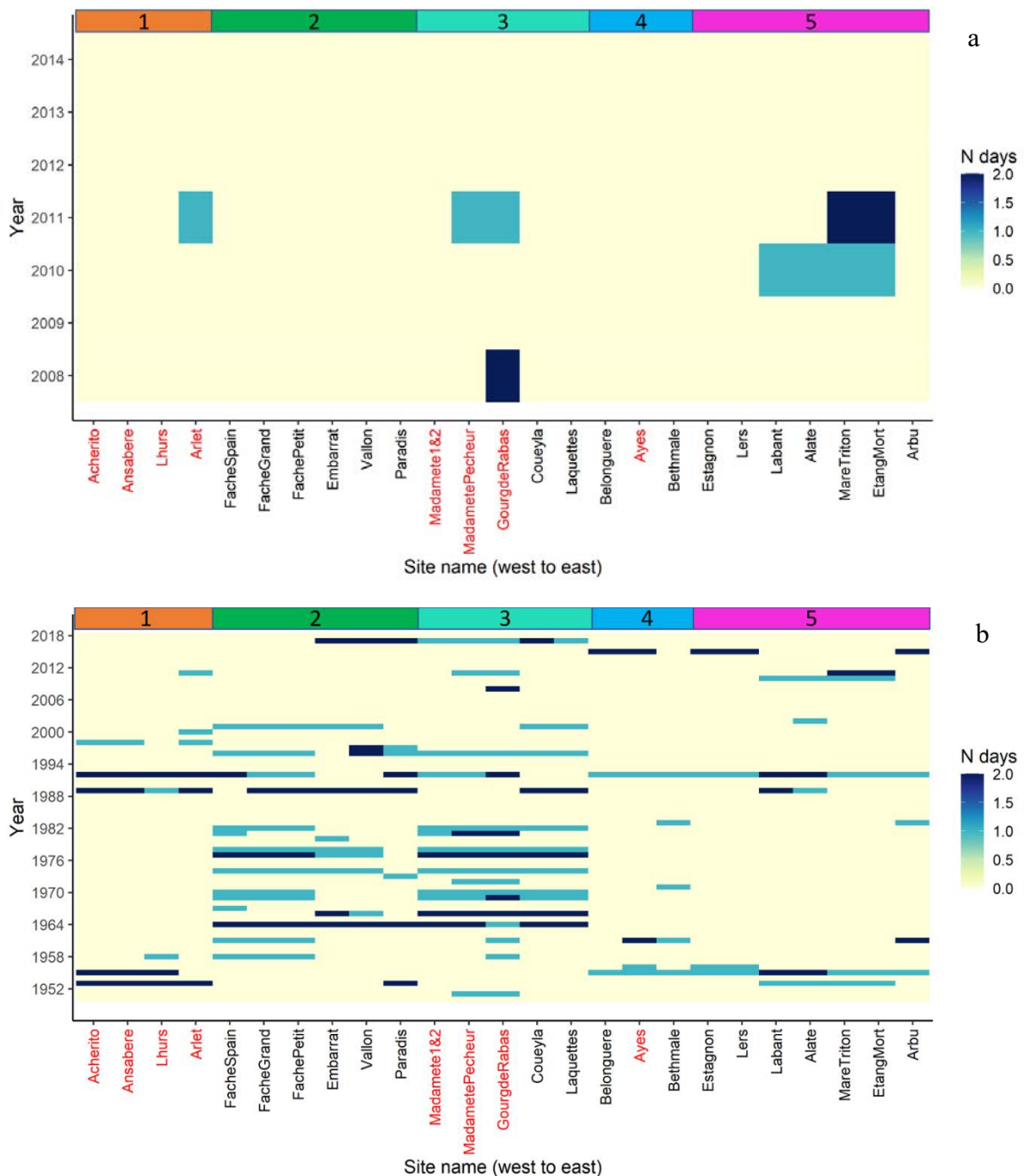


Figure 36 a-b: Heatmap time series for post spring start (DOY simulated spring start +60 days) number of days below 4 °C, all sites, a) 2008-2014, b) 1950-2018. Sites with high *Bd*-prevalence are marked in red. Elevation gradient number indicated in bars above the heatmap.

6.2.2.2 Extreme climate & Hydrology

Only 2 out of 14 annual extreme climate indices, maximum annual wet spell length (CWD) and annual total precipitation on wet days (PRCPTOT), showed to be significantly related to spring start at lake Arlet between 2008 and 2014 ($p < 0.05$, Kendall's tau test) (Table 7). Both

CWD and PRCPTOT are lower in years with earlier spring start (Figure 37). In the 7-year period of 2008-2014 all study sites showed shorter wet spells in the western sites in 2011 and 2012 of EG 1 and 2 and generally shorter wet spells in eastern sites (EG 4 and 5), which are under a Mediterranean climate influence. Further, 2012, but especially 2013 and 2014 are characterized by longer wet spell duration in the western of EG 1 to 3. PRCPTOT reflects the general precipitation gradient with higher precipitation in the west and at higher elevations. Finally, 2013 and 2014 show generally higher annual precipitation and 2011 the lowest amounts in the 7-year period.

As discussed in Chapter 5 for the 68-year period 1950-2018, CWD shows a period with longer wet spells starting around 2012 in the western EGs (and starting earlier in EG 3) and an earlier such period between 1970 and 1980 (Figure 24, Chapter 5). PRCPTOT shows a clear tendency towards lower annual precipitation, reflecting decreasing annual snowfall, the inter-annual variability of precipitation amounts as well as the previously mentioned longitudinal and elevation gradient of precipitation (Figure 25, Chapter 5).

The years with earlier spring start at lake Arlet 2008-2014 were characterized by lower simulated runoff amounts and lower soil moisture levels, as evidenced by significant results for 4 out of 6 tested hydrology variables (Table 7, Figure 38 and Figure 39).

Table 7: Kendall's tau test results for annual extreme climate indices.

Variable category	Variable	Estimate (r ²)	Test statistic	p-value (sign. level= 0.05)
climdex	CWD	0.75	2.31	0.021
	PRCPTOT	0.71	18.00	0.030
	R10mm	0.62	17.00	0.069
	SDII	0.52	16.00	0.136
	SU	-0.39	-1.22	0.224
	WSDI	-0.33	-0.95	0.342
	GSL	0.29	0.91	0.362
	R10mm	-0.25	-0.77	0.442
	r99ptotal	0.20	0.61	0.543
	CSDI	0.20	0.57	0.568
	FD	0.14	12.00	0.773
	r95pTOT	-0.14	9.00	0.773
	CDD	0.05	0.15	0.878
	ID	-0.05	10.00	1.000
mhm	Q (routed)	0.71	18.00	0.030
	Soil moisture	0.62	17.00	0.069
noahmp	Q	0.81	19.00	0.011
	Q (routed)	0.71	18.00	0.030
	Soil moisture	0.71	18.00	0.030
	ET	-0.05	10.00	1.000

Chapter 6: Climate and hydrology impacts on Bd prevalence in the Pyrenees

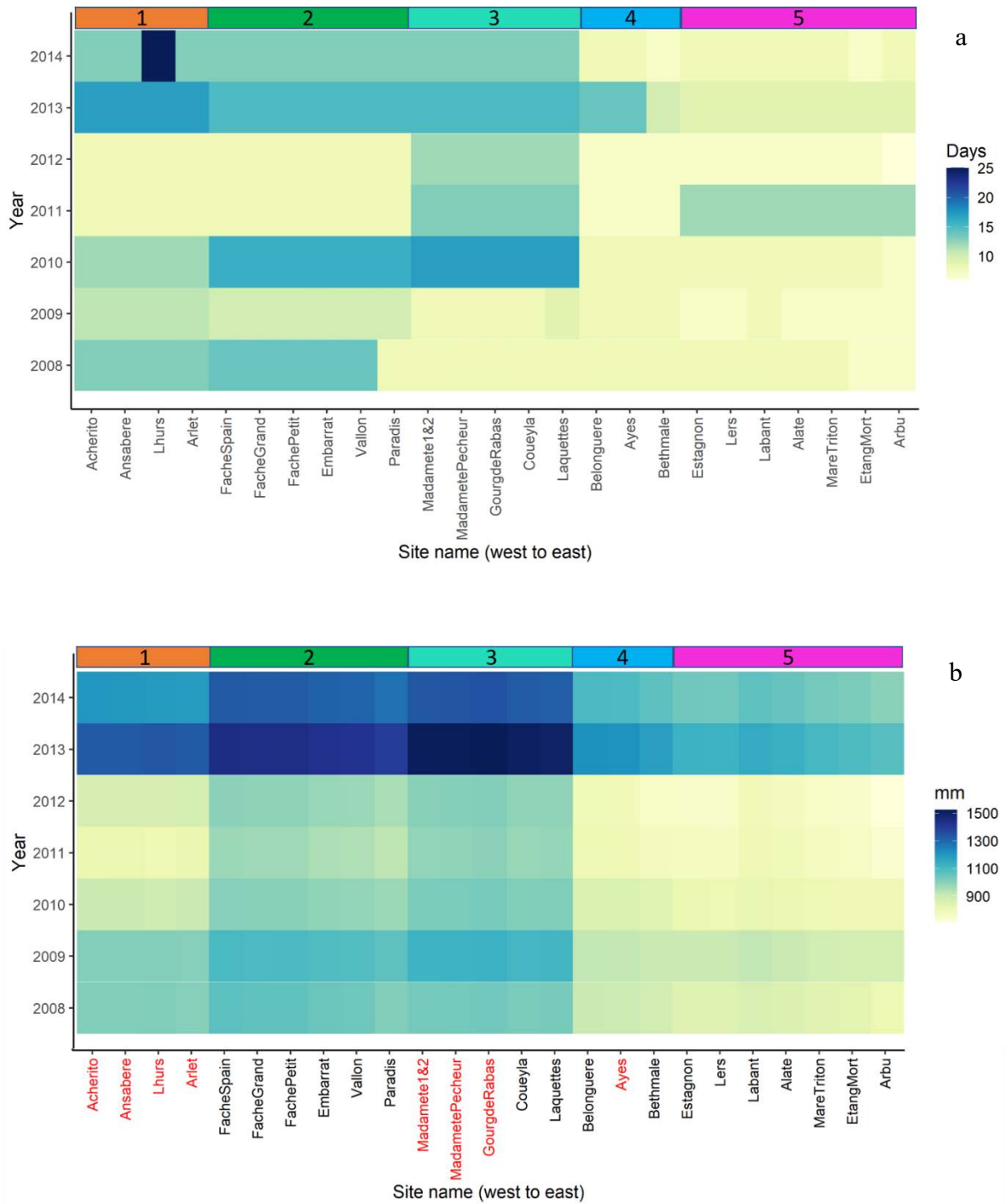


Figure 37 a-b: Time series of a) maximum annual wet spell length (CWD) and b) annual total precipitation on wet days (PRCPTOT) for all sites, from 2008-2014. Elevation gradient number indicated in bars above the heatmap.

a

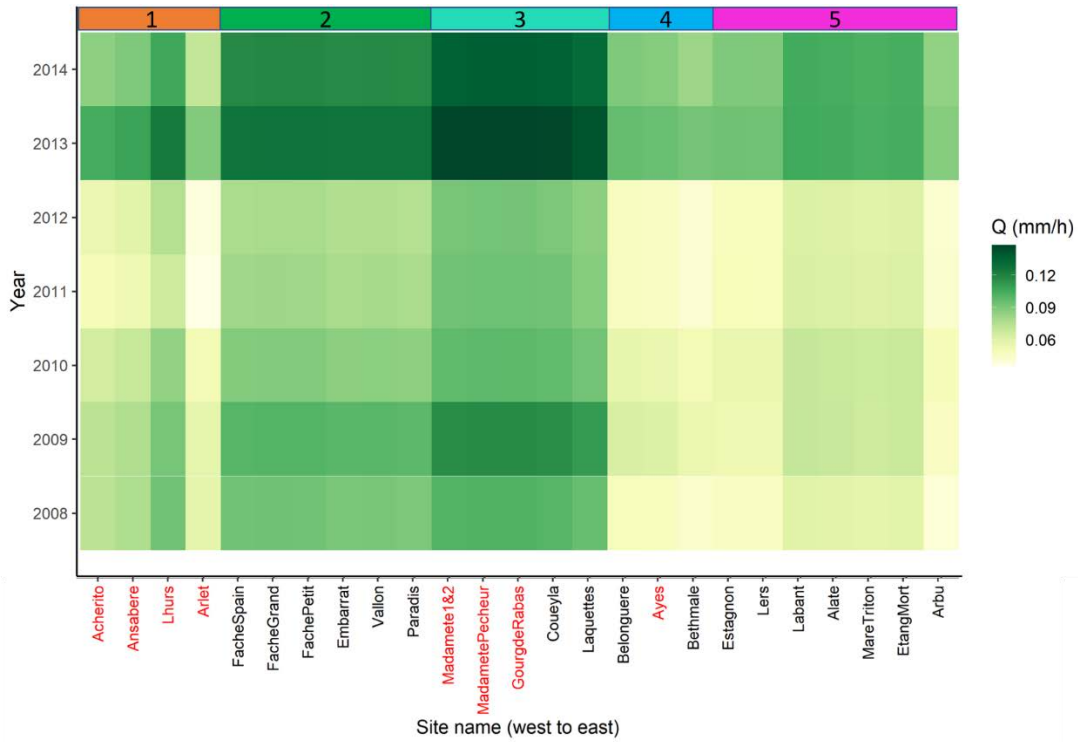


Figure 38: Time series of mean annual runoff Q (noahmp model), for all sites, from 2008-2014. Elevation gradient number indicated in bars above the heatmap.

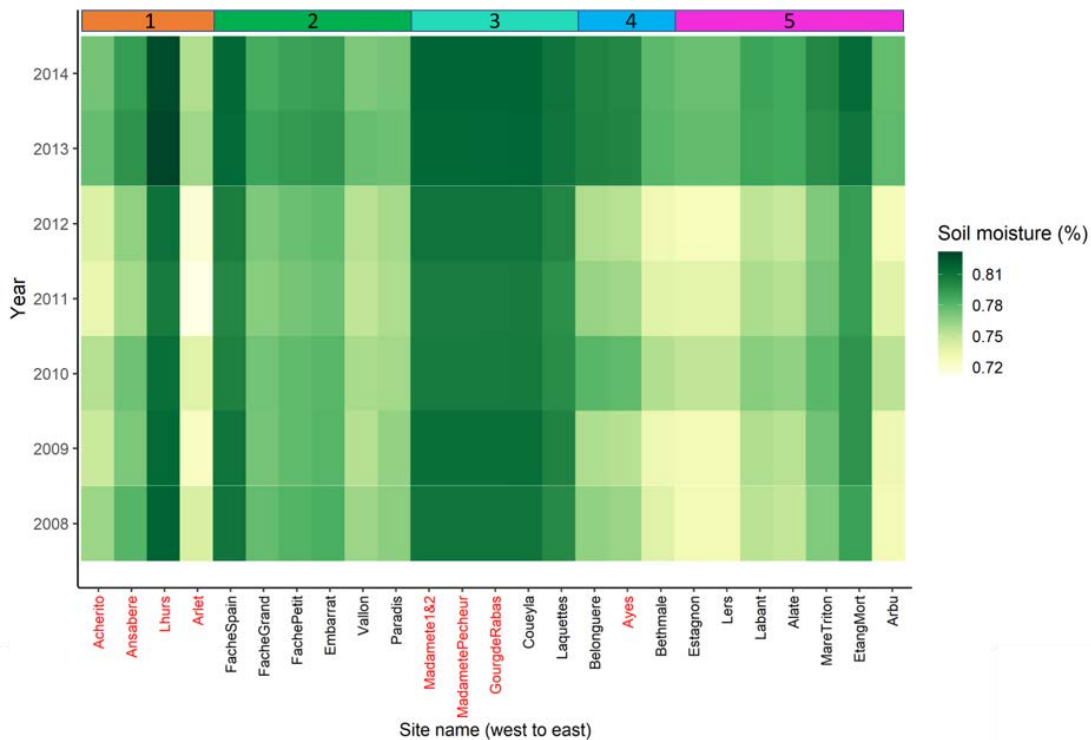


Figure 39: Time series of mean annual soil moisture, simulated as fraction of saturation (all soil layers), (noahmp-model) for all sites, from 2008-2014. Elevation gradient number indicated in bars above the heatmap.

6.3 Discussion

The results for observed and simulated climatic and hydrologic variables for 2008-2014 and their correlation with *Bd* prevalence data at lake Arlet suggest drier climate, less snow and generally colder early growing season (here: period of two months after spring start) characterize years with early spring start and higher observed *Bd* infections in amphibians. For mountains such as the Pyrenees, where snow and lake ice cover are important drivers of freshwater species' life cycles, these results point at important abiotic variables which should be included as predictors of inter-annual infection risk for amphibians.

6.3.1 Inter-annual infection dynamics & the importance of snow on ice phenology based on the example of lake Arlet

In the year period 2008-2014, a rise in *Bd* prevalence and chytridiomycosis infection intensity in the two amphibian species *R. temporaria* and *B. spinosa* was observed at lake Arlet, and explained by an advancement of ice-out (Clare *et al.*, 2016). These results were remarkable and alarming, as those two species generally were known to show low susceptibility for *Bd* (Clare *et al.*, 2016). Within the seven year study period, infections in *R. temporaria* and *B. spinosa* started to increase from 2009 and peaked in 2011 when the earliest ice-out at lake Arlet was observed (Clare *et al.*, 2016). After 2011, prevalence in the two species *R. temporaria* and *B. spinosa* returned to a lower level in the following year 2012, before slightly rising in 2013 in *A. obstetricans*. and *R. temporaria* and local extinction of *B. spinosa* observed in that year.

On the one hand, the effect of snow cover, which insulates the lake ice cover and thus delays ice melt, and inter-annual differences in snow amounts can for a large part explain the observed differences in ice-out timing at lake Arlet between 2008-2014 (Figure 29). In the highest prevalence year 2011, the results from the analysis presented here show that an early start of the snow-free season and low temperatures characterized the early growing season. Indeed, climate reanalysis data for snow show that 2011 and 2012 were years with low snowpack and shorter snow cover duration in the Pyrenees (López-Moreno *et al.*, 2020) (Chapter 4, Figure 15).

On the other hand, the effect of snow cover duration cannot explain the rise in infection prevalence observed for 2009 and 2010 (Clare *et al.*, 2016). 2008, with low observed *Bd* prevalence, was characterized by a longer lasting snow cover, followed by 2009 with the longest snow cover duration in the seven year period (López-Moreno *et al.*, 2020). It should

be noted that both results for snow cover and air temperature related spring start presented here point at a later 2010 spring start than the estimated date published in Clare *et al.*, 2016. Thus, in addition to snow cover, estimated spring start also does not explain sufficiently the rise in infection before 2011, as it was also discussed in the Clare *et al.* article. Clare *et al.* found the highest proportional mortality in *A. obstetricans* for 2010, which they suggested could have been a reason of the sharp rise in *Bd* prevalence in the two other amphibian species *R. temporaria* and *B. spinosa*. However, prevalence in *A. obstetricans* was no significant predictor for prevalence in *R. temporaria* and *B. spinosa* in the other years, whereas year was a significant predictor for prevalence in all three species, suggesting the importance of other drivers (e.g. climate, ice-out) of infections (Clare *et al.*, 2016). The question of which is the main driving mechanism of rising infections in 2009 and 2010 underlines the importance of including other potential drivers of infection risk, such as inter-annual variations and changes in the biotic variables plankton species abundance or composition of the host microbiome, as these are also highly responsive to changes in climate and hydrology (Clare *et al.*, 2016; Bates *et al.*, 2018; Bernardo-Cravo *et al.*, 2020).

Annual snowpack is a crucial variable in ice phenology of high-elevation mountain lakes. For lakes in different mountains of the Western United states, the timing of ice-out was shown to be mainly controlled by (winter and spring-) air temperatures and annual snow amounts (Preston *et al.*, 2016; Sadro *et al.*, 2019; Caldwell *et al.*, 2021). Which variables will have the overriding impact on ice cover phenology in mountain lakes may depend on the size of geographic area taken into account (Caldwell *et al.*, 2021). On a large geographic scale, spring and winter cumulated air temperature, accumulated snowpack and lake size combined are good predictors of lake ice-out date (Caldwell *et al.*, 2021). For smaller mountain areas, such as the Pyrenees, snow was found to be the most important driver of lake ice phenology (Sánchez-López *et al.*, 2015; Preston *et al.*, 2016; Caldwell *et al.*, 2021). In the Pyrenees, the inter-annual differences of the effect of the North Atlantic Oscillation (NAO) on precipitation amounts, and thus on winter snowpack, was found to be a significant driver of ice phenology in the central range (Sánchez-López *et al.*, 2015). No study or model of lake ice phenology at the larger scale of the Pyrenees exists at present, but for studying inter-annual infection dynamics in high-elevation lakes of mountains with lake ice regimes, such as it is the case for the Pyrenees, it is crucial to take variability and changes in annual snow cover into account.

