Pathogen pollution across spatial scales

A primer on disease ecology of *Batrachochytrium salamandrivorans* in Europe

Wouter Beukema

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Contents

Glossary

Acclimation	Adjustment process of an organism to its environment.
Assemblage (ecology)	Group of species that share an attribute of habitat or taxonomic similarity, or taxon.
AUC	Area Under the Curve; metric to evaluate performance of (among others) ecological niche models.
Bd	*Batrachochytrium dendrobatidis*; an amphibian-pathogenic chytrid fungus.
Behavioural fever	Change in thermal preference driven by pathogen recognition, occurring in various invertebrates and ectothermic vertebrates.
Bioactive	Having an effect on a living organism, tissue or cell.
Bioclimatic niche	Approximation of the realized niche based on climatic parameters.
Biotic interactions	Interactions between organisms, either within or between species.
Bivariate	Composed of two variables.
Bsal	*Batrachochytrium salamandrivorans*; a salamander-pathogenic chytrid fungus.
Chytrid	Zoosporic organisms in the kingdom Fungi, officially known as Chytridiomycota.
Chytridiomycosis	Amphibian disease caused by *Batrachochytrium* fungi.
Community (ecology)	Group of interacting species that inhabit a particular location at a particular time.
Ecosystem	Community of living organisms in conjunction with the nonliving components of their environment, interacting as a system.
Ecological niche	Approximation of the realized niche created by an ecological niche model.
Ecological niche model	Multivariate models fitting a species ecological niche by relating occurrence records with environmental variables measured at the same sites, and projected over a whole study area.
Ectotherm	Organism that uses external heat sources to regulate its body temperature, i.e. a ‘cold-blooded’ animal.
Emerging infectious disease	Infectious disease has that recently appeared or those of which incidence or geographic range is rapidly increasing or threatens to increase in the near future.
Empirical	Originating in, or based on observation or experience; i.e. during an experiment one gathers empirical evidence.
ENFA	Environmental Niche Factor Analysis; a presence-only ecological niche model.
Enzootic (disease)	Disease maintained in an animal population without the need for external inputs, usually only affecting a limited number of individuals at any one time.

Ephemeral (water)	Temporary water body.
Epidemiology	Study and analysis of the distribution (who, when, and where), patterns and determinants of health and disease conditions in defined populations.
Epidermis	The outer, protective layer of the skin.
Epizootic (disease)	Disease event that temporarily affects a large number of animals in a given area.
ESM	Ensemble of Small Models; performance-based weighed average of *n* bivariate ecological niche models.
Evaporative cooling	In amphibians, evaporation of skin moisture to cool down.
Flagellum	Tail-like appendage of certain bacteria and eukaryotic cells used for locomotion or as sensory organelle.
Functional ecology	Branch of ecology that focuses on functions that species play in the community or ecosystem in which they occur.
FWO	Research Foundation – Flanders; a Belgian public research council based in Brussels, aiming to sponsor ground-breaking research and innovation.
GAA	Global Amphibian Assessment, directed at assessing the conservation status of amphibians worldwide.
GAM	Generalized Additive Model; a variant of GLM that tests relationships between a non-linearly related response variable and multiple predictor variables by smoothing the predictors to improve data fitting.
GAMM	Generalized Additive Mixed Model a variant of GAM that includes a grouping factor (random effect).
Germling	The organism produced by germination of an algal or fungal spore.
GIS	Geographic Information System; system designed to capture, store, manipulate, analyze, manage, and present spatial or geographic data.
GLM	Generalized Linear Model; a statistical procedure that tests relationships between a non-linearly related response variable and multiple predictor variables through use of a link function.
GLMM	Generalized Linear Mixed Model; a variant of GLM that includes a grouping factor (random effect).
Habitat suitability model	See ‘ecological niche model’.
Homeostasis (ecology)	State of steady internal physical and chemical conditions in an organism.
Innate	A quality or ability that