6.3.2 General trends towards earlier spring start and higher infection risk

At lower elevation sites (EG 1, 4 and 5), advances in spring start for the time period 1950-2018 (Figure 30b), and the related recent trend in increase in simulated infection risk for *R. temporaria* and *B. spinosa* (Figure 31b - Figure 32b) reflect trends in increasing air temperatures from around 1980 in the Pyrenees (Chapter 5, OPCC_CTP, 2018). A different picture is observed for the higher elevation sites belonging to EG 2 and 3, with no clear advancing trend of spring start, and thus lower simulated infection risk. The high *Bd* prevalence at the high elevation sites belonging to EG 3 can thus not be explained by changing air temperatures, at least based on the data used for this study. A possible explanation for a less visible trend of increased spring-start related risk at higher elevations might be elevation-dependent warming, which can lead to lower velocities of temperature change above a certain elevation threshold, also called the snowline, where the cooling effect of snow becomes more important (snow-albedo⁸) (Kotlarski *et al.*, 2012; Scherrer *et al.*, 2012; Mountain Research Initiative EDW Working Group, 2015). Due to global warming, the snowline in mountains is expected to continue to move upslope, which will lead to increased surface warming (and earlier spring start at lakes) at increasingly higher elevation (Scherrer *et al.*, 2012).

An additional factor to be considered for the Pyrenees is that seasonal warming was found to be stronger in spring, summer and autumn, than in winter (OPCC_CTP, 2018) which leads to the spring snowpack that persists at higher elevations being stronger affected by warming (López-Moreno *et al.*, 2020). As a consequence, above a certain elevation, the faster changes in snow and ice cover might lead to more intense change in freshwater ecosystems, which will affect freshwater species and potentially chytridiomycosis disease dynamics.

Advances in spring start timing due to generally higher spring temperatures and global warming effects intensifying since the 1980s, combined with decreases in annual snow (OPCC_CTP, 2018; López-Moreno *et al.*, 2020) may be straightforward in explaining the increased infection prevalence at the lower-elevation study sites, belonging to EG 1 and 4. Contrarily, at the higher elevation study sites of EG 3, which are above 2000 m elevation (thus likely experience high snow sensibility to warming) (López-Moreno *et al.*, 2020),

⁸ Snow-albedo describes the effect of snow on reflecting incoming radiation, which is the reason for lower surface warming due to snow cover compared to a surface without snow (e.g. 0.4 °C difference on a spring day in the Alps) (Scherrer *et al.*, 2012).

changes in temperatures are much less informative, but changes in snow depth and snow duration over the recent decades could have led to increased *Bd* prevalence.

Elevational and longitudinal gradients in spring-start related infection risk for amphibians in the Pyrenees might thus reflect combined effects of long-term trends in surface temperature warming and changes in snow.

Besides its effect on ice-out timing and thus spring start in lake ecosystems, snow in mountain catchments is also an important control on temperatures experienced by freshwater species. Higher water temperatures in spring and summer can be a consequence of a winter with low snow amounts (drought years) (Sadro *et al.*, 2019). Combined with spring and summer warming, this may lead to improved pathogen growth in some locations, when temperatures rise into more optimal growth conditions for *Bd* (17 °C – 25 °C (Piotrowski *et al.*, 2004)). Additionally, future advances in spring start timing can impact species growth and species community composition (pathogen, host, plankton growth) and host immunity in the early growing season, via increased exposure to low temperatures in early spring (see next section 6.3.3).

Despite elevational differences in effects of global warming on lake ecosystems, the risk of increasingly early lake ice-out is likely to increase over the entire Pyrenean range on a long term, due to both rising spring temperatures and decreasing winter snow accumulation. Based on these changes, an increase in exposure and vulnerability of amphibians to disease can be expected.

6.3.3: Early growing season temperatures & their potential importance for inter-annual infection dynamics

In addition to spring start timing, infection dynamics could be impacted by the temperatures after spring start. Temperature impacts not only directly pathogen growth and host development and immunity, but also plankton community composition (Jiang *et al.*, 2019) or the host microbiome (Catenazzi *et al.*, 2018), both potential important controls on *Bd* prevalence or chytridiomycosis disease expression. (Bernardo-Cravo *et al.*, 2020) (see also Chapter 2, section 2.2.2.1).

At lake Arlet, the early spring start of 2011 was followed by the lowest average temperature in the two months afterwards, considering the year period 2008-2014 (Table 6, Figure 33, Figure 35a). In addition, the same year of 2011 at lake Arlet counted 3 days of air temperatures between 4 and 5 °C and one day below 4 °C, which were the highest counts of

these metrics within these 7 years as well (Table 6, Figure 33, Figure 35a and Figure 36a). The temperatures and their fluctuations in this low range might have impacted infection dynamics by delaying amphibian metabolism and immune response, but already allowing the pathogen to grow sufficiently well to create an advantage over its hosts.

Temperatures and temperature variations are important controls in chytridiomycosis infection dynamics, as they affect both host and pathogen (Chapter 2). Low temperatures are decreasing the capability of amphibians to fight pathogen infections due to their temperature-dependent immune response (Maniero & Carey, 1997; Rollins-Smith *et al.*, 2011). Additionally, *Bd* is well adapted to temperatures below its growth optimum (Woodhams *et al.*, 2008), see also Chapter 2, Figure 7. Further, when sudden temperature drops occur, a time lag of immune response in hosts, which need to acclimatize to a new temperature, may play in favour of *Bd* and lead to higher host susceptibility to *Bd* and more severe infections (Raffel *et al.*, 2006, 2013, 2015). Amphibians are likely to be negatively affected by early spring start and following low temperatures. This was suggested by a recent study linking trends towards earlier snowmelt to higher frost risk for amphibians at high elevations in the European Alps (Bison *et al.*, 2021). Amphibians living in mountain environment are adapted to snow and cold winters, but when the insulating winter snow cover decreases or disappears early, they are exposed to stronger temperature variation and danger of freezing (Fitzpatrick *et al.*, 2020).

Generally, and naturally, temperatures are lower when spring start, thus lake ice-out, is earlier in the year, although this relationship is not as strong at higher elevations (above 2000m), where spring start is generally later in spring (Suppl. Figure 60a). Thus, with limitations due to different importance at different elevations, average air temperatures in the two months after ice-out can be a predictor of increased annual chytridiomycosis infection risk in mountain lakes.

As additional predictors of higher infection risk may serve the occurrence of daily average temperature drops and fluctuations below 5 °C in the early growing season. Such temperature metrics describe ecologically-relevant thermal conditions and variability around the 4 °C to 5 °C threshold, which define the metabolic activities of freshwater organisms, including host, pathogen and planktonic species. As higher counts of these metrics showed to mark the 2011 spring start at lake Arlet, but were not strongly correlated to spring start timing over all study sites and years (Suppl. Figure 60 b-c), they might indicate infection risk independent of

elevation, as opposed to the more elevation-dependent metric early growing season average temperature.

Early lake ice-out, and thus increased exposure of amphibians to low temperatures, will be increasingly occurring due to global warming and the effect of reduced snow cover, thus, *Bd* infection risk may increase as well. The risk of negative effects on amphibian immunity is higher in early spring, when low temperatures are more frequent/likely to occur. Thus, early growing season temperatures extend the infection risk of changes in inter-annual spring start, formulated before by Clare *et al.* 2016. However, the discussed temperature metrics might be informative for annual infection risk independent from spring start timing, as infection dynamics might be affected generally when cold temperatures characterize the early growing season.

Both the importance of lake ice-out timing, as well as air temperatures in the early growing season and their fluctuations will need to be validated for inter-annual infection dynamics, using more lake ice phenology data from a longer and /or more geographically diverse dataset (in terms of longitude and elevation), in addition to multi-year observations on *Bd* infection prevalence.

6.3.4 Climate extremes and hydrology point towards increased infection risk related to increased conditions of (snow-) drought

As a third potential infection driver category, annual climate extremes and hydrology were studied for the 25 study sites, based on daily-resolution climate extreme indices and annual hydrologic variables. The results from this analysis point towards drier conditions, or more precisely, “less wet” conditions, in years with higher observed infections. Shorter wet spells (CWD) and lower annual total precipitation (PRCPTOT) characterize years with earlier spring start and higher infections (Table 7, Figure 37). No significantly longer dry spells, as defined by the climdex dry spell index (CDD) are found for these years (Table 7). Meteorological drought, caused by an annual precipitation deficit, is thus not indicated for these years. However, on a longer time scale, annual total precipitation (PRCPTOT) shows a decreasing trend towards the present (Suppl. Figure 55), which can be due to decreases in both annual snow and rain. This evolution of decreases in precipitation in the Pyrenees may cause decreases in annual water balance, and thus increased drought, in Pyrenean catchments. The results for hydrology point towards hydrological drought conditions in years with early spring start at lake Arlet, with lower mean annual runoff and soil moisture levels in these

years of higher *Bd* prevalence (Table 7, Figure 38 & Figure 39). Hydrological drought is a consequence of meteorological drought, but integrates also land surface processes, such as evapotranspiration, and is indicated generally by reduced streamflow (Vicente-Serrano *et al.*, 2021). Hydrological drought can be detrimental for freshwater species, vegetation and wildlife, and compromise water availability for human societies (Vicente-Serrano *et al.*, 2021). Due to the fact that snow is the driver of the annual water balance the Pyrenees (García-Ruiz *et al.*, 2011), snow deficits will drive hydrological drought in the form of so-called “snow drought” (Huning & AghaKouchak, 2020).

As discussed in Chapter 5, a long-term trend towards lower runoff and lower soil moisture levels is evident for the study sites (Figure 26, Figure 27, Suppl. Figure 56 and Figure 58). Results from other previous studies confirm the evolution towards increased hydrological drought in the Pyrenees, combined effects of less snow, reforestation and temperature increase (López-Moreno *et al.*, 2008, 2020; García-Ruiz *et al.*, 2011; OPCC_CTP, 2018).

Changes in annual snow accumulation in combination with seasonal climate, in terms of temperature and precipitation and the timing of extreme climatic events, can be important present and future drivers of disease dynamics via several mechanisms.

First, decreased accumulated winter snow may lead to earlier ice-out, caused by the lower insulation effect of a smaller snowpack on lake ice. This earlier spring start may then induce exposure of amphibians to low temperatures (or even frost) and lead to hampered immune response to pathogen infection. Recent results from the Sierra Nevada (California, USA), relate higher mortality rates of amphibian embryos to years with extended freezing of shallow wetlands that are thought to have facilitated spread of pathogen infections (water mould and flatworm) (Sadinski, Gallant, & Cleaver, 2020). In the latter study, it was also observed that the potential of sub-zero overnight temperatures after snowmelt was higher at lower elevation sites (due to earlier snowmelt).

Second, the control of snow cover duration on breeding season length was found to regulate annual infection dynamics (Barrile, Chalfoun, & Walters, 2021). In western Wyoming, USA, lower *Bd* prevalence were observed in years with higher snowpack, when breeding seasons start at a later date due to later snowmelt. In that case, lower pathogen prevalence was explained by the shorter exposure of tadpoles to *Bd* in shorter breeding season years. In the same study, an additional effect of cooler summer temperatures was found to lead to higher host mortalities (Barrile *et al.*, 2021). The control of the combined effects of annual snow and

summer temperatures on infection risk can be a deciding factor in mountains with snow regimes, and thus also in the Pyrenees, while the impact of individual variables is likely to vary depending on breeding phenology of concerned amphibian species.

Third, *Bd* infections can be expected to rise due to drought stress in amphibians and habitat loss caused by dry-out of shallow freshwater habitat. Snow drought in their mountain habitat can lead to drought stress for amphibians during the later spring season or during summer, induced by drying of shallow habitat or low soil moisture levels. Development under drought conditions was found to have negative effects on amphibian immune fitness, and together with thermal stress might explain higher susceptibility to *Bd* and development of more severe chytridiomycosis (Brannelly *et al.*, 2019; Lertzman-Lepofsky *et al.*, 2020).

Shorter hydroperiod of seasonal (snowmelt-) ponds and wetlands, or the dry -out of shallow freshwater habitat as a consequence of lower winter snowpack signifies the loss of important (potentially pathogen-free) amphibian habitat. Shallow, temporary or seasonal freshwaters are an important amphibian habitat in mountains, and they are very sensitive to drought (Kissel *et al.*, 2019; Carlson *et al.*, 2020). Amphibians have been relying on shallow freshwater habitat, as a consequence of fish-stocking in larger lakes (Knapp & Matthews, 2000; Wilkins *et al.*, 2019). Such temporary ponds are also important habitat for amphibians in the Pyrenees (Oromi *et al.*, 2015), and their importance as alternative fish-free habitat most probably has also increased since the introduction of salmonids for sport fishing in many Pyrenean high elevation lakes. When shallow or temporary freshwaters dry out, or their water levels lower too much, tadpoles may suffer mortalities or adult amphibians may crowd together in remaining ponds or lakes, which increases risk for *Bd* transmission (Scheele *et al.*, 2015; Wilkins *et al.*, 2019). Drought as a consequence of reduced winter snowpack might thus increase susceptibility for *Bd* in amphibians in affected freshwater ecosystems in the Pyrenees.

Snow, with its role as major driver in annual catchment water balance, buffering overwintering species from extreme temperatures, and controlling ice phenology can thus be seen as a crucial variable in freshwater species health and life cycles in mountains with snow regimes (Figure 40). The recent studies from the Sierra Nevada (Wilkins *et al.*, 2019; Sadinski *et al.*, 2020), discussed above, integrate snow as a variable and significant driver in relation to observed amphibian mortalities and pathogen infections. The Sierra Nevada can be seen as climatically and hydrologically similar to the Pyrenees, with Mediterranean climate and hydrology governed by the winter snowpack. While snow is of great importance in many

hot spots of chytridiomycosis disease globally, the integration of snow hydrology is still a research gap in disease ecology of amphibians in mountains.

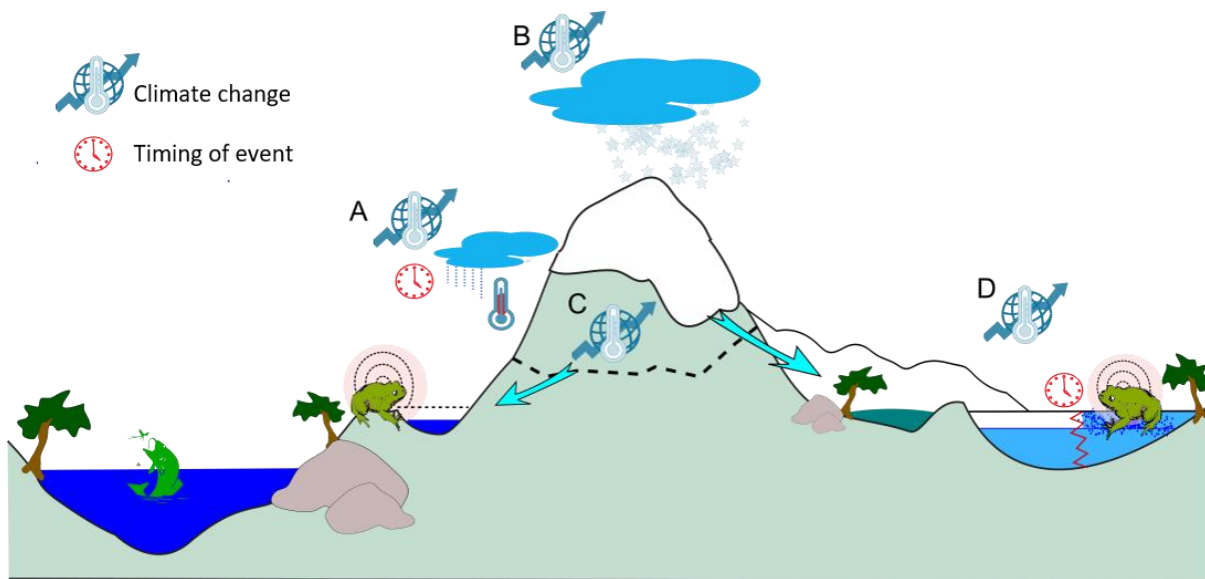


Figure 40: Schematic illustration of processes and variables that may impact annual chytridiomycosis dynamics in mountains with snow regimes, and which are sensitive to climate change. A- (Seasonal) temperatures & precipitation / timing of climate extremes; B- Amount of annual winter snow accumulation (SWE - important control on drought risk); C-Snowmelt timing (important control on D, lake ice-out); D-Timing of ice-out = spring start at the lake = start of the growing/activity/breeding season of freshwater organisms.

6.3.5 Limitations and future perspectives

The presented results and those discussed from other recent studies are indicative of the importance of seasonal temperatures and snow in driving disease in mountains. However, the limited data and time period considered for this study in the Pyrenees call for a validation of these results and extension to other sites across the mountain range, for which data on *Bd* prevalence are available.

6.3.5.1 Limitations of available climate data

A main challenge met when studying effects of climate change on mountain species, like discussed here, arise from reduced data availability in mountains. In many global mountains, including the Pyrenees, climate data from in situ measurements are spatially sparse (Scherrer, 2020) and can often be retrieved only relatively far from study sites and/or at a different elevation. In situ climate data are a crucial part of climate monitoring frameworks, but they

do not account for the pronounced terrain-effects on climate in mountains (Thornton *et al.*, 2021).

Gridded interpolated climate data, such as used here, and gridded climate reanalysis products offer the advantage of spatially continuous data, which are provided at increasingly higher spatiotemporal resolution (Scherrer, 2020). For macroecological studies, such as at the spatial scale of a mountain range, such gridded data products often are the only available option for obtaining climate data and thus, they are often used in practice (Scherrer, 2020).

Nevertheless, gridded data products come with their limitations as well, for example is their uncertainty naturally higher in data-sparse regions such as mountains, as less observations are available for interpolation (Kotlarski *et al.*, 2012; Scherrer, 2020; Thornton *et al.*, 2021). Further, a spatial grain of several kilometres grid size is integrating much more topographic heterogeneity in mountains than in flat areas. This may for example lead to sites at different elevation being considered climatically identical if they are sharing the same climate grid cell due to their relative spatial proximity. Concerning the results presented here, the climate data grid size of 10 km resolves general climatic differences between sites at different elevation and climatic differences along the longitudinal gradient across the Pyrenees. For sites that group together relatively close spatially, the resolved climatic differences may be minimal and lower than in reality, if their catchments share the same grid cell (or neighbouring data grid cells due to averaging between cells over a catchment).

Another general limitation that needs to be taken into consideration is the failure to account for topoclimate and microclimate effects acting on the studied species and ecosystems. These effects can be important especially in complex mountain terrain (Faye *et al.*, 2014), and a combination of in-situ monitoring and high-resolution remote sensing would be necessary to describe climate at such small scales. However, high spatial resolution assessments of climate are no suitable approach when the intention is to relate ecological processes to long term climate change effects, due to high costs and efforts of high-resolution remote sensing. That said, high resolution assessments of surface temperatures for example can offer a general insight on climate heterogeneity and potential thermal refuges that may shield species such as amphibians and pathogens from long-term climate warming (Isaak *et al.*, 2016), and impacts on disease dynamics are possible due to such thermal refuges (Haider *et al.*, 2017).

6.3.5.2 *Directions for future studies on mountain-scale infection risk*

For future comparable studies on the interactions between climate change and wildlife disease dynamics, regional climate reanalysis and climate models may offer the advantage of higher resolution, and a better representation of terrain effects (e.g. due to vegetation or other land cover) on climate (Amblar-Frances *et al.*, 2020; Thornton *et al.*, 2021). Although the spatial resolution of many currently available reanalysis products may be as well arguably coarse (5-50km grid size) for sufficiently resolving mountain climate heterogeneity, especially at the scale of small mountain catchments, they may be a better option than interpolated-only data and are increasingly improved in their quality for application in mountains (Scherrer, 2020). The setbacks of reanalysis climate data are that often a shorter time period is covered by the data and they may not be available open access, or made available only after a while when ready for the larger public (as their production is time-consuming and computational expensive) (Thornton *et al.*, 2021). Concerning limitations of gridded climate data should be noted that a certain caution should be applied when analysing climatic trends, as the number of stations used for interpolation or simulation has increased for most areas since the start of the considered time period (Simmons *et al.*, 2017; Cornes *et al.*, 2018; Scherrer, 2020). Due to individual and shared disadvantages of available data, data assimilation can lead to improved results and lower result uncertainty, combining the assets of in-situ climate data, models and reanalysis (Thornton *et al.*, 2021).

Within the last two decades, the monitoring of snow distribution and snow cover duration in mountains greatly profited from new satellite remote sensing products, namely from the MODIS and Copernicus Sentinel missions. For example, MODIS data were used for a climatology of annual snow cover at the scale of the Pyrenees, at daily-resolution and 500m spatial resolution (Gascoin *et al.*, 2015). Further, calculated fractional snow cover retrieved from MODIS images can even give snow cover time series at sub-pixel resolution (Masson *et al.*, 2018). From 2015 onwards, high spatial resolution data from the Sentinel satellite mission (5-day temporal and 20m spatial resolution) are available, which represent a greatly improved opportunity to monitor snow cover dynamics, for example for use in freshwater ecology (Carlson *et al.*, 2020). In addition, Sentinel data could also serve to monitor ice-cover dynamics (Tom *et al.*, 2020), which presents an opportunity for monitoring recent and future changes in ice phenology and related disease risk. Both remote sensing products have the limitation that they only allow only studies limited to the last two decades at maximum (MODIS data available from the year 2000, Sentinel from 2015). Consequently, studies of

more long term past climate change impacts on snow largely rely on the use of gridded products, such as e-obs, reanalysis data and data assimilation (Largeron *et al.*, 2020; Thornton *et al.*, 2021).

Further, proxy data for informing on lacking observational data may complement this kind of studies, for example, air temperature or water temperature data may be used for estimating snow or ice cover disappearance at study sites (Clare *et al.*, 2016; Bison *et al.*, 2021). In the case of the framework discussed in this chapter, using a larger database of such in-situ water temperature data would facilitate improving the model of infection risk based on lake ice phenology and early growing season temperatures at the scale of the Pyrenees.

In summary, all currently available methods and data for monitoring climatic variables in mountains, including the monitoring of snow, have both their advantages and limitations. It follows that one must assess which data are most suitable in terms of spatiotemporal resolution and covered time period for the individual research question, and if reproducibility is important. In the context of currently available data for the Pyrenees, the e-obs dataset can still be considered a good compromise of spatiotemporal resolution and accessibility. In some mountain regions, such as the Alps, e-obs might be completed with other gridded data at higher spatial resolution, if available (Kotlarski *et al.*, 2012).

6.4 Conclusion

The presented results for climate extreme indices and simulated hydrology point at precipitation deficits and hydrological drought as potential abiotic driver variables in high *Bd* prevalence in amphibians at lake Arlet in the Western Pyrenean *Bd* hot spot. In the early spring start year 2011 at lake Arlet, additional abiotic stressors, low winter snow accumulation, and low temperatures in the following early growing season may have been deciding variables that have led to the highest observed infections during the seven-year study period.

The simulated infection risk, based on air-temperature defined spring start at the study sites, shows an increasing trend at lower-to mid-elevation sites, which can be seen as a signal of global warming. However, for higher elevation sites (EG 2 and 3, above around 2000m elevation), the changes in annual snow (Figure 15) might better explain observed mortalities of amphibians due to disease in recent decades.

Especially variables and processes related to mountain snow cover are an important research gap, and they need to be included in future research on abiotic drivers of chytridiomycosis in

mountains with snow regimes. Building on the presented study, improved models of annual infection risk for amphibians in mountains may be developed, taking into account recently made available climate reanalysis data and remote sensing data. The study of disease dynamics in amphibians, serving as sentinel species for change, can further help to formulate essential mountain biodiversity variables and thus aid in conservation efforts for mountain species threatened by global change. While there are considerable uncertainties in scenarios for future snow and temperatures (Sadinski *et al.*, 2020), hydrological drought and snow drought are likely to be more and more frequent at high elevations in global mountain areas (Huning & AghaKouchak, 2020; Hatchett, Rhoades, & McEvoy, 2021) and rising temperatures and lower annual snow are also expected in the future for the Pyrenees (OPCC_CTP, 2018). This scenario paints a dire picture for amphibians that are adapted to thermal conditions and snow in their mountain habitat.

Chapter 7: Conclusion & Perspectives

7.1 General conclusion

The aim of this thesis is a contribution to a better understanding of the globally decreasing health status of mountain freshwater ecosystems, which can be linked to ongoing species extinctions. The overall aim of this thesis is to contribute to finding essential variables, that inform on changes in freshwaters by linking climate and hydrology to emerging disease such as caused by fungal pathogens.

Climate change is causing accelerated change in mountain ecosystems and threatens survival of numerous species, among them amphibians. The manifestation of disease in amphibians, caused by the fungal pathogen *Bd*, has been related to complex interactions of biotic and abiotic variables, and recent climate change in mountains. The case of amphibian epidemics in mountain freshwater ecosystems should not be considered separable from a more global concept of ecosystem health (“One Health”, see thesis introduction) as amphibians are seen as sentinels, and thus warning system, for underlying change. Understanding drivers of wildlife disease caused by pathogens is urgent, and has to be approached interdisciplinary, as stressed in this thesis.

Here, disease expression in amphibians is approached from the top corner of the disease pyramid (Bernardo-Cravo *et al.*, 2020), the abiotic environment. Potential abiotic drivers in a mountain context were defined to be largely grouped into the categories climate, hydrology and water chemistry. For a step towards understanding current and past climatic and hydrologic stressors to amphibians at the scale of a mountain range, climate extremes and simulated hydrology variables were analysed for 25 study sites in the Pyrenees. Further, annual ice-out, extreme climate and hydrology were studied in more detail for one heavily infected amphibian community in the Western Pyrenees. The results allowed to simulate past infection risk for amphibians in the Pyrenees, across gradients of elevation and longitude. Further, the analysis led to the identification of an important research gap, which is snow hydrology and changes in snow due to climate change, which is still understudied in chytridiomycosis research.

The main climatic and hydrologic drivers that impact infection dynamics can be different between mountain ranges due to different bioclimatic contexts, different sensitivities of amphibian species to change, the simultaneous effects of other stressors and the complex

interactions between the different species of the disease pyramid (Fisher & Garner, 2020; Bernardo-Cravo *et al.*, 2020) Pollutant introduction or mobilization, land use changes such as reforestation or climate-related changes in microbiome and plankton communities are only a few examples of potential drivers of disease which can act in synergy with climate change on locally observed infection prevalence (Kiesecker *et al.*, 2001; Blaustein *et al.*, 2011; Fisher & Garner, 2020; Bernardo-Cravo *et al.*, 2020). Lastly, local abiotic and biotic variables and climate change are very likely to play an important role in observed infection dynamics, but they have to be seen in a context of pathogen introduction history (Walker *et al.*, 2010; Bates *et al.*, 2018).

While this framework for disease risk was developed for the Pyrenees, the same approach may be transferred to other mountain regions and to the future, taking projections of future climate into account.

7.2 Conclusions by Chapter

7.2.1 Main conclusions from Chapter 1

Abiotic variables in mountains & their response to global change

- *Mountain ecosystems are characterized by strong gradients in abiotic variables which are the result of the interplay of the atmosphere and the complex land surface.*
- *A main driver of change in mountain ecosystems is anthropogenic climate change, which has been leading to shifts in abiotic variables in mountain freshwater ecosystems and affected health and survival of mountain species.*

Mountain freshwater ecosystems are embedded into the complex terrain at high elevations, where steep gradients and extreme climatic and hydrologic conditions create distinct dynamics in abiotic variables. This complexity in abiotic variables creates a great variety of habitats, which are the reason for mountains being global biodiversity hot spots and habitats for specialist species (Rahbek *et al.*, 2019).

However, anthropogenic climate change has directly or indirectly led to changes in abiotic variables, which include alterations in mountain temperatures and precipitation regimes, mountain hydrology, UVR levels, altered water chemistry and pollution levels. Mountain freshwater species are especially exposed to these alterations, which is indicated by changes in species abundance, community composition, species health and species mortalities or even extinctions.

7.2.2 Main conclusions from Chapter 2

A mountain perspective on abiotic variables driving disease expression in a globally emerged fungal pathogen

- *Main abiotic variables that impact chytridiomycosis disease dynamics in mountains can be grouped into the categories climate, hydrology and water chemistry.*
- *Future research on abiotic variables in disease expression in mountain freshwaters should be including increased temporal resolution of data, take into account processes related to mountain hydrology as well as promote interdisciplinary studies.*

The interplay of biotic and abiotic variables drives chytridiomycosis disease expression in amphibians. Abiotic variables in disease expression can be categorized, based on the current literature in: Climate and hydrology variables, such as temperature, precipitation, hydrology and UVR, as well as variables related to water chemistry, such as pH, salinity and pollutant levels in water. These abiotic variables are important direct or indirect controls on host-pathogen dynamics and also affect microbiome and plankton, which all may play a role in disease expression. In mountains, distinct climatic and hydrologic processes shape amphibian exposure to stressors related to extreme climate or pollution exposure. The responses and adaptation capacity of pathogen and host to global change in mountains have the potential of changing disease dynamics, and this likely in favour of *Bd*.

The complex web of environment-species interactions calls for interdisciplinary studies in conservation of freshwater biodiversity, and this review aims at helping to bring together expertise from climatology, hydrology, disease ecology, and conservation management.

7.2.3 Main conclusions from Chapter 4

Pyrenees under global change: Changes in climate and hydrology & responses of freshwater species

- *Recent studies evidence of accelerated climate change in the Pyrenees.*
- *Main observations related to changes in Pyrenean climate and hydrology are a significant rise in annual average temperatures and reductions in annual precipitation, mostly related to reductions in annual snow cover and to a lesser extent to lower annual rainfall.*

- *A potential response of freshwater species to global change in the Pyrenees is the occurrence of chytridiomycosis disease, which has caused amphibian mortalities in the Western Pyrenees in the recent decades.*

Temperatures in the Pyrenees have been rising with 0.28 °C on average per decade since 1959, while seasonal warming is stronger, and strongest in summer, followed by spring (+0.4 °C and +0.2 °C per decade respectively) (OPCC_CTP, 2018). Annual precipitation decreases with -2.5 % per decade (OPCC_CTP, 2018), while the trend of decrease in annual snow cover and depth is significant at above 2100m elevation (López-Moreno *et al.*, 2020). Hydrologic data evidence of reduced annual runoff amounts in Pyrenean rivers, which can be related to changes in precipitation and land use within the last century (García-Ruiz *et al.*, 2011).

Freshwater species are expected to respond to these global and profound abiotic changes. The reports of fatal chytridiomycosis in Pyrenean amphibians date back to the year 2002, and since, abiotic and biotic factors have been suggested as drivers of infection (Walker *et al.*, 2010; Clare *et al.*, 2016; Bates *et al.*, 2018). Climate and hydrology are likely to impact on disease dynamics in the Pyrenees along geographic and elevation gradients, but the exact mechanisms of inter-annual disease dynamics still need to be determined.

7.2.4 Main conclusions from Chapter 5

Changes in extreme climate & hydrology in Pyrenean freshwater ecosystems

- *Climate extremes and hydrology variables for the 25 freshwater study sites show a general picture of warming and increasing meteorological and hydrological drought towards the present.*
- *Elevational and longitudinal gradients in climate extremes and hydrology are visible. Eastern sites show more pronounced extremes in heat and drought.*

The analysis of daily-resolution climate extreme indices and annual hydrologic variables from two hydrologic models for the 25 freshwater study sites confirm previous evidence of climate change trends in the Pyrenees (OPCC_CTP, 2018) from the mid-last century. The study sites show a generally increasing intensity and length in warm and dry climate extremes with a pronounced signal in the eastern and lower elevation study sites from around 1980, and a decreasing intensity of wet extremes across the entire mountain range and time period.

Increasing conditions of hydrological drought are indicated by the variables mean annual runoff and soil moisture over all study sites and the entire time period from 1960.

7.2.5 Main conclusions from Chapter 6

*Climate and hydrology impacts on *Bd* prevalence in the Pyrenees*

- The high *Bd*-prevalence year 2011 at lake Arlet was characterized by early spring start (as defined by observed lake ice-out), a short snow cover duration and low temperatures and temperature fluctuations in the low (4-5 °C, <4 °C)
- Spring start, based on air temperatures above 4 °C, simulated for all study sites in order to predict annual infection risk, shows increasing risk of infection for amphibian species at sites below 2000m (EG 1, 4 and 5) from the 1980's towards the present.
- Temperature metrics for the early growing season, after annual simulated spring start at the study sites, show no trends of warming but may be indicative of spring thermal conditions as additional stressors contributing to inter-annual infection dynamics.
- Climate extremes and hydrology variables which were significantly correlated to spring start timing at lake Arlet (2008-2014), show high *Bd*-prevalence years were characterized by lower annual precipitation and shorter wet spells.
- Annual snow accumulation and snow cover duration is retained as crucial variable in infection dynamics for future research, and in the case of the Pyrenean high-elevation *Bd* hotspots (EG 3, >2000m) climate-change impacts on snow could serve as explanation for recent increase in disease in these sites.

Observed *Bd* prevalence and ice-out data for seven years (2008-2014) at lake Arlet, in the Western Pyrenean *Bd* hotspot, allowed to study and extrapolate infection risk for amphibians at all 25 freshwater study sites, based on interannual climate and hydrology. Climate extreme indices were found to be less informative for annual infection risk, but together with hydrological variables runoff and soil moisture the results point at precipitation and water balance deficits as mechanisms which can reinforce annual disease dynamics in the Pyrenees. Snow cover duration was exceptionally low in the year of highest infections (2011), which is confirmed by recent snow reanalysis data (López-Moreno *et al.*, 2020). In addition, recent studies from other mountains show the importance of snow for amphibian health and life cycles in mountains, by its control on frost risk, start and length of the breeding period, and risk of drought (Wilkins *et al.*, 2019; Sadinski *et al.*, 2020; Bison *et al.*, 2021; Barrile *et al.*, 2021). With snow as a main driver in infection risk for amphibians in mountains, infection

risk can be expected to increase further due to current trends of decreasing annual snow, which is very likely to continue.

As main future directions, snow hydrology and seasonal temperature dynamics should be included for improved models of disease risk in mountain freshwater ecosystems, and results from such studies can inform important conservation policies and also be included in indicators for EMBV⁹s.

7.3 Perspectives

Increasingly available data of climate and hydrology offer improved opportunities for macroecological studies in freshwater ecosystem health in global mountain regions. In mountains with snow regimes, such as the Pyrenees, potential additive effects of snow and temperatures in disease dynamics should be studied.

To start with, an improved infection risk model for the Pyrenees built on the work presented in this chapter, would take into account recent dynamics in snow cover duration as already available from the MODIS satellite mission (for studies starting after 2000). The availability of Sentinel data could make additionally the observation of annual lake ice-out timing feasible (Tom *et al.*, 2020) (after 2015).

Such an improved model of annual infection risk would include seasonal temperature dynamics, more precisely winter and spring (accumulated) air temperatures to model annual lake ice-out, such as done in Caldwell *et al.*, 2021 and temperatures after ice-out.

Additionally important to include in analyses and models for future infection risk and general health of freshwater ecosystems, would be the timing of seasonally occurring extreme events. Climate and hydrologic extremes and short-term variability of temperatures can be important variables in disease dynamics and general amphibian health (Walls *et al.*, 2013; Raffel *et al.*, 2015). Extreme events in spring and summer might have different effects on immunity or development of host and pathogen development, due to the distinct species life cycles, metabolism and different responses to thermal and hydric stress. These effects could be included for example by calculation of monthly-resolution climdex and other custom-defined extreme indices of temperature and precipitation.

⁹ Essential mountain biodiversity variables, see Introduction and (Pereira *et al.*, 2013; Schmeller *et al.*, 2017)

Lastly, the combination of distributed data on climate with either catchment-scale or ecosystem-scale hydrological models (for lake-scale e.g. with the open-source model WET (Nielsen *et al.*, 2017)) should be considered for future research. The feasibility of such modelling relies on at least some successive years of in-situ data of variables to be modelled (e.g., water temperature). Such models would offer the opportunity to include other processes related to climate and hydrology dynamics, such as pathogen transport in runoff, hydrologic connectivity between sites depending on runoff levels or levels of other stressors such as pollutants or nutrients in water and their import from catchment surfaces.

While not yet accessible at the time of data analysis for this thesis, recently made available regional reanalysis data offer climate data at the spatial scale of Pyrenean mountain massifs and for 300 m elevation bands (Durand *et al.*, 2009; Vernay *et al.*, 2019). These reanalysis data were for example used to study past evolution of the Pyrenean snowpack (López-Moreno *et al.*, 2020) and they are a good candidate for future studies of ecological effects of climate change at the scale of the Pyrenees and for testing the framework presented in this chapter.

Results from future improved models for infection risk could then be used to test formulated hypotheses on snow and temperature impacts on infection risk and their longitudinal and elevational gradients. This can be achieved by correlating time series results of simulated infection risk (or simulated ice-out timing) with *Bd* occurrence data from the Pyrenean infection hotspots (here, study sites in EG 1, EG 3 and EG 4). Finally, simulated infection risk across the mountain range, and for the past, may be compared with data on historic prevalence of *Bd*, such as obtained from conserved amphibian individuals from museum collections (H. Butler, personal communication, June 2021). As such, past infection prevalence, in combination with data on past climate, may provide additional insights that can inform on current climatic drivers of infection.

7.4 Conclusions générales en français

L'objectif de cette thèse est de contribuer à une meilleure compréhension de l'état de santé globalement déclinant des écosystèmes d'eau douce de montagne, qui peut être lié aux extinctions d'espèces en cours. L'objectif général de cette thèse est de contribuer à trouver des variables essentielles, qui informent sur les changements dans les eaux douces en reliant le climat et l'hydrologie aux maladies émergentes.

Le changement climatique provoque une évolution accélérée des écosystèmes de montagne et menace la survie de nombreuses espèces, dont les amphibiens. La manifestation de la maladie chez les amphibiens, causée par le champignon pathogène *Bd*, a été mise en relation avec des interactions complexes de variables biotiques et abiotiques, et avec le récent changement climatique en montagne. Le cas des épidémies d'amphibiens dans les écosystèmes d'eau douce de montagne ne doit pas être considéré comme séparable d'un concept plus global de la santé des écosystèmes ("One Health", voir l'introduction de la thèse) car les amphibiens sont considérés comme des sentinelles, et donc un système d'alerte, pour le changement sous-jacent. Il est urgent de comprendre les moteurs des maladies de la faune sauvage causées par des agents pathogènes, et cette compréhension doit être abordée de manière interdisciplinaire, comme le souligne cette thèse.

Ici, l'expression de la maladie chez les amphibiens est abordée à partir du coin supérieur de la pyramide des maladies (Bernardo-Cravo *et al.*, 2020), l'environnement abiotique. Les facteurs abiotiques potentiels dans un contexte de montagne ont été définis comme étant largement regroupés dans les catégories climat et hydrologie et chimie de l'eau. Afin de mieux comprendre les facteurs de stress climatiques et hydrologiques actuels et passés pour les amphibiens à l'échelle d'une chaîne de montagne, les extrêmes climatiques et les variables hydrologiques simulées ont été analysés pour 25 sites d'étude dans les Pyrénées. En outre, le dégel annuel, le climat extrême et l'hydrologie ont été étudiés plus en détail pour une communauté d'amphibiens fortement infectée dans les Pyrénées occidentales. Les résultats ont permis de simuler le risque d'infection passé pour les amphibiens dans les Pyrénées, à travers les gradients d'altitude et de longitude. En outre, l'analyse a permis d'identifier une importante lacune dans la recherche, à savoir l'hydrologie de la neige et les changements dans la neige dus au changement climatique, qui sont encore peu étudiés dans la recherche sur la chytridiomycose.

Les principaux facteurs climatiques et hydrologiques qui ont un impact sur la dynamique de l'infection peuvent être différents d'une chaîne de montagnes à l'autre en raison de contextes bioclimatiques différents, de sensibilités différentes des espèces d'amphibiens au changement, des effets simultanés d'autres facteurs de stress et des interactions complexes entre les différentes espèces de la pyramide de la maladie (Fisher & Garner, 2020 ; Bernardo-Cravo *et al.*, 2020). L'introduction ou la mobilisation de polluants, les changements d'utilisation des sols tels que la reforestation ou les changements liés au climat dans le microbiome et les communautés de plancton ne sont que quelques exemples de facteurs potentiels de maladie qui peuvent agir en synergie avec le changement climatique sur la prévalence des infections observées localement (Kiesecker *et al.*, 2001 ; Blaustein *et al.*, 2011 ; Fisher & Garner, 2020 ; Bernardo-Cravo *et al.*, 2020). Enfin, il est très probable que les variables abiotiques et biotiques locales et le changement climatique jouent un rôle important dans la dynamique d'infection observée, mais ils doivent être considérés dans le contexte de l'historique d'introduction des agents pathogènes (Walker *et al.*, 2010 ; Bates *et al.*, 2018).

Bien que ce cadre pour le risque de maladie ait été développé pour les Pyrénées, la même approche peut être transférée à d'autres régions de montagne et à l'avenir, en tenant compte des projections du climat futur.

7.5 Conclusions par chapitre en français

7.5.1 Principales conclusions du chapitre 1

Variables abiotiques en montagne et leur réponse au changement global

- Les écosystèmes de montagne sont caractérisés par de forts gradients de variables abiotiques qui sont le résultat de l'interaction entre l'atmosphère et la surface terrestre complexe.
- L'un des principaux facteurs de changement dans les écosystèmes de montagne est le changement climatique anthropique, qui a entraîné des modifications des variables abiotiques dans les écosystèmes d'eau douce de montagne et a affecté la santé et la survie des espèces de montagne.

Les écosystèmes d'eau douce de montagne sont intégrés dans le terrain complexe à haute altitude, où les gradients abrupts et les conditions climatiques et hydrologiques extrêmes créent des dynamiques distinctes dans les variables abiotiques. Cette complexité des variables abiotiques crée une grande variété d'habitats, qui sont la raison pour laquelle les montagnes

sont des points chauds de la biodiversité mondiale et des habitats pour les espèces spécialisées (Rahbek *et al.*, 2019).

Cependant, le changement climatique anthropique a entraîné directement ou indirectement des changements dans les variables abiotiques, qui comprennent des modifications des températures et des régimes de précipitations des montagnes, de l'hydrologie des montagnes, des niveaux d'UVR, de la chimie altérée de l'eau et des niveaux de pollution. Les espèces d'eau douce de montagne sont particulièrement exposées à ces altérations, ce qui se traduit par des changements dans l'abondance des espèces, la composition des communautés, la santé des espèces et leur mortalité, voire leur disparition.

7.5.2 Principales conclusions du chapitre 2

Une perspective montagnarde sur les variables abiotiques qui déterminent l'expression de la maladie chez un pathogène fongique émergent à l'échelle mondiale

- Les principales variables abiotiques qui ont un impact sur la dynamique de la maladie de la chytridiomycose en montagne peuvent être regroupées dans les catégories suivantes : climat, hydrologie et chimie de l'eau.
- Les recherches futures sur les variables abiotiques dans l'expression de la maladie dans les eaux douces de montagne devraient inclure une résolution temporelle accrue des données, prendre en compte les processus liés à l'hydrologie de montagne ainsi que promouvoir les études interdisciplinaires.

L'interaction des variables biotiques et abiotiques détermine l'expression de la maladie de la chytridiomycose chez les amphibiens. Les variables abiotiques dans l'expression de la maladie peuvent être catégorisées, sur la base de la littérature actuelle en : Les variables climatiques et hydrologiques, comme la température, les précipitations, l'hydrologie et les UVR, ainsi que les variables liées à la chimie de l'eau, comme le pH, la salinité et les niveaux de polluants dans l'eau. Ces variables abiotiques sont des contrôles directs ou indirects importants de la dynamique hôte-pathogène et affectent également le microbiome et le plancton, qui peuvent tous jouer un rôle dans l'expression de la maladie. En montagne, des processus climatiques et hydrologiques distincts façonnent l'exposition des amphibiens aux facteurs de stress liés au climat extrême ou à l'exposition à la pollution. Les réponses et la capacité d'adaptation de l'agent pathogène et de l'hôte au changement global en montagne ont le potentiel de modifier la dynamique de la maladie, et ce probablement en faveur de *Bd*.

Le réseau complexe d'interactions entre l'environnement et les espèces nécessite des études interdisciplinaires pour la conservation de la biodiversité des eaux douces, et cette revue a pour but d'aider à rassembler l'expertise de la climatologie, de l'hydrologie, de l'écologie des maladies et de la gestion de la conservation.

7.5.3 Principales conclusions du chapitre 4

Les Pyrénées sous le changement global : Changements dans le climat et l'hydrologie et réponses des espèces d'eau douce

- Des études récentes mettent en évidence une accélération du changement climatique dans les Pyrénées.
- Les principales observations liées aux changements du climat et de l'hydrologie des Pyrénées sont une augmentation significative des températures moyennes annuelles et des réductions des précipitations annuelles, principalement liées à des réductions de la couverture neigeuse annuelle et, dans une moindre mesure, à des précipitations annuelles plus faibles.
- Une réponse potentielle des espèces d'eau douce au changement global dans les Pyrénées est l'apparition de la maladie de la chytridiomycose, qui a provoqué des mortalités d'amphibiens dans les Pyrénées occidentales au cours des dernières décennies.

Les températures dans les Pyrénées augmentent avec 0,28 °C en moyenne par décennie depuis 1959, tandis que le réchauffement saisonnier est plus fort, et le plus fort en été, suivi du printemps (+0,4 °C et +0,2 °C par décennie respectivement) (OPCC_CTP, 2018). Les précipitations annuelles diminuent de 2,5 % par décennie (OPCC_CTP, 2018), tandis que la tendance à la diminution de la couverture et de l'épaisseur de la neige annuelle est significative au-dessus de 2100 m d'altitude (López-Moreno *et al.*, 2020). Les données hydrologiques mettent en évidence une réduction des quantités de ruissellement annuel dans les rivières pyrénéennes, qui peut être liée à des changements dans les précipitations et l'utilisation des terres au cours du siècle dernier (García-Ruiz *et al.*, 2011).

On s'attend à ce que les espèces d'eau douce réagissent à ces changements abiotiques mondiaux et profonds. Les rapports de chytridiomycose mortelle chez les amphibiens pyrénéens remontent à l'année 2002, et depuis, des facteurs abiotiques et biotiques ont été suggérés comme moteurs de l'infection (Walker *et al.*, 2010 ; Clare *et al.*, 2016 ; Bates *et al.*, 2018). Le climat et l'hydrologie ont probablement un impact sur la dynamique de la maladie

dans les Pyrénées le long des gradients géographiques et d'altitude, mais les mécanismes exacts de la dynamique interannuelle de la maladie doivent encore être déterminés.

7.5.4 Principales conclusions du chapitre 5

Changements dans le climat et l'hydrologie extrêmes dans les écosystèmes d'eau douce pyrénéens

- Les extrêmes climatiques et les variables hydrologiques pour les 25 sites d'eau douce étudiés montrent une image générale de réchauffement et de sécheresse météorologique et hydrologique croissante vers le présent.
- Des gradients d'altitude et longitudinaux dans les extrêmes climatiques et l'hydrologie sont visibles. Les sites de l'Est montrent des extrêmes plus prononcés en matière de chaleur et de sécheresse.

L'analyse des indices climatiques extrêmes à résolution quotidienne et des variables hydrologiques annuelles provenant de deux modèles hydrologiques pour les 25 sites d'étude d'eau douce confirme les preuves précédentes des tendances du changement climatique dans les Pyrénées (OPCC_CTP, 2018) depuis le milieu du siècle dernier. Les sites d'étude montrent une augmentation générale de l'intensité de la durée des extrêmes climatiques chauds et secs, avec un signal prononcé dans les sites d'étude orientaux et de plus faible altitude à partir d'environ 1980, et une diminution de l'intensité des extrêmes humides dans l'ensemble de la chaîne de montagnes et de la période. Des conditions croissantes de sécheresse hydrologique, comme l'indiquent les variables hydrologiques simulées, le ruissellement annuel et la neige sur tous les sites d'étude et sur toute la période à partir de 1960.

7.5.5 Principales conclusions du chapitre 6

Impacts du climat et de l'hydrologie sur la prévalence de Bd dans les Pyrénées

- L'année 2011, année de forte prévalence de *Bd* au lac d'Arlet, a été caractérisée par une fonte des glaces de lac précoce, une courte durée de la couverture neigeuse et des températures basses et des fluctuations de température dans le bas (4-5 °C, < 4 °C)
- Le début du printemps, basé sur des températures de l'air supérieures à 4 °C, simulé pour tous les sites d'étude afin de prédire le risque d'infection annuel, montre un risque d'infection croissant pour les espèces d'amphibiens sur les sites situés en dessous de 2000m (EG 1, 4 et 5) depuis les années 1980 jusqu'à aujourd'hui.

Chapter 7: Conclusion & Perspectives

- Les mesures de température pour le début de la saison de croissance, après la simulation annuelle du début du printemps sur les sites d'étude, ne montrent aucun signal de réchauffement global mais peuvent être indicatives des conditions thermiques printanières en tant que facteurs de stress supplémentaires contribuant à la dynamique interannuelle des infections.
- Les extrêmes climatiques et les variables hydrologiques qui ont été significativement corrélés au moment du début du printemps au lac d'Arlet (2008-2014), montrent que les années à forte prévalence de *Bd* sont caractérisées par des précipitations annuelles plus faibles et des périodes humides plus courtes.
- L'accumulation annuelle de neige et la durée de la couverture neigeuse sont retenues comme des variables cruciales dans la dynamique de l'infection pour les recherches futures, et dans le cas des points chauds de *Bd* à haute altitude des Pyrénées (EG 3, >2000m), les impacts du changement climatique sur la neige pourraient servir d'explication à la récente augmentation de la maladie dans ces sites.

L'observation de la prévalence de *Bd* et des données de fonte des glaces pendant sept ans (2008-2014) au lac d'Arlet, dans le hotspot de *Bd* des Pyrénées occidentales, a permis d'étudier et d'extrapoler le risque d'infection pour les amphibiens dans les 25 sites d'eau douce étudiés, en fonction du climat et de l'hydrologie interannuels. Les indices climatiques extrêmes se sont avérés moins informatifs pour le risque d'infection annuel, mais avec les variables hydrologiques de ruissellement et d'humidité du sol, les résultats indiquent que les précipitations et les déficits du bilan hydrique sont des mécanismes qui peuvent renforcer la dynamique annuelle de la maladie dans les Pyrénées. La durée de l'enneigement était exceptionnellement faible l'année des plus fortes infections (2011) (López-Moreno *et al.*, 2020). De plus, des études récentes menées dans d'autres montagnes montrent l'importance de la neige pour la santé et les cycles de vie des amphibiens en montagne, par son contrôle sur le risque de gel, le début et la durée de la période de reproduction, et le risque de sécheresse (Wilkins *et al.*, 2019 ; Sadinski, Gallant, & Cleaver, 2020 ; Bison *et al.*, 2021 ; Barrile, Chalfoun, & Walters, 2021). La neige étant le principal facteur de risque d'infection pour les amphibiens en montagne, on peut s'attendre à ce que le risque d'infection augmente encore en raison des tendances actuelles de diminution de la neige annuelle, qui vont très probablement se poursuivre.

Comme principales orientations futures, l'hydrologie de la neige et la dynamique des températures saisonnières devraient être incluses pour améliorer les modèles de risque de maladie dans les écosystèmes d'eau douce de montagne, et les résultats de ces études peuvent informer les politiques de conservation importantes et également être inclus dans les indicateurs pour les EMBVs.

7.6 Perspectives en français

Les données de plus en plus disponibles sur le climat et l'hydrologie offrent de meilleures possibilités d'études macroécologiques sur la santé des écosystèmes d'eau douce dans les régions montagneuses du monde. Dans les montagnes au régime neigeux, comme les Pyrénées, les effets additifs potentiels de la neige et des températures sur la dynamique des maladies doivent être étudiés.

Pour commencer, un modèle amélioré du risque d'infection pour les Pyrénées, basé sur le travail présenté dans ce chapitre, prendrait en compte la dynamique récente de la durée de la couverture neigeuse, déjà disponible grâce à la mission satellitaire MODIS (pour les études commençant après 2000). La disponibilité des données Sentinel pourrait rendre possible l'observation de la période annuelle de dégel des lacs (Tom *et al.*, 2020) (après 2015).

Un tel modèle amélioré du risque d'infection annuel inclurait la dynamique des températures saisonnières, plus précisément les températures de l'air (accumulées) en hiver et au printemps pour modéliser le dégel annuel des lacs, comme l'ont fait Caldwell *et al.* en 2021, et les températures après le dégel.

Il serait également important d'inclure dans les analyses et les modèles relatifs aux risques d'infection futurs et à la santé générale des écosystèmes d'eau douce, le moment où se produisent les événements extrêmes saisonniers. Les extrêmes climatiques et hydrologiques et la variabilité à court terme des températures peuvent être des variables importantes dans la dynamique des maladies et la santé générale des amphibiens (Walls *et al.*, 2013 ; Raffel *et al.*, 2015). Les événements extrêmes au printemps et en été pourraient avoir des effets différents sur l'immunité ou le développement de l'hôte et du pathogène, en raison des cycles de vie distincts des espèces, du métabolisme et des différentes réponses au stress thermique et hydrique. Ces effets pourraient être inclus, par exemple, par le calcul de climdex à résolution mensuelle et d'autres indices extrêmes de température et de précipitations définis sur mesure.

Enfin, la combinaison de données distribuées sur le climat avec des modèles hydrologiques à l'échelle du bassin versant ou de l'écosystème (pour l'échelle du lac, par exemple avec le

modèle open-source WET (Nielsen *et al.*, 2017)) devrait être envisagée pour les recherches futures. La faisabilité d'une telle modélisation repose sur au moins quelques années successives de données in-situ des variables à modéliser (par exemple, la température de l'eau). De tels modèles offriraient la possibilité d'inclure d'autres processus liés à la dynamique du climat et de l'hydrologie, tels que le transport d'agents pathogènes dans le ruissellement, la connectivité hydrologique entre les sites en fonction des niveaux de ruissellement ou des niveaux d'autres facteurs de stress tels que les polluants ou les nutriments dans l'eau et leur importation depuis les surfaces du bassin versant.

Bien que non encore accessibles au moment de l'analyse des données pour cette thèse, les données de réanalyse régionales récemment mises à disposition offrent des données climatiques à l'échelle spatiale des massifs montagneux pyrénéens et pour des bandes d'altitude de 300 m (Durand *et al.*, 2009 ; Vernay *et al.*, 2019). Ces données de réanalyse ont par exemple été utilisées pour étudier l'évolution passée du manteau neigeux pyrénéen (López-Moreno *et al.*, 2020) et elles constituent un bon candidat pour de futures études des effets écologiques du changement climatique à l'échelle des Pyrénées et pour tester le cadre présenté dans ce chapitre.

Les résultats des futurs modèles améliorés du risque d'infection pourraient alors être utilisés pour tester les hypothèses formulées sur les impacts de la neige et de la température sur le risque d'infection et leurs gradients longitudinaux et altimétriques. Cela peut être réalisé en corrélant les résultats des séries chronologiques du risque d'infection simulé (ou le moment simulé de la fonte des glaces) avec les données d'occurrence de la maladie de *Bd* dans les points chauds d'infection des Pyrénées (ici, les sites d'étude de EG 1, EG 3 et EG 4). Enfin, le risque d'infection simulé à travers la chaîne de montagnes, et pour le passé, peut être comparé aux données sur la prévalence historique de *Bd*, telles que celles obtenues à partir d'individus amphibiens conservés dans les collections des musées (H. Butler, communication personnelle, juin 2021). En tant que telle, la prévalence de l'infection dans le passé, en combinaison avec des données sur le climat passé, peut fournir des informations supplémentaires qui peuvent informer sur les moteurs climatiques actuels de l'infection.

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Supplementary Figures

Chapter 4

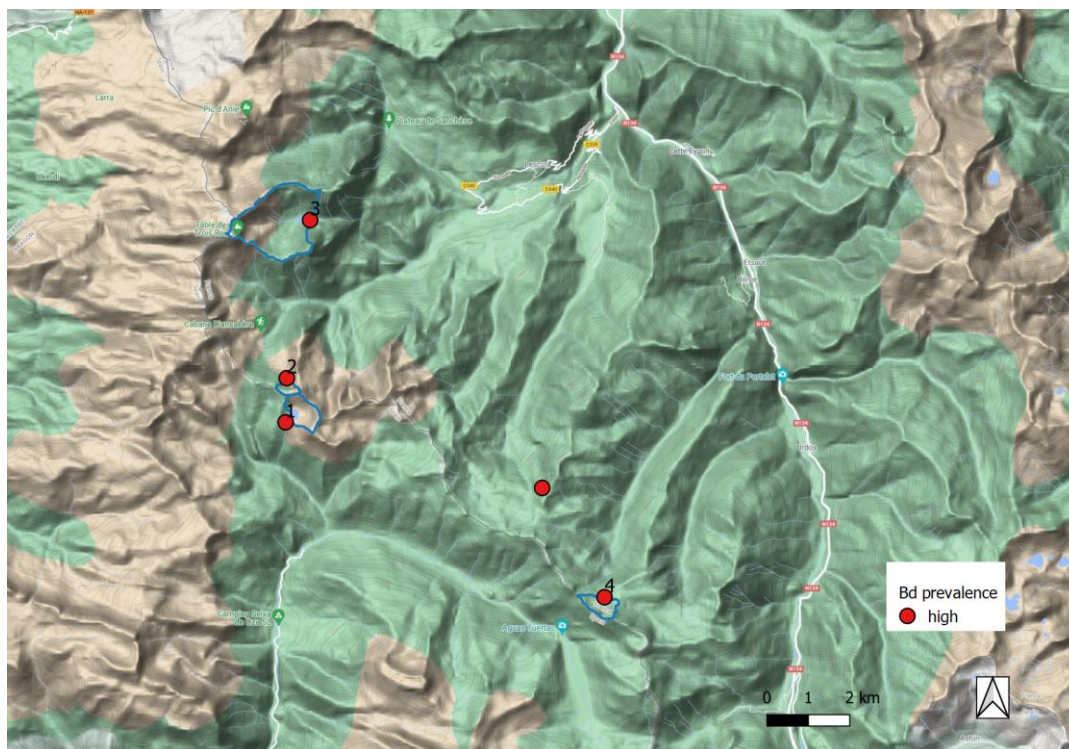


Figure 41: Map of EG 1, sites 1 -4. Red points: High *Bd* prevalence; Blue polygons: catchments of study sites. (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid)

Supplementary Figures

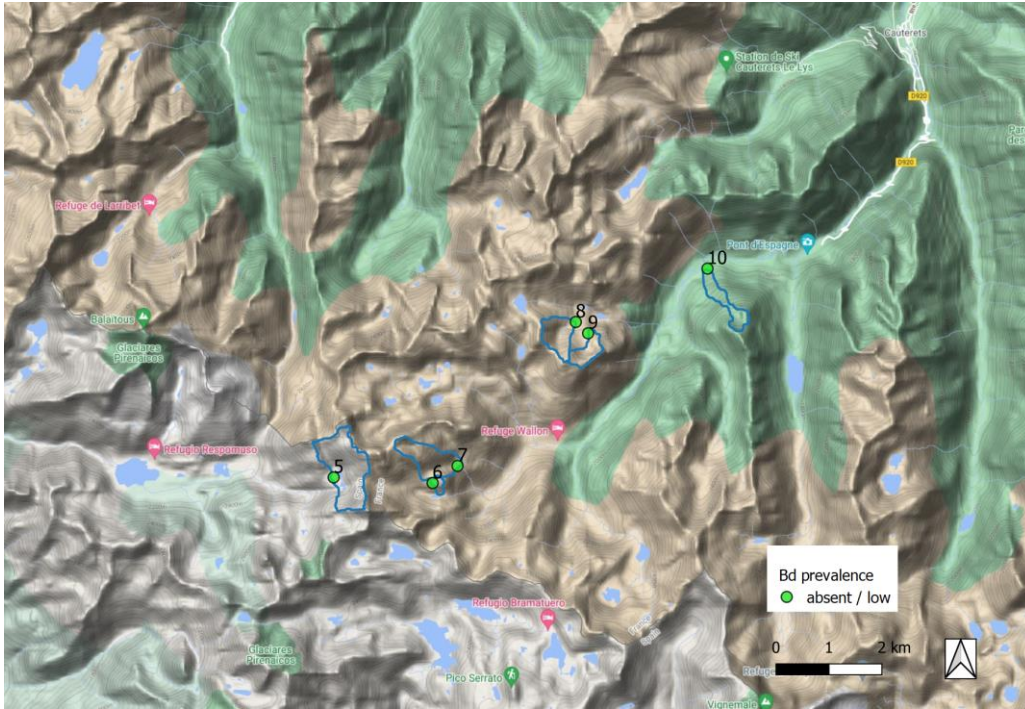


Figure 42: Map of EG 2, study sites 5 -10. Green points: Low *Bd* prevalence or absence. Blue polygons: catchments of study sites. (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid)



Figure 43: Map of EG 3, sites 11-15. Red points: High *Bd* prevalence; Green points: Low *Bd* prevalence or absence. Blue polygons: catchments of study sites. (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid)

Supplementary Figures

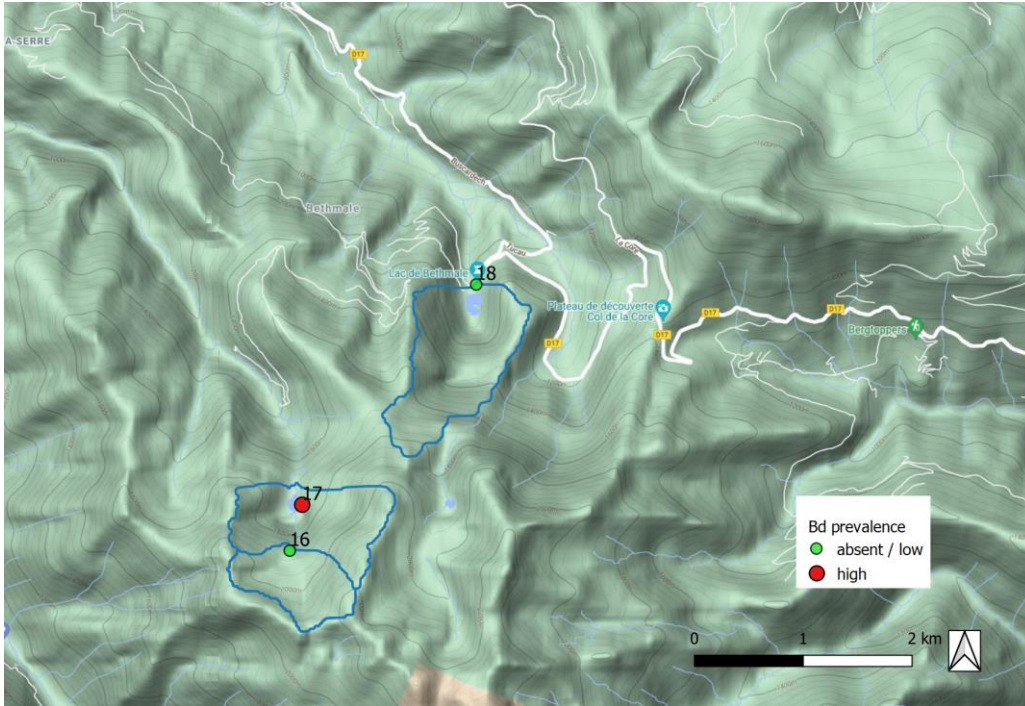


Figure 44: Map of EG 4, sites 16-18. Red points: High *Bd* prevalence; Green points: Low *Bd* prevalence or absence. Blue polygons: catchments of study sites (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid)

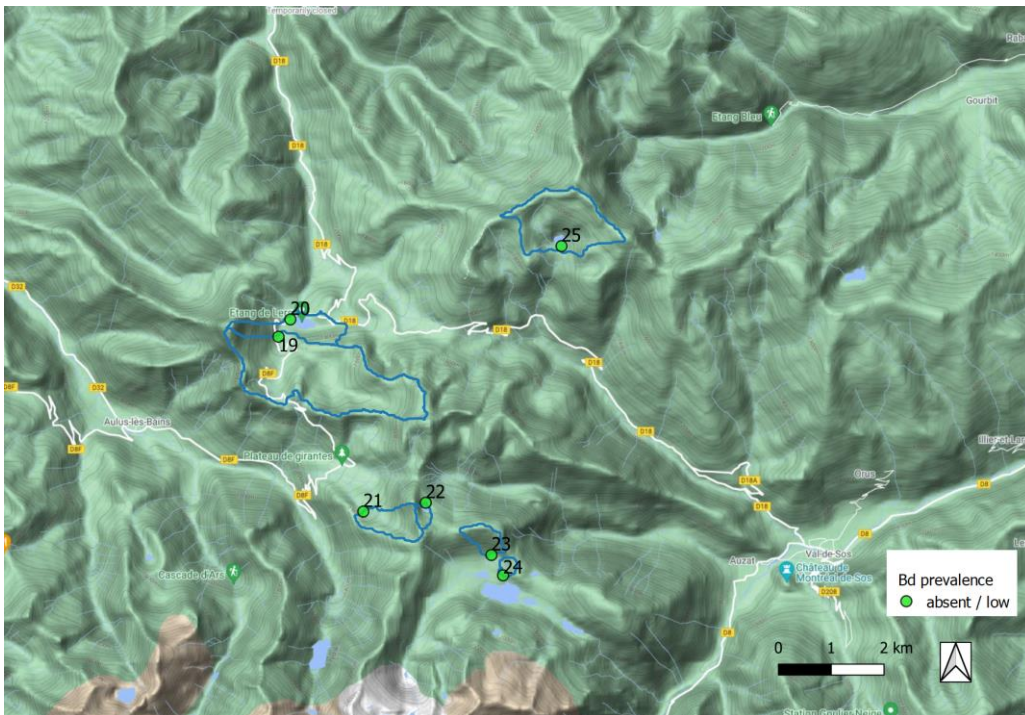


Figure 45: Map of EG 5, sites 19-25. Green points: Low *Bd* prevalence or absence. Blue polygons: catchments of study sites. (Basemap: OMS / WebMapService (WMS) Google Terrain Hybrid).

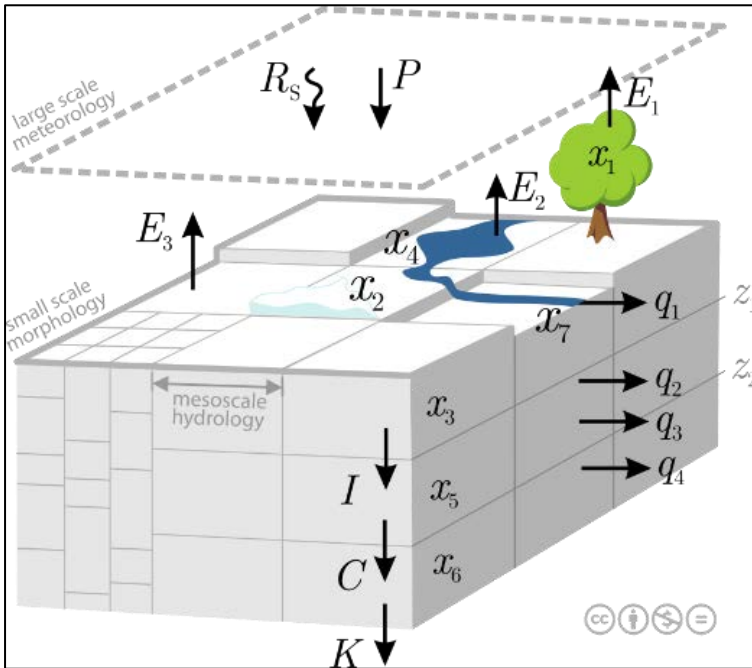


Figure 46: mhm model schematic illustration (letters/arrows represent processes/fluxes or input/output data, cubes or rectangles represent resolution levels), for more detailed information see: Samaniego et al., 2010.

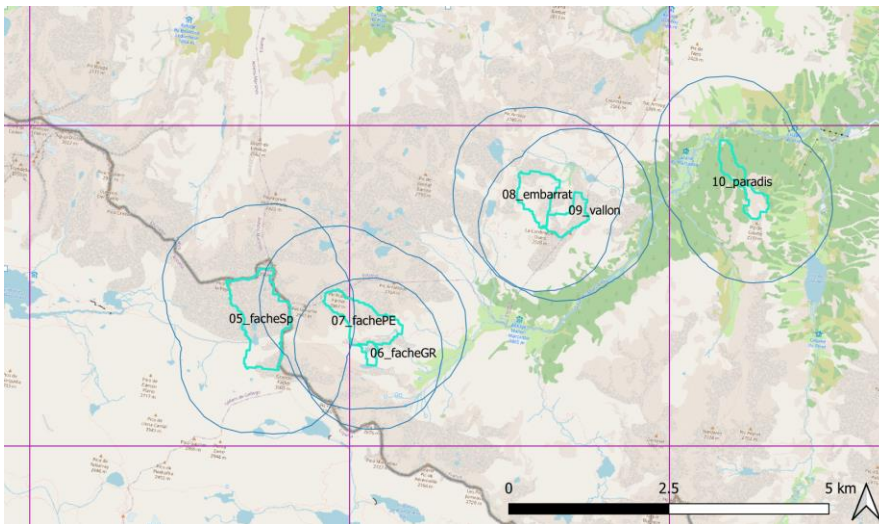


Figure 47: Example of catchments (bright blue polygons) and 1km buffer (dark blue polygons) for data extraction from gridded data (here: 5km grid for hydrology variables). Basemap: WMS OSM standard map service.

Chapter 5

Additional Figures: Climate extreme indices – Temperature

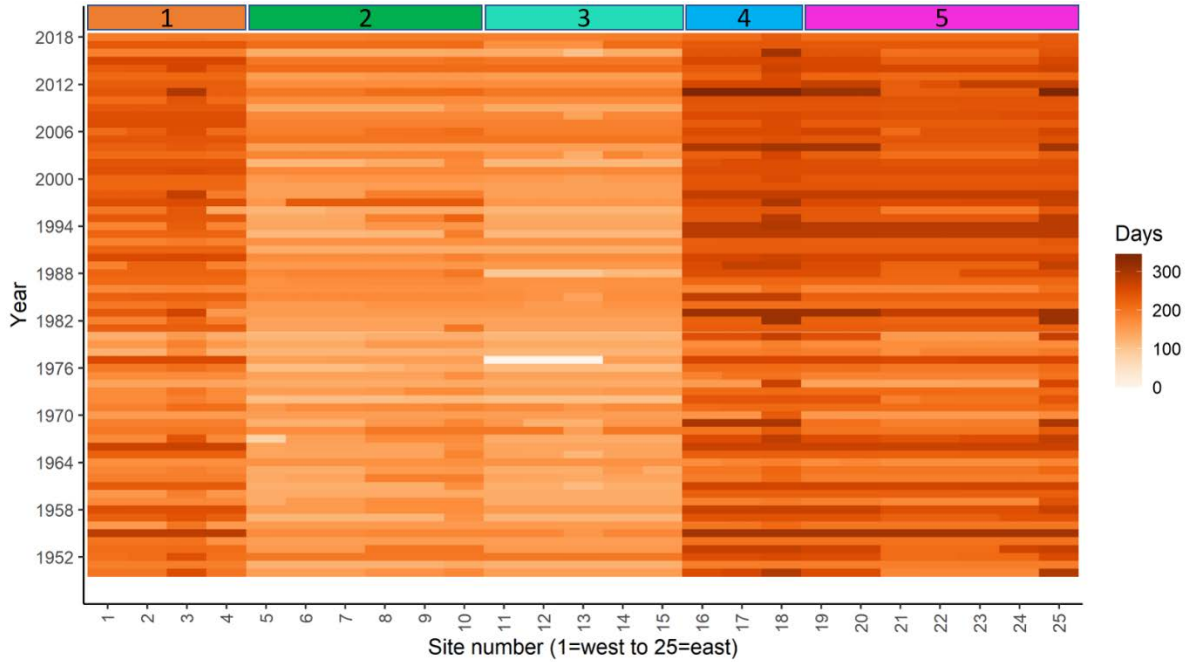
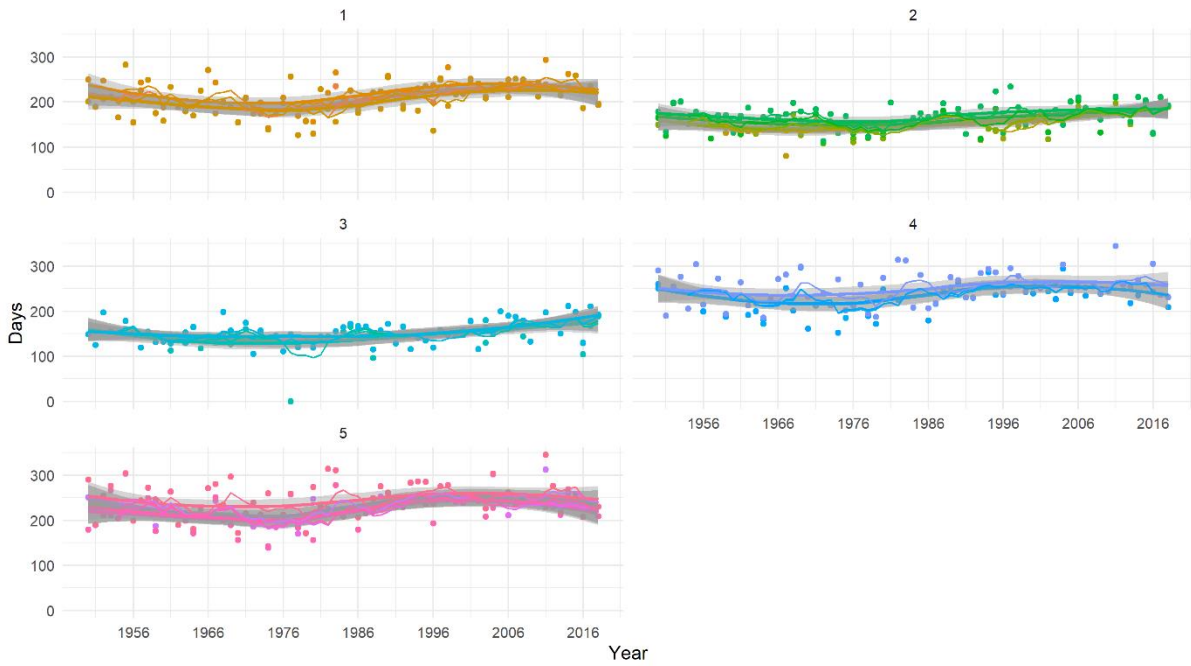


Figure 48: Annual length of growing season length (GSL), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.



Supplementary Figures

Figure 49: Trends in annual growing season length (GSL), in days, for the years 1960-2014, all sites separated by gradients 1 – 5 (5 panels), plotted together with the 5-year running average (thin lines) and the temporal trend with 95% confidence interval (grey band).

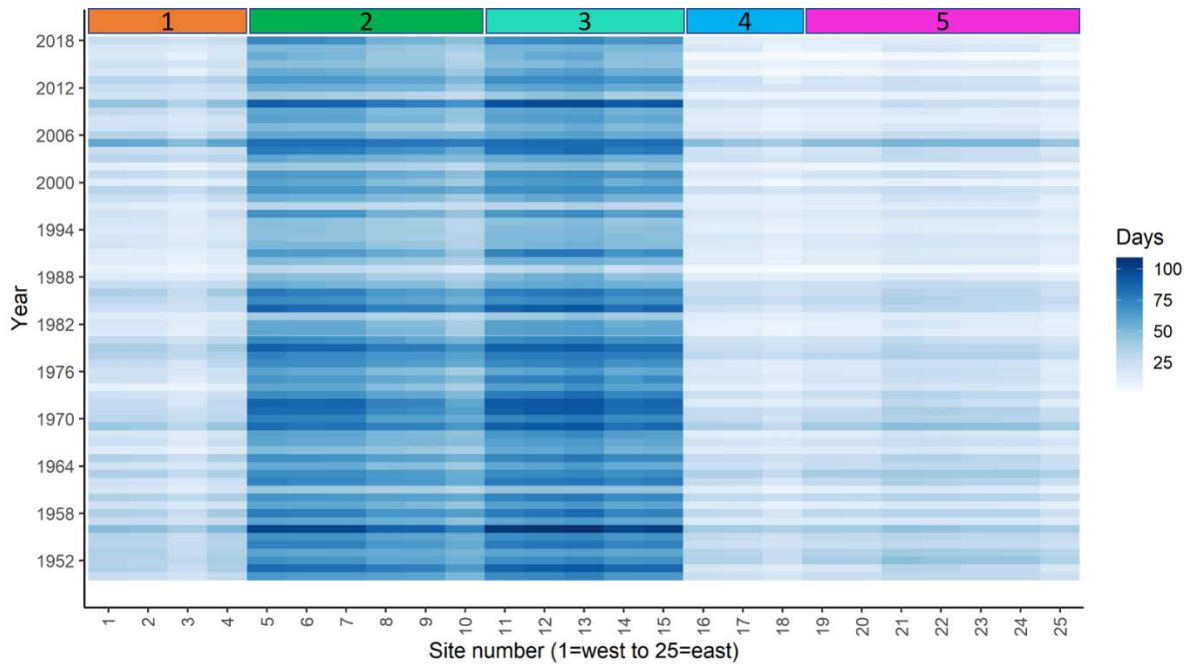
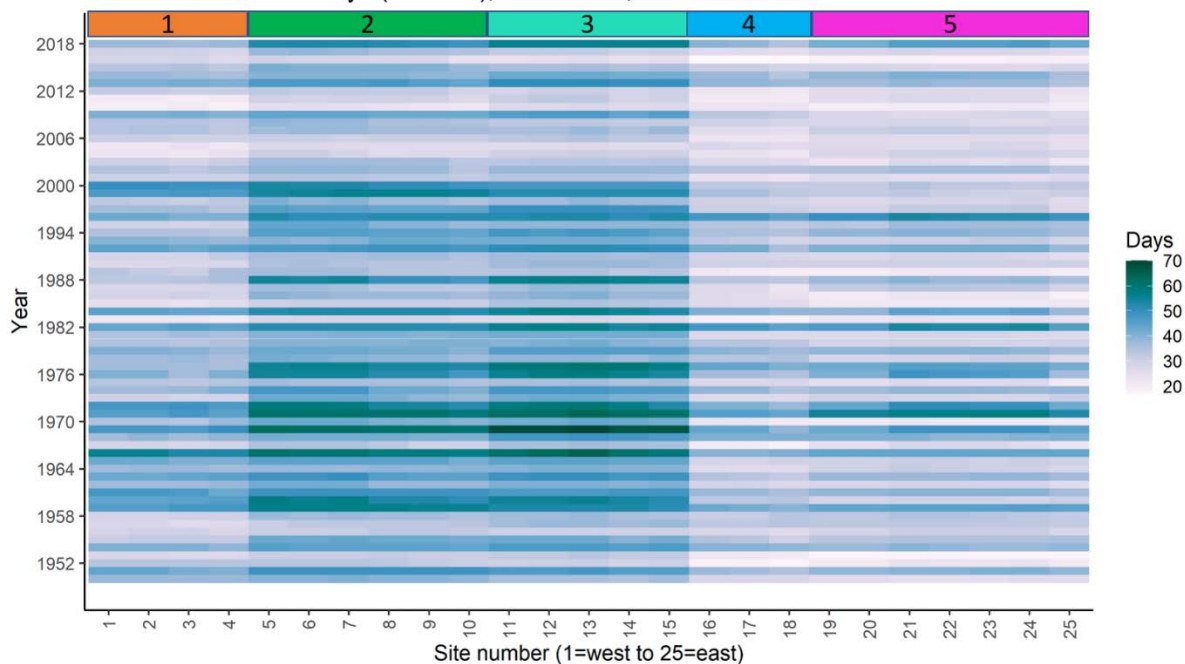


Figure 50: Annual count of icing days (ID), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

Additional Figures: Climate extreme indices – Precipitation



Supplementary Figures

Figure 51: Annual count of wet days (R10mm), in days, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

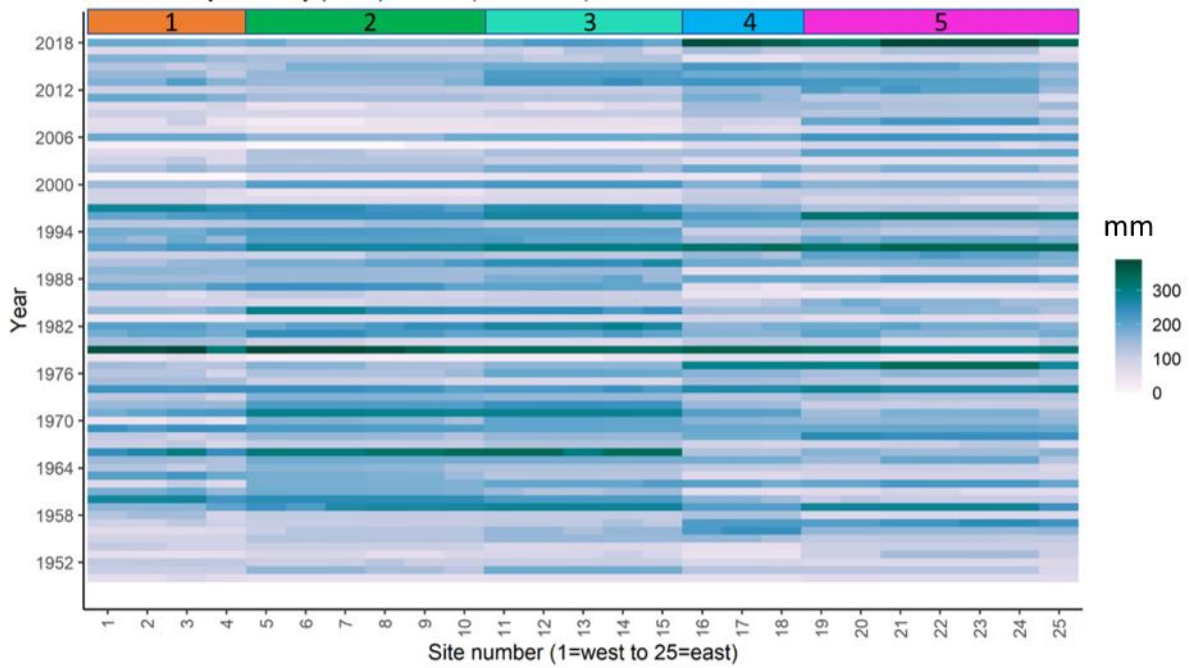
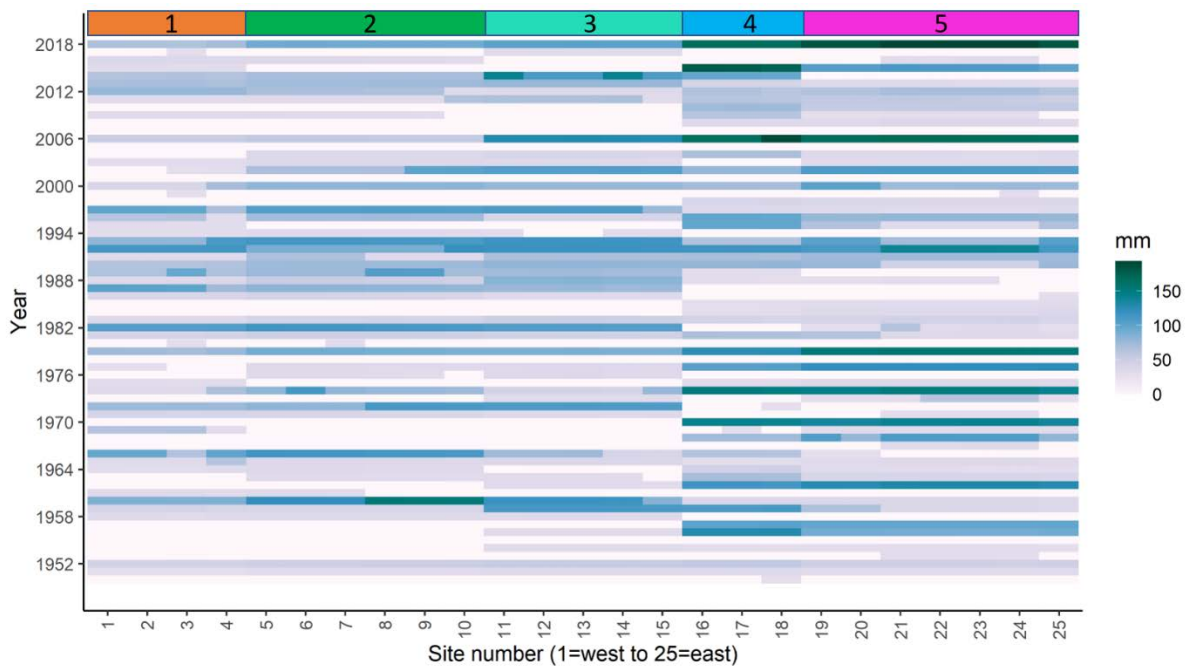


Figure 52: Annual very wet day precipitation (R95ptotal), in mm, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.



Supplementary Figures

Figure 53: Annual extremely wet day precipitation (R99ptotal), in mm, for the years 1950-2018, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

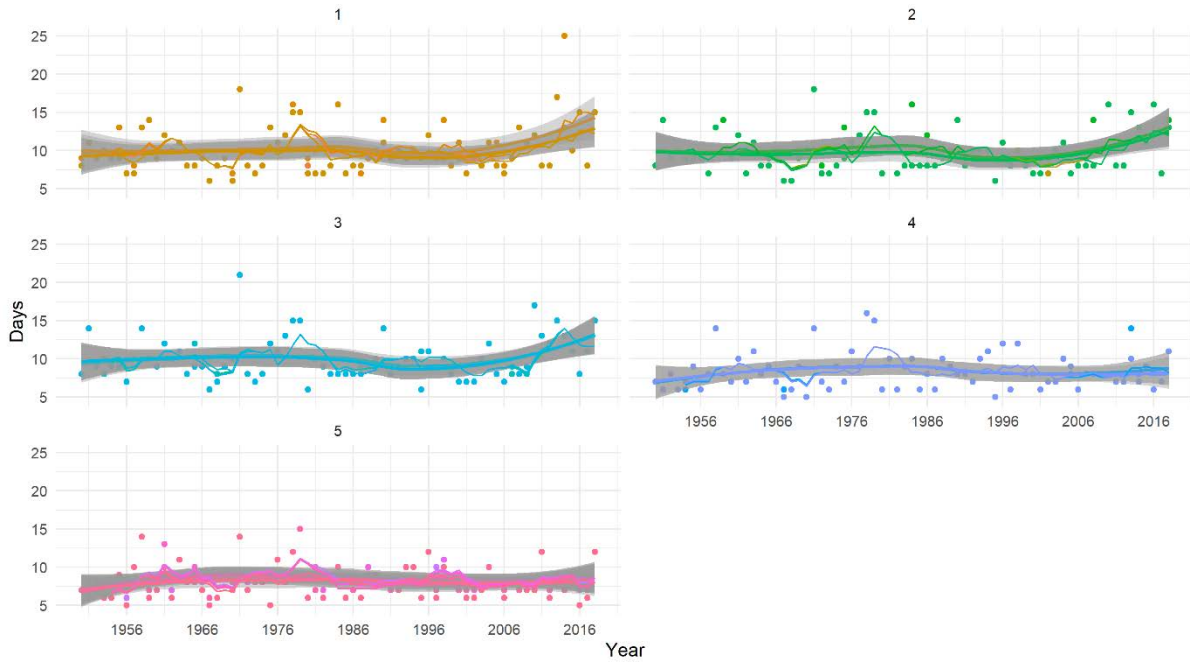


Figure 54: Trends in annual maximum wet spell duration (CWD), in days, for the years 1960-2014, all sites separated by gradients 1 – 5 (5 panels), plotted together with the 5-year running average (thin lines) and the temporal trend with 95% confidence interval (grey band).

Supplementary Figures

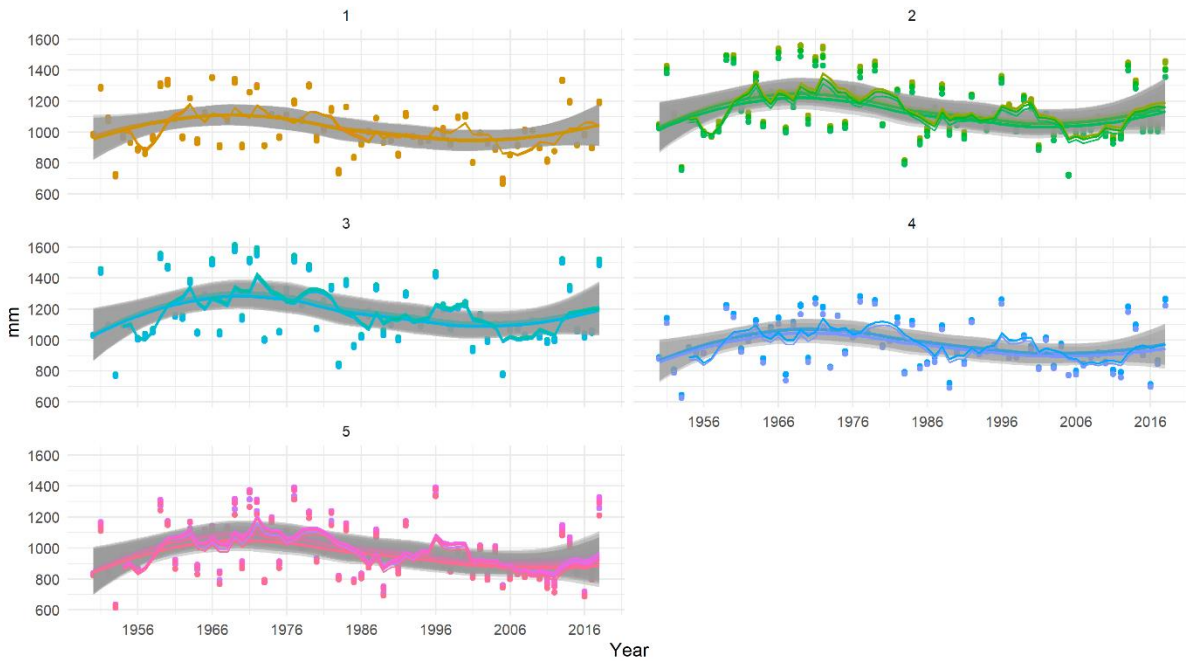


Figure 55: Trends in annual total precipitation on wet days (PRCPTOT), in mm, for the years 1960-2014, all sites separated by gradients 1 – 5 (5 panels), plotted together with the 5-year running average (thin lines) and the temporal trend with 95% confidence interval (grey band).

Additional Figures: Hydrology

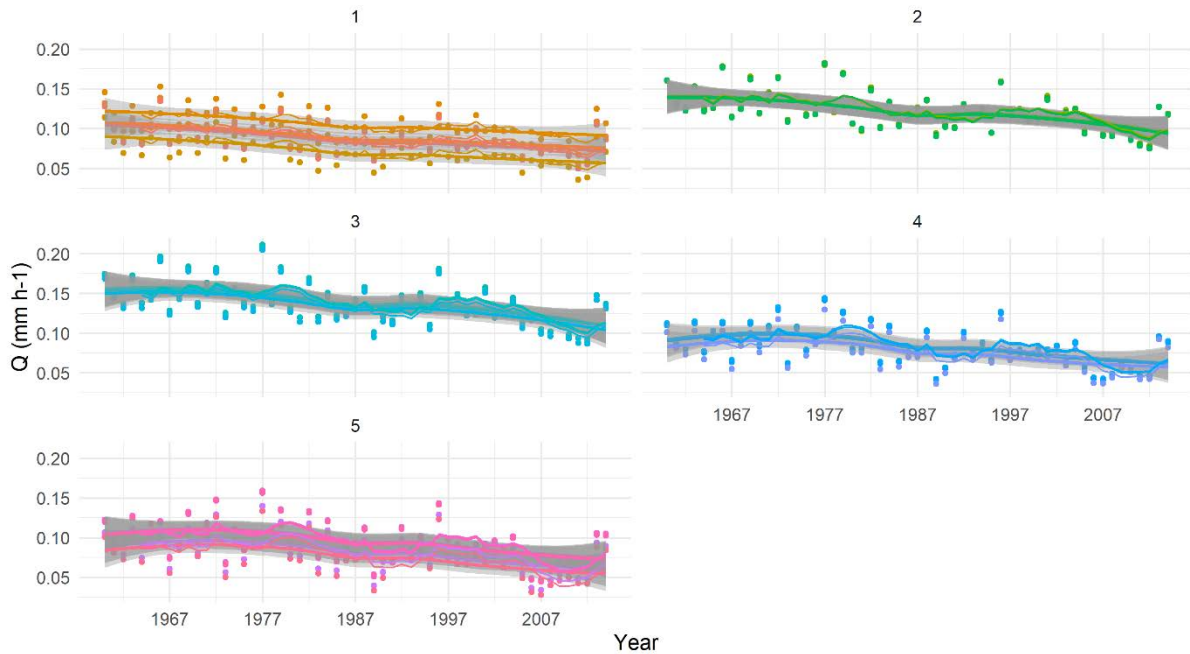


Figure 56: Trends in annual runoff (Q noahmp model), in mm per hour, for the years 1960-2014, all sites separated by gradients 1 – 5 (5 panels), plotted together with the 5-year running average (thin lines) and the temporal trend with 95% confidence interval (grey band).

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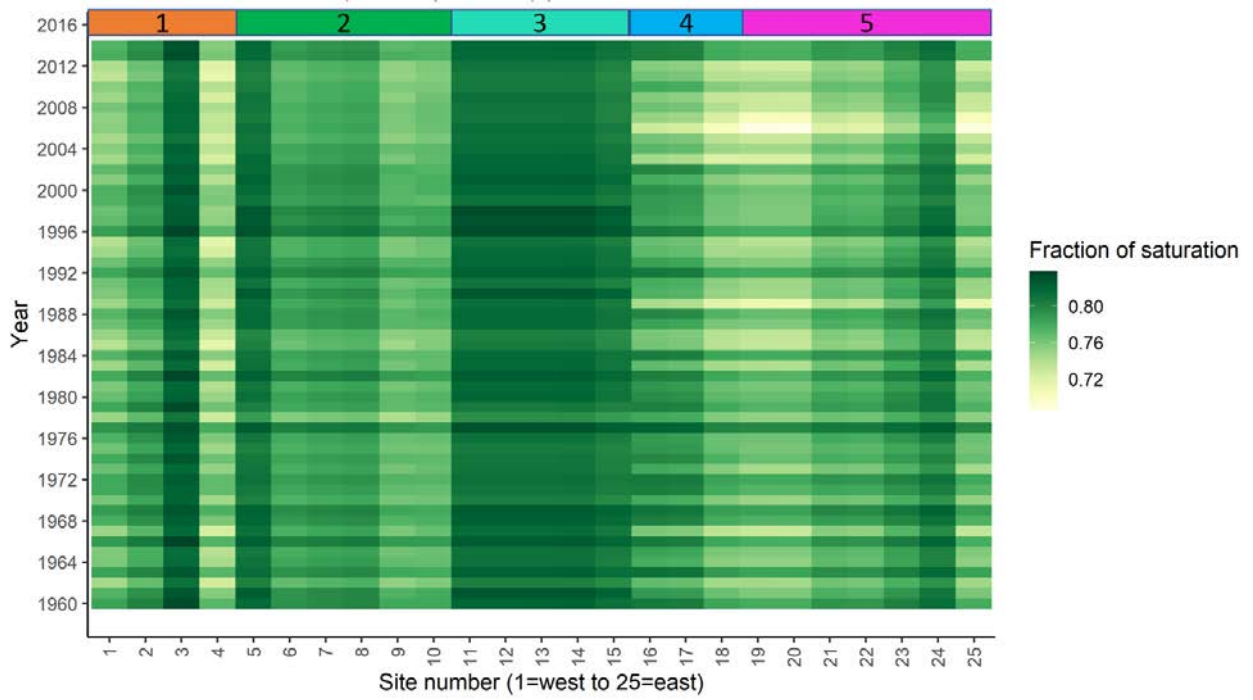


Figure 57: Annual soil moisture (noahmp model) for the years 1960-2014, in fraction of saturation, per site 1-25. Elevation gradient (EG) number marked in colours above the time series.

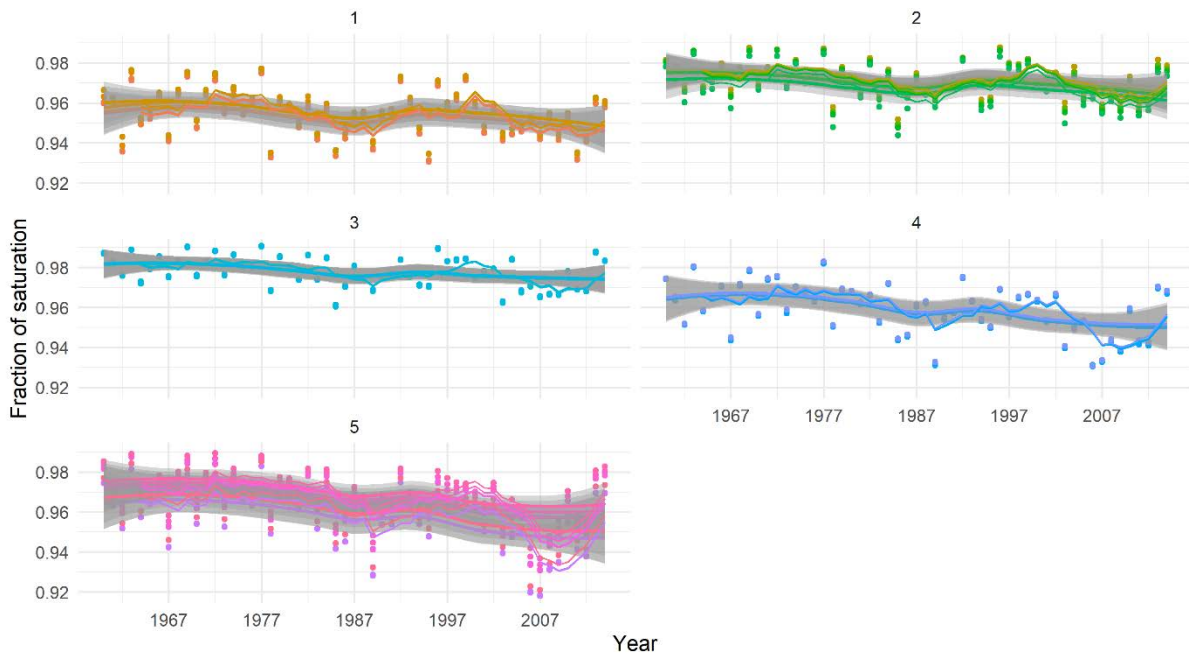


Figure 58: Trends in annual soil moisture levels (mhm model), in fraction of saturation, for the years 1960-2014, all sites separated by gradients 1 – 5 (5 panels), plotted together with the 5-

Supplementary Figures

year running average (thin lines) and the temporal trend with 95% confidence interval (grey band).

Supplementary Figures

Chapter 6

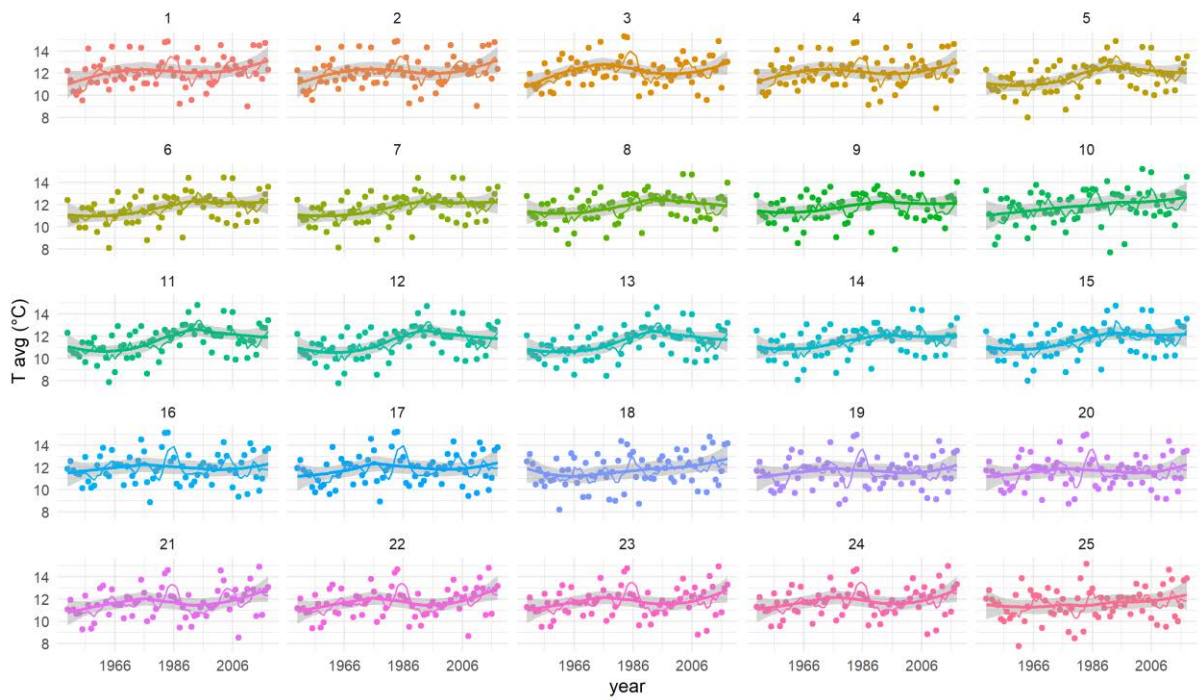
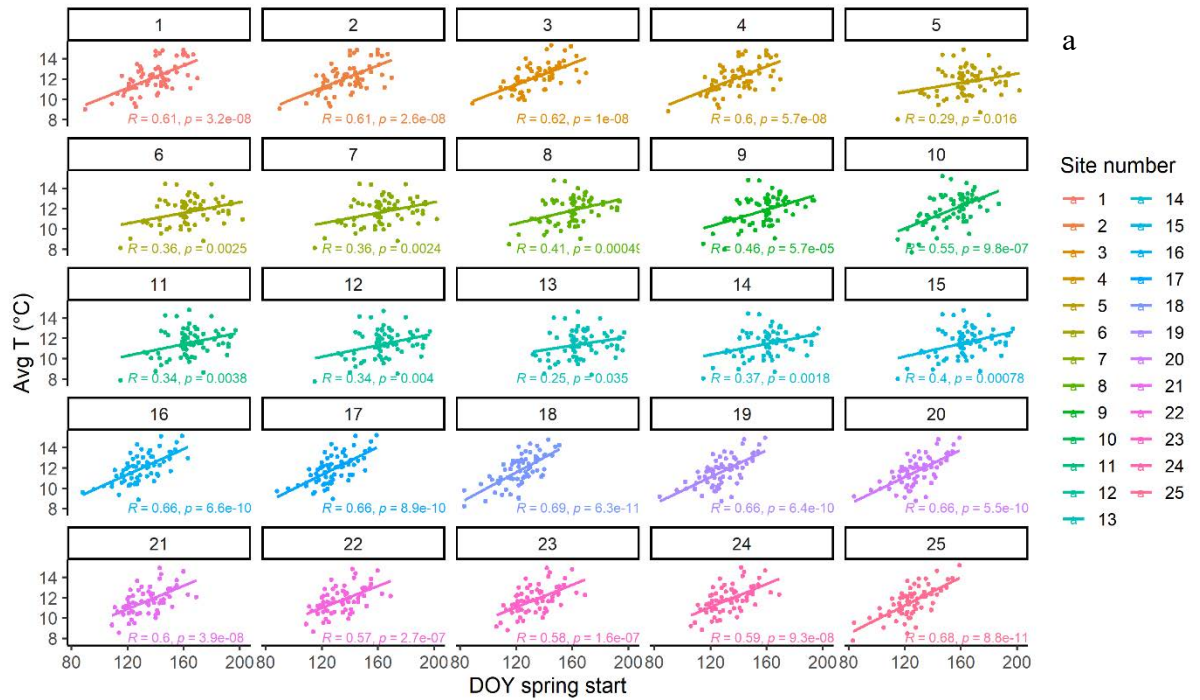


Figure 59: Trends in average temperatures (°C) after annual spring start (DOY simulated spring start +60 days) for all sites and the years 1950-2018, per site



Supplementary Figures

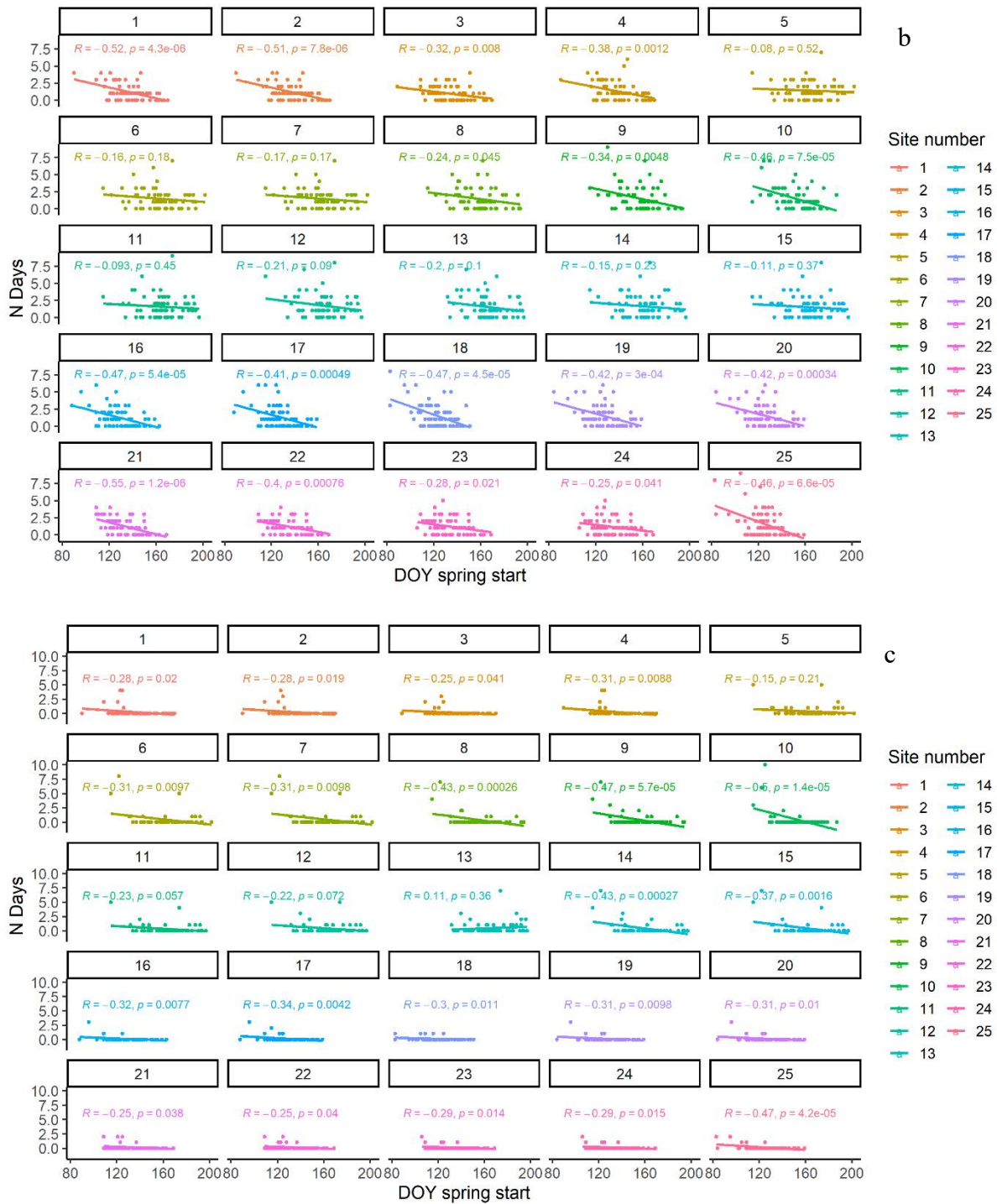


Figure 60 a -c: Linear regression plots for spring start (DOY simulated spring start +60 days) versus 3 temperature metrics, for all sites and the years 1950-2018: a) Average temperature (°C), b) Number of days between 4 and 5 °C and c) Number of days below 4 °C.

Appendix

Appendix A: Co-author publications

A.1 Modifications in biological composition of aquatic ecosystems in the Pyrenees (OPCC-report, *section 2.6.5, pp. 74-76*)

Contribution to the chapter 2.6: Water cycle and water resources, Pyrenean Climate Change Observatory, OPCC

ISBN: 978-84-09-06268-3, see full report online: <https://www.opcc-ctp.org/fr>

English version: <https://www.opcc-ctp.org/en/documentos>

Abstract (for Chapter 2.6, OPCC_CTP, 2018)

The Pyrenees play a fundamental role in providing water resources to the territories located on both sides of the hydrological boundary, and this influence goes well beyond the strict limits of the mountain range, since its headwaters generate a very important share of the surface and sub-surface resources of important river basins such as the Ebro, Adour, Garonne, etc.

The analysis of time series of streamflow data shows a global trend towards reduced annual river flows in the last decades. This reduction of streamflow can be attributed in part to climate change, but an important contribution to this trend can be attributed to changes in land use and in vegetation cover, most notably in the southern part of the mountain range. There have been changes also in the monthly regime of streamflow, related to a change in the ratio snow / rainfall in winter that led to reduced snow accumulation and earlier snowmelt. This has translated into changes in the river regimes, which have now a more pluvial character with higher streamflow in winter, earlier and reduced snowmelt-related high flows in spring, and longer and more pronounced summer lows. No clear sign has been found, on the other hand, about statistically significant changes in the frequency of extreme flows.

Most future climate simulations indicate that the Pyrenean region will experience a trend towards higher temperatures and reduced precipitation during the 21st century. These changes will represent increased stress to the vegetation and will enhance evapotranspiration, which in turn will affect the other components of the water balance (surface runoff, base streamflow and groundwater recharge). The overall consequence will be less water in the

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soils and less frequent soil saturation conditions, which will be limited to shorter periods in winter and spring.

In addition to changes in the climatic forcing, the water balance of the Pyrenees will be affected by changes in the vegetation cover, driven by a continuation of re-vegetation processes already observed since the middle of the 20th century as a consequence of the abandonment of agricultural activities and as a response to global warming that implies an upward movement of the vegetation altitudinal belts. Human activity, in the meantime, will concentrate on a few places such as ski resorts and touristic villages. The observed and foreseen changes in the annual and seasonal streamflow levels may imply a reduction in water quality, due to the dilution effect of contaminants. The most agreed scenario, therefore, is towards a continuation of the trends already observed in the last decades, which lead towards reduced water resources. On the demand side, despite foreseen improvements in distribution and use of water resources, there are also predictions of increasing water demand, most notably due to the planned expansion of irrigated agriculture thanks to the development of new infrastructures. Considering that irrigated agriculture is a major water consumer in the region, this may lead to increased competition for water resources across sectors and regions.

A.2 People, pollution and pathogens – Global change impacts in mountain freshwater ecosystems

Dirk S. Schmeller, Adeline Loyau, Kunshan Bao, Werner Brack, Antonis Chatzinotas, Francois De Vleeschouwer, Jan Friesen, Laure Gandois, Sophia V. Hansson, Marilen Haver, Gaël Le Roux, Ji Shen, Roman Teisserenc, Vance T. Vredenburg:

People, pollution and pathogens – Global change impacts in mountain freshwater ecosystems. *Science of the Total Environment*, Elsevier, 2018, 622-623, pp.756-763.

HAL Id: hal-02333545, version 1

DOI: 10.1016/j.scitotenv.2017.12.006

Abstract

Mountain catchments provide for the livelihood of more than half of humankind, and have become a key destination for tourist and recreation activities globally. Mountain ecosystems are generally considered to be less complex and less species diverse due to the harsh environmental conditions. As such, they are also more sensitive to the various impacts of the Anthropocene. For this reason, mountain regions may serve as sentinels of change and provide ideal ecosystems for studying climate and global change impacts on biodiversity. We here review different facets of anthropogenic impacts on mountain freshwater ecosystems. We put particular focus on micropollutants and their distribution and redistribution due to hydrological extremes, their direct influence on water quality and their indirect influence on ecosystem health via changes of freshwater species and their interactions. We show that those changes may drive pathogen establishment in new environments with harmful consequences for freshwater species, but also for the human population. Based on the reviewed literature, we recommend reconstructing the recent past of anthropogenic impact through sediment analyses, to focus efforts on small, but highly productive waterbodies, and to collect data on the occurrence and variability of microorganisms, biofilms, plankton species and key species, such as amphibians due to their bioindicator value for ecosystem health and water quality. The newly gained knowledge can then be used to develop a comprehensive framework of indicators to robustly inform policy and decision making on current and future risks for ecosystem health and human well-being.

A.3 Trace metal legacy in mountain environments: a view from the Pyrenees mountains.

Le Roux, G., Hansson, S.V., Claustres, A., Binet, S., De Vleeschouwer, F., Gandois, L., Mazier, F., Simonneau, A., Teisserenc, R., Allen, D., Rosset, T., Haver, M., Da Ros, L., Galop, D., Durantez, P., Probst, A., Sánchez-Pérez, J.M., Sauvage, S., Laffaille, P., Jean, S., Schmeller, D.S., Camarero, L., Marquer, L. and Lofts, S. (2020):

Trace Metal Legacy in Mountain Environments. In *Biogeochemical Cycles* (eds K. Dontsova, Z. Balogh-Brunstad and G. Le Roux)

HAL Id : hal-02358447, version 1

DOI : 10.1002/9781119413332.ch9

Abstract

The mineral reserves of mountain environments have been exploited since the beginning of metallurgy and legacy contamination from activities such as mining persist to this day. This is particularly the case in the soils of the European mountains where potential harmful trace elements (such as Pb, Sb, As, and Hg) of anthropogenic origin have accumulated since Antiquity. The French Pyrenees are no exception to this, as many mine sites in the region date back to the Bronze Age, resulting in landscape alternations and anthropogenic environmental impacts on a millennial scale. The mountain critical zone is sensitive both to human-induced environmental changes (e.g., agriculture, mining, clear-cutting) as well as to climate-induced rapid environmental fluctuations. The legacy of trace metal contamination in other environments has been documented at individual sites in Europe and around the world, however, the fate of such legacy metals over time, in particular within mountainous regions, is poorly understood. This is despite the fact that a large proportion of metals was deposited and stored before 1800 CE in these areas. Using a case study from the Central French Pyrenees as a specific example, we here show that legacy metal (e.g., Pb) contamination in mountain environments is still persistent and a potential threat to mountain ecosystem health. We emphasize methods that aim to understand, in an interdisciplinary and coordinated way, the fate of legacy metals in the Central Pyrenees and beyond. We highlight the importance of research in the mountain critical zone for the whole of Europe, as mountains are the source of water and provide regional economic and socio-ecological resources. The goal of this chapter

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is, therefore, to draw attention to and provide fellow researchers with, the background information and methodologies needed to address the problem of legacy metal accumulation, transport, storage, remobilization, and redeposition in mountain watersheds, as well as potential subsequent environmental impacts downstream.

A.4 Climate and introduced fish influence amphibian skin microbiome in mountain lakes

Adriana P. Bernardo-Cravo, Adeline Loyau, Canan Karakoç, Marilen Haver, Vance T. Vredenburg, Antonis Chatzinotas, Dirk S. Schmeller

Climate and introduced fish influence amphibian skin microbiome in mountain lakes

Article **submitted** to *Scientific Reports* on 22 September 2021

Abstract

Microbiomes are important elements for the health of multicellular host organisms, yet it remains unclear whether host microbiomes are affected by environmental disturbances and climatic factors. Here we examine whether climatic, temporal, spatial, and anthropogenic factors affect bacterial skin microbiomes of larval amphibians (tadpoles) from 3 host species (*Alytes obstetricans*, *Rana temporaria* and *Bufo bufo*). We sequenced the V3-V4 region of the 16S rRNA gene from 516 tadpole samples collected from 19 montane lakes across the French Pyrenees. The sites are situated along an altitudinal gradient, and differ in the level of anthropogenic activities (e.g. tourism, fish introduction), and climatic conditions. Our results show that climatic variables (length of growing season) and the presence of fish were driving the microbial community composition on amphibian tadpole skin. The combined effects of climate change and fish stocking on the tadpole skin microbiome may increase the susceptibility to external pressures, including pathogens, with important consequences for amphibian populations and mountain freshwater ecosystems.

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Appendix B: Student projects (Water temperature data for the Pyrenees)

B.1: Student project on historic water temperature data for the Pyrenees (Clément Rudelle)

Student project, Year 1 Master (M-1), Université de Perpignan Via Domitia, Master mention « Sciences de la Mer »

French title: Etude bibliographique des données de température des masses d'eaux situés en altitude dans les Pyrénées durant la période 1900 à 2000.

English title: Water temperature data from a literature study of freshwater sites in the Pyrenees for the period 1900 to 2000.

Abstract

Climate change is a process that is increasingly influencing the world's environments. Here the subject is the altitude area more precisely how this process influences these areas. The ecosystems of these areas are very sensitive to global change impacts, e.g. on mountain water balance. It is therefore necessary to know how to observe changes in these ecosystems. As a step towards monitoring these changes, a bibliographic study was performed on water temperatures of freshwaters in the Pyrenees. During this research many single observation points and some annual time series of temperature were found, among the wealth of articles published on the subject of the Pyrenees. Past temperature data found reach back to the year 1913.

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Carte du nombre d'échantillonnage de température par site d'étude

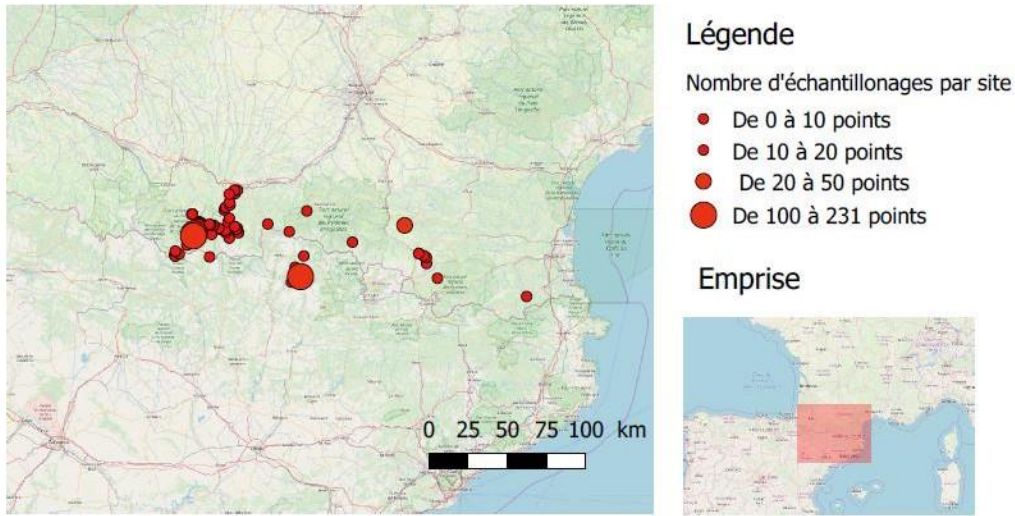


Figure 61: Map of temperature data points from the literature.

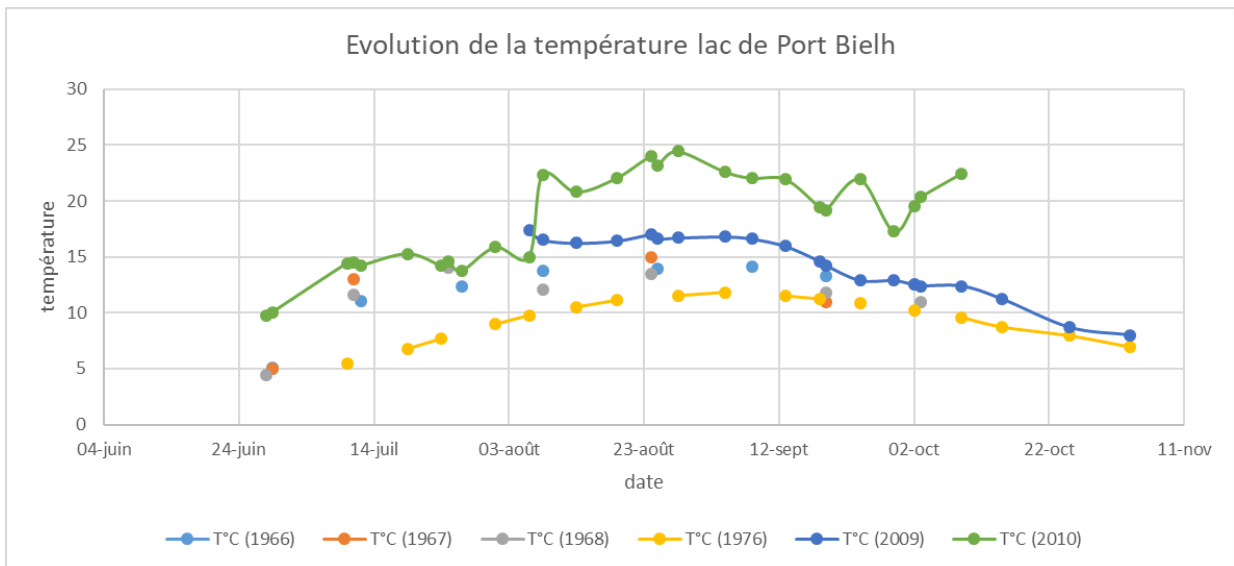


Figure 62: Surface temperature time series at Lac de Port Bielh for years of data found in the literature.

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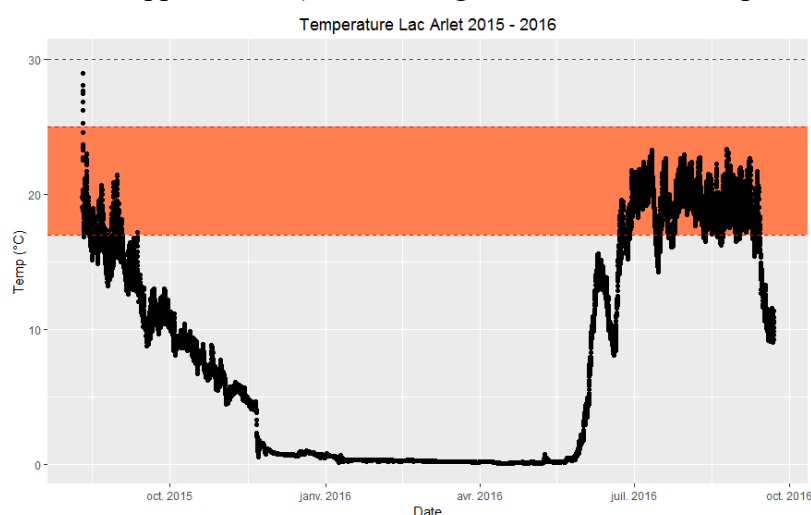
B.2: Student project on water temperature data for the Pyrenees: Annual water temperature dynamics with potential consequences for *Bd* growth and chytridiomycosis dynamics in Pyrenean lakes

French title: Quantification et effets du réchauffement climatique sur le développement et la propagation du pathogène fongique des amphibiens *Batrachochytrium dendrobatidis* : recherche bibliographique et traitement de données de température autour de lacs des Pyrénées

English title: Quantification and effects of global warming on the development and spread of the amphibian fungal pathogen *Batrachochytrium dendrobatidis*: bibliographic research and processing of temperature data around Pyrenean lakes

Abstract

Long term effects of climate change on ecosystems are still uncertain. In order to better understand its repercussions, environmental studies are achieved in specific sensitive environments such as mountain environments. These are the hot spot of the P³ project's concern to preserve four mountain biotopes while making case studies to better predict future variation across the world. Within the framework of this project, the bibliographical studies carried out during my internship at the end of my Bachelor degree aim to contribute to a database of water temperatures at the scale of the Pyrenees dating back to early twentieth century see even further if possible. This database can serve researchers who need such data which cannot be accessed without tedious research. This internship is also intended to study temperature dynamics in mountain lakes as possible causes of the expansion of an extremely virulent pathogen in amphibians, *Batrachochytrium dendrobatidis*. The primary outcomes of this work are the formatting of the water temperature database (previous work done by C. Rudelle, Appendix B.1), the writing of the R code for import, formatting and visualization of



water temperature of P³ lakes
(see example Figure 63).

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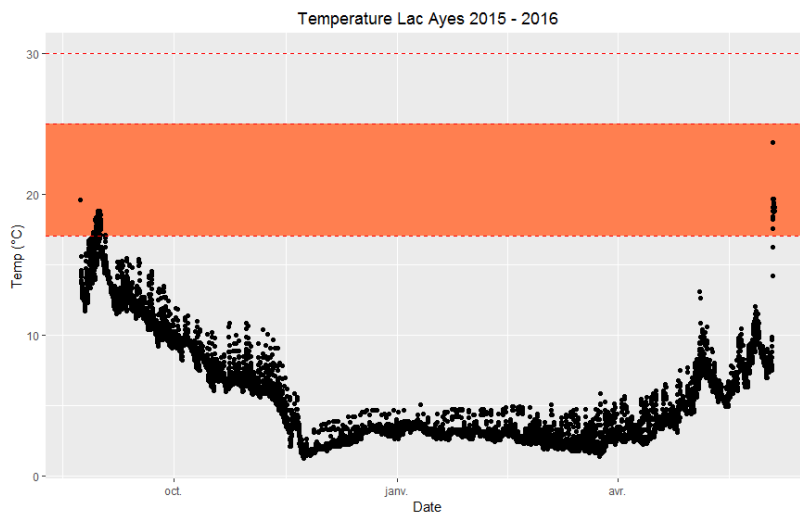


Figure 63 a-b: Water temperature time series for two lake sites in the Pyrenees 2015-2016 (Data source: D.S. Schmeller).

Appendix C: R Code

Note:

- C.1 – C.2: The following data/files are needed: daily-resolution climatic data for maximum, minimum temperature and precipitation (here: e-obs tn, tx, rr in .nc-file format), study sites shapefiles (here: 25 study site catchment polygons)
- Best practice: create R project that contains all R scripts, all input data files as well as output data and figures created with those R scripts
- Copy and paste the following scripts in R studio editor and execute line by line, adapt the code to your input data (shapefiles, climate data, date vectors etc.)
- Feel free to review code and make it more efficient, this is just a suggestion
- Not all code, e.g. for creating the plots, can be included here due to length, but can be made available by request (e.g. code to create climate extreme / hydrology heatmap plots or infection risk heatmap plots (Chapter 5 and Chapter 6) or for hydrology variable extraction

C.1 Script for extracting of climate data for annual climdex indices

Note:

- input nc-files: tx, tn and rr (maximum, minimum temperature and precipitation)
 - a) e-obs Version 19.0e (1950-2018)
 - b) e-obs Version 23.1e (1950-2020)
- Execute the following script for one input climate file at a time (e.g. first for tn and export tn, then start over for tx, rr)

```
library(ncdf4)
library(raster)
library(rgdal)
library(sp)
library(lubridate)
library(ggplot2)
```

```
### LOAD DATA -----
```

```
# load variables from nc file one after the other and create raster brick
```

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```
# min T
s <- brick("tn_ens_mean_0.1deg_reg_v19.0e.nc")
s
# s <- brick("tn_ens_mean_0.1deg_reg_v23.1e.nc")

# max T

s <- brick("tx_ens_mean_0.1deg_reg_v19.0e.nc")
# s <- brick("tx_ens_mean_0.1deg_reg_v23.1e.nc")

# precipitation
s <- brick("rr_ens_mean_0.1deg_reg_v19.0e.nc")
# s <- brick("rr_ens_mean_0.1deg_reg_v23.1e.nc")

# cropped new
s2 <- crop(s, extent(-2.3, 3.5, 41.9, 43.6))
s2

## visualize resolution of data (point or polygon grid)
# x <- s2[[1]]
# plot(x)
## show projection
# proj4string(s2)

# set path to catchment shapefiles, can be in the same R project CHANGE HERE to your folder

shape_path = "./Catchments/catchments_Bu5km_new"

#list all files in directory
# add full.names = T because of relative path
all_shapes_in_mydir <- list.files(path = shape_path, pattern = "shp", full.names=T)
all_shapes_in_mydir

### EXTRACT FUNCTION -----
# define extract function, to extract data for all catchment shapefiles, transform shapefiles into same
projection as # the raster brick:

extract_raster_shp <- function(i) {
  #read in shp
  the_shape <- readOGR(i)
```

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```
# project shapefile: transform to the same projection as r
the_shape <- spTransform(the_shape, proj4string(s2))
val <- raster::extract(s2, the_shape, weights = T, normalizeWeights = T, fun = mean, na.rm = TRUE)
#val <- raster::extract(s2, the_shape, method= "bilinear", weights = T, normalizeWeights = T, fun =
mean, na.rm = TRUE)
#val <- raster::extract(s2, the_shape, method = "simple", weights = T, normalizeWeights = T, fun =
mean, na.rm = TRUE)
#val <- extract(r, the_shape, fun = mean, na.rm = TRUE) # takes only cell corresponding to
catchment location
#val <- extract(r, the_shape, fun = mean, method= "bilinear", na.rm = TRUE) # take 4 nearest cells ,
method bilinear
return(val)
}
```

EXTRACT AND FORMAT DATA

```
# in case the tidyverse package was loaded before this script, execute the following lines:
#onload tidy as package conflict with extract raster, etc...!!! reload later
#.rs.unloadPackage("tidyverse")
```

```
# apply function to raster stack
extracted2 <- lapply(all_shapes_in_mydir, FUN=extract_raster_shp)
str(extracted2)
```

```
# reload package tidyverse
library(tidyverse)
```

```
# name columns with catchment numbers CHANGE HERE FOR YOUR SITES/NUMBERS
# catchment order according to names/numbering of catchment files in the shapefile folder
# and from West to East in the Pyrenees -> 1 = Acherito ... 25 = Arbu
catchment_numbers <- c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25)
names(extracted2) <- catchment_numbers
```

```
## get hold of the days list in extracted object
# days_eobs <- attributes(extracted2[[1]])$dimnames[2]
```

```
# convert to sequence of dates in date format, then to dataframe
date <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="day")
# date <- seq.Date(as.Date("1950/1/1"), as.Date("2020/12/31"), by="day")
```

```
## create dataframe from extracted list
.rs.unloadPackage("dplyr")
.rs.unloadPackage("plyr")
library(plyr)
library(dplyr)
```

```
df_me2 <- ldply(extracted2, data.frame, .id = NULL)
```

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```
# transpose dataframe
df_me2 <- t(df_me2)

# change column names to catchment/site names
colnames(df_me2) <- catchment_numbers

# convert into dataframe
df_me2 <- as.data.frame(df_me2)
# add date vector
df_me2 <- bind_cols(date, df_me2)

# for all analyses
library(tidyverse)

### EXPORT DATAFILES FOR FURTHER USE: -----
# # Tavg
dailyTavgcatchments <- df_me2 %>%
# pivot_longer(-date, names_to = "catchment", values_to = "tavg")
# save(dailyTavgcatchments, file="dailyTavgcatchments_eobs.RData")

# FORMAT DATA FOR CLIMDEX INDICES CREATION (Script C.2):

# Tmin
dailyTmincatchments <- df_me2 %>%
  pivot_longer(-date, names_to = "catchment", values_to = "tmin")

save(dailyTmincatchments, file="dailyTmincatchments_eobs_v19.0e.RData")
#save(dailyTmincatchments, file="dailyTmincatchments_eobs_v23.1e.RData")

# Tmax
dailyTmaxcatchments <- df_me2 %>%
  pivot_longer(-date, names_to = "catchment", values_to = "tmax")

save(dailyTmaxcatchments, file="dailyTmaxcatchments_eobs_v19.0e.RData")
#save(dailyTmaxcatchments, file="dailyTmaxcatchments_eobs_v23.1e.RData")

# Precipitation
dailyPPcatchments <- df_me2 %>%
  pivot_longer(-date, names_to = "catchment", values_to = "precip")

save(dailyPPcatchments, file="dailyPPcatchments_eobs_v19.0e.RData")
#save(dailyPPcatchments, file="dailyPPcatchments_eobs_v23.1e.RData")
```

C.2 Script for creating annual climdex indices

```
##### CREATE CLIMDEX INPUT LIST -----

# load packages
library(tidyverse)
library(climdex.pcic)
library(PCICt)

# results needed from other scripts: run script C.1 for input climate data

# load input climate data
load("dailyPPcatchments_eobs_v19.0e.RData")
load("dailyTmaxcatchments_eobs_v19.0e.RData")
load("dailyTmincatchments_eobs_v19.0e.RData")

# rename data
pp <- dailyPPcatchments
tmax <- dailyTmaxcatchments
tmin <- dailyTmincatchments

# function to get variables for each catchment (ignore the names, they are just placeholders)
for(i in 1:25) {
  nam <- paste("ppc", i, sep = "")
  pete <- filter(pp, catchment==i)
  assign(nam, pete[[3]])

  nam2 <- paste("tmaxc", i, sep = "")
  claus <- filter(tmax, catchment==i)
  assign(nam2, claus[[3]])

  nam3 <- paste("tminc", i, sep = "")
  lola <- filter(tmin, catchment==i)
  assign(nam3, lola[[3]])

  rm(pete, claus, lola)
}

# format date for climdex
#days.pos <- seq.POSIXt(as.POSIXct("1950/1/1"), by="day", length.out=23922)
days.pos <- seq.POSIXt(as.POSIXct("1950/1/1"), by="day", length.out=25202)
# for data until 2020:
#days.pos <- seq.POSIXt(as.POSIXct("1950/1/1"), by="day", length.out=25933)

#head(days.pos)
days.pcict <- as.PCICt(days.pos, cal = "gregorian")
```

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```
#length(days.pcict)
```

```
# create climdex input objects for all catchments (ugly code, but it works)
```

```
ci_cat_a <- climdexInput.raw(tmaxc1, tminc1, ppc1, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_b <- climdexInput.raw(tmaxc2, tminc2, ppc2, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_c <- climdexInput.raw(tmaxc3, tminc3, ppc3, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_d <- climdexInput.raw(tmaxc4, tminc4, ppc4, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_e <- climdexInput.raw(tmaxc5, tminc5, ppc5, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_f <- climdexInput.raw(tmaxc6, tminc6, ppc6, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_g <- climdexInput.raw(tmaxc7, tminc7, ppc7, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_h <- climdexInput.raw(tmaxc8, tminc8, ppc8, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_i <- climdexInput.raw(tmaxc9, tminc9, ppc9, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_j <- climdexInput.raw(tmaxc10, tminc10, ppc10, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_k <- climdexInput.raw(tmaxc11, tminc11, ppc11, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_l <- climdexInput.raw(tmaxc12, tminc12, ppc12, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_m <- climdexInput.raw(tmaxc13, tminc13, ppc13, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_n <- climdexInput.raw(tmaxc14, tminc14, ppc14, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_o <- climdexInput.raw(tmaxc15, tminc15, ppc15, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_p <- climdexInput.raw(tmaxc16, tminc16, ppc16, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_q <- climdexInput.raw(tmaxc17, tminc17, ppc17, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_r <- climdexInput.raw(tmaxc18, tminc18, ppc18, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_s <- climdexInput.raw(tmaxc19, tminc19, ppc19, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_t <- climdexInput.raw(tmaxc20, tminc20, ppc20, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_u <- climdexInput.raw(tmaxc21, tminc21, ppc21, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_v <- climdexInput.raw(tmaxc22, tminc22, ppc22, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_w <- climdexInput.raw(tmaxc23, tminc23, ppc23, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))  
ci_cat_x <- climdexInput.raw(tmaxc24, tminc24, ppc24, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))
```

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```
ci_cat_y <- climdexInput.raw(tmaxc25, tminc25, ppc25, days.pcict, days.pcict, days.pcict,  
base.range=c(1971, 2000))
```

```
# list those ci input objects, this list is used in the following code to create the indices  
ci_catlist <- ls(pattern = "ci_cat[[:punct:]]")  
ci_catlist
```

```
### CREATE INDICES -----
```

```
# get list of available indices based on input data, using the first list object created before:  
climdex.get.available.indices(ci_cat_a)
```

```
# Create all indices -----
```

```
# gives list of 25 with each 69 icing days (yearly icing days per catchment)  
icingdays <- lapply(lapply(ci_catlist, get), FUN=climdex.id)
```

```
# convert to dataframe and change column names  
icingdays <- as.data.frame(icingdays[1:25])  
icingdays <- icingdays %>% `colnames<-`(c(1:25))  
# add year as a column  
year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")  
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2020/12/31"), by="year")  
icingdays$year <- year  
# create a tidy format  
# for all analyses except for climdex  
icingdays <- icingdays %>%  
  pivot_longer(-year, names_to = "catchment", values_to = "icingdays")
```

```
### do the above now for all variables and in the end combine data:
```

```
# ***** growing season *****  
growingseason <- lapply(lapply(ci_catlist, get), FUN=climdex.gsl)
```

```
# convert to dataframe and change column names  
growingseason<- as.data.frame(growingseason[1:25])  
growingseason<- growingseason %>% `colnames<-`(c(1:25))  
# add year as a column  
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")  
growingseason$year <- year
```

```
# create a tidy format  
# for all analyses except for climdex  
growingseason <- growingseason %>%  
  pivot_longer(-year, names_to = "catchment", values_to = "growingseason")
```

```
# ***** summer days ***** #
```


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```
summerdays <- lapply(lapply(ci_catlist, get), FUN=climdex.su)
# convert to dataframe and change column names
summerdays <- as.data.frame(summerdays[1:25])
summerdays <- summerdays %>% `colnames`->`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
summerdays$year <- year

# create a tidy format
# for all analyses except for climdex
summerdays <- summerdays %>%
  pivot_longer(-year, names_to = "catchment", values_to = "summerdays")

# ***** warm spell ***** #
warmspell <- lapply(lapply(ci_catlist, get), FUN=climdex.wsgi)
# convert to dataframe and change column names
warmspell <- as.data.frame(warmspell[1:25])
warmspell <- warmspell %>% `colnames`->`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
warmspell$year <- year

# create a tidy format
# for all analyses except for climdex
warmspell <- warmspell %>%
  pivot_longer(-year, names_to = "catchment", values_to = "warmspell")

# ***** cold spell ***** #
# csdi: cold spell duration index
coldspell <- lapply(lapply(ci_catlist, get), FUN=climdex.csdi)
# convert to dataframe and change column names
coldspell <- as.data.frame(coldspell[1:25])
coldspell <- coldspell %>% `colnames`->`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
coldspell$year <- year

# create a tidy format
# for all analyses except for climdex
coldspell <- coldspell %>%
  pivot_longer(-year, names_to = "catchment", values_to = "coldspell")

# ***** frost days ***** #
frostdays <- lapply(lapply(ci_catlist, get), FUN=climdex.fd)
# convert to dataframe and change column names
frostdays <- as.data.frame(frostdays[1:25])
frostdays <- frostdays %>% `colnames`->`(c(1:25))
```

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```
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
frostdays$year <- year

# create a tidy format
# for all analyses except for climdex
frostdays <- frostdays %>%
  pivot_longer(-year, names_to = "catchment", values_to = "frostdays")

# ***** consecutive dry days ***** #
consdrydays <- lapply(lapply(ci_catlist, get), FUN=climdex.cdd)
# convert to dataframe and change column names
consdrydays <- as.data.frame(consdrydays[1:25])
consdrydays <- consdrydays %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
consdrydays$year <- year

# create a tidy format
# for all analyses except for climdex
consdrydays <- consdrydays %>%
  pivot_longer(-year, names_to = "catchment", values_to = "consdrydays")

#***** consecutive wet days *****
conswetdays <- lapply(lapply(ci_catlist, get), FUN=climdex.cwd)
# convert to dataframe and change column names
conswetdays <- as.data.frame(conswetdays[1:25])
conswetdays <- conswetdays %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
conswetdays$year <- year

# create a tidy format
# for all analyses except for climdex
conswetdays <- conswetdays %>%
  pivot_longer(-year, names_to = "catchment", values_to = "conswetdays")

#***** total precipitation *****
pcpttotal <- lapply(lapply(ci_catlist, get), FUN=climdex.prcptot)
# convert to dataframe and change column names
pcpttotal <- as.data.frame(pcpttotal[1:25])
pcpttotal <- pcpttotal %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
pcpttotal$year <- year
```

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```
# create a tidy format
# for all analyses except for climdex
pcptotal <- pcptotal %>%
  pivot_longer(-year, names_to = "catchment", values_to = "pcptotal")

#***** # r10mm: nb of days pp over 10 mm\ *****
pcp_days_10mm <- lapply(lapply(ci_catlist, get), FUN=climdex.r10mm)
# convert to dataframe and change column names
pcp_days_10mm <- as.data.frame(pcp_days_10mm[1:25])
pcp_days_10mm <- pcp_days_10mm %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
pcp_days_10mm$year <- year

# create a tidy format
# for all analyses except for climdex
pcp_days_10mm <- pcp_days_10mm %>%
  pivot_longer(-year, names_to = "catchment", values_to = "pcp_days_10mm")

# *****r20mm
pcp_days_20mm <- lapply(lapply(ci_catlist, get), FUN=climdex.r20mm)
# convert to dataframe and change column names
pcp_days_20mm <- as.data.frame(pcp_days_20mm[1:25])
pcp_days_20mm <- pcp_days_20mm %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
pcp_days_20mm$year <- year

# create a tidy format
# for all analyses except for climdex
pcp_days_20mm <- pcp_days_20mm %>%
  pivot_longer(-year, names_to = "catchment", values_to = "pcp_days_20mm")

#***** r95ptot: total daily pp exceeding 95 percentile threshold for
base period\
r95pttotal <- lapply(lapply(ci_catlist, get), FUN=climdex.r95ptot)
# convert to dataframe and change column names
r95pttotal <- as.data.frame(r95pttotal[1:25])
r95pttotal <- r95pttotal %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
r95pttotal$year <- year

# create a tidy format
# for all analyses except for climdex
r95pttotal <- r95pttotal %>%
  pivot_longer(-year, names_to = "catchment", values_to = "r95pttotal")
```

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```
##### r99ptot
r99ptotal <- lapply(lapply(ci_catlist, get), FUN=climdex.r99ptot)
# convert to dataframe and change column names
r99ptotal <- as.data.frame(r99ptotal[1:25])
r99ptotal <- r99ptotal %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
r99ptotal$year <- year

# create a tidy format
# for all analyses except for climdex
r99ptotal <- r99ptotal %>%
  pivot_longer(-year, names_to = "catchment", values_to = "r99ptotal")

##### # sdii: simple precipitation intensity index
sdindex <- lapply(lapply(ci_catlist, get), FUN=climdex.sdii)
# convert to dataframe and change column names
sdindex <- as.data.frame(sdindex[1:25])
sdindex <- sdindex %>% `colnames<-`(c(1:25))
# add year as a column
#year <- seq.Date(as.Date("1950/1/1"), as.Date("2018/12/31"), by="year")
sdindex$year <- year

# create a tidy format
# for all analyses except for climdex
sdindex <- sdindex %>%
  pivot_longer(-year, names_to = "catchment", values_to = "sdindex")

### COMBINE INTO ONE DATAFRAME -----
library(dplyr)
library(purrr)

df_climdex <- list(coldspell, consdrydays, conswetdays, frostdays, growingseason, icingdays,
pcp_days_10mm, pcp_days_20mm, pcptotal, r95ptotal, r99ptotal, sdindex, summerdays, warmspell)
%>%
  reduce(left_join, by=c("year", "catchment"))
df_climdex

### EXPORT DATA -----
save(df_climdex, file="df_climdex_v19.0.RData")
```

C.3 Script for spring start calculation from air temperature (Chapter 6)

```

library(heatwaveR)
library("dplyr")
library(lubridate)
library(tidyverse)
library(ggplot2)
library(viridis)
library(viridisLite)
library(plotly)
library(ggpubr)
library(RColorBrewer)

# create df_all -----
# load data
load("dailyTavgcatchments_eobs.RData")
tavg <- dailyTavgcatchments

tavg <- tavg %>%
  pivot_longer(-date, names_to = "catchment", values_to = "temp")
tavg$catchment <- factor(tavg$catchment,levels=c(1:25))

# calculate spring start from air temperature -----
# from exceedance script
exceed_calc_air <- function(i) {
  ex <- exceedance(
    i,
    x = date,
    y = temp,
    threshold =4,
    below = FALSE,
    minDuration =30,
    joinAcrossGaps = F,
    maxGap = 0, # 0,5 (5 used so far, but 0 doesn't make a difference for doy start only for duration,
    thus use 0)
    maxPadLength = FALSE
  )
  d <- as.data.frame(ex$exceedance)
  return(d)
}

# apply exceedance function to all catchments
#### air_so: calculate spring onset all catchments from air temperature ----
exceed_air <- tavg %>%
  group_by(catchment) %>%

```

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```
group_modify(~ exceed_calc_air(.x))

# formatting of results -----
# select columns of exceed_df
exceed_air <- exceed_air %>% select(catchment, duration, date_start, date_end)
# add doy_start column
# exceed_air$doy_start <- strptime(exceed_air$date_start, format = "%j")
# exceed_air$doy_start <- as.numeric(exceed_air$doy_start)
exceed_air
# select catchments
#exceed_air <- exceed_air %>% filter(catchment == 4) %>% filter(year(date_start) >= "2008" &
year(date_start) <= "2016")

# Arlet calibration: set limit
#exceed_air <- exceed_air %>% filter(year(date_start) >= "2008" & year(date_start) <= "2016")

# add doy_start column
exceed_air$doy_spring <- strptime(exceed_air$date_start, format = "%j")
exceed_air$doy_spring <- as.numeric(exceed_air$doy_spring)
exceed_air$year <- year(exceed_air$date_start)
# filter first event longest duration
air_so <- exceed_air %>%
  group_by(year,catchment) %>%
  filter(date_start == min(date_start)) %>%
  filter(duration == max(duration))
air_so

load("df_climdex_v19.0.RData ")
df <- df_climdex

# format air_so -----
air_so

# add variables: geogr, climatic
## gradient
air_so$gradient[air_so$catchment %in% 1:4]<-1
air_so$gradient[air_so$catchment %in% 5:10]<-2
air_so$gradient[air_so$catchment %in% 11:15]<-3
air_so$gradient[air_so$catchment %in% 16:18]<-4
air_so$gradient[air_so$catchment %in% 19:25]<-5

# combine dfs
air_so$catchment <- as.numeric(air_so$catchment)
df$catchment <- as.numeric(df$catchment)
```

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```
df_all <- left_join(df, air_so, by= c("year", "catchment"))  
df_all
```

C.4 Script for calculation of temperature metrics after annual spring start

```
# calculate temperatures post annual spring-start
```

```
library(heatwaveR)  
library("dplyr")  
library(lubridate)  
library(tidyverse)  
library(ggplot2)  
library(viridis)  
library(viridisLite)  
library(plotly)  
library(ggpubr)  
library(RColorBrewer)
```

```
load("dailyTavgcatchments_eobs.RData")  
tavg <- dailyTavgcatchments
```

```
tavg  
tavg$year <- year(tavg$date)  
tavg
```

```
# select from df_all dates spring start and create +x dates
```

```
df_all$doy_post30 <- df_all$doy_spring + 30  
df_all$doy_post14 <- df_all$doy_spring + 14  
df_all$doy_post60 <- df_all$doy_spring + 60
```

```
# formatting  
df_all$date <- df_all$date_start
```

```
d <- df_all %>% select(year, catchment, doym_spring, doym_post30, doym_post14, doym_post60, date,  
gradient, Bd_stat, catchment_name, date_start)
```

```
dt <- d
```

```
tavg$catchment <- as.numeric(tavg$catchment)
```

```
# join dataframes tavg and dt with spring start dates (df_all)  
g <- full_join(tavg, dt, by= c("date", "catchment"))
```

```
# create new columns with end dates  
g <- g %>% mutate(start = date_start, end_30= ymd(date_start + days(30)), end_14 =ymd(date_start  
+ days(14)), end_60=ymd(date_start + days(60)))
```

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```
g$year <- year(g$date)

dates_for_avgs <- g %>% select(year, catchment, start, end_30, end_14, end_60) %>%
  drop_na(start)

dates_for_avgs <- dates_for_avgs %>%
  group_by(year, catchment) %>%
  filter(start == min(start))

years <- c(dates_for_avgs$year)

# for 60 days post spring start:

# choose the dates that we want to average over, define as a dataframe, group by catchment
dates_filter <- dates_for_avgs %>%
  rowwise() %>%
  do(data.frame(catchment= .$catchment, datelist = seq(.$start,
                                                    .$end_60, by = '1 day')) %>%
  group_by(catchment)

dates_filter$date <- ymd(dates_filter$datelist)

# join temperatures with dates
u <- inner_join(g, dates_filter, by=c("catchment","date"))

# create dataframe with different temperature metrics (60 days post spring start)

post_60 <- u %>%
  group_by(catchment, year)%>%
  summarise(days_below5 = sum(temp <=5), days_betw4_5 = sum(temp >= 4 & temp <=5),
  days_below4 = sum(temp <= 4), trange = max(temp)- min(temp), meanT = mean(temp))
post_60
# formatting of results

post_60$catchment <- factor(post_60$catchment,levels=c(1:25))
df_all$catchment <- factor(df_all$catchment,levels=c(1:25))
df_all <- left_join(df_all, post_60, by= c("catchment", "year"))

df_all <- df_all %>% mutate(date = lubridate::ymd(year, truncated = 2L))

df_all$catchment <- factor(df_all$catchment,levels=c(1:25))
df_all$gradient <- factor(df_all$gradient,levels=c(1:5))

# end
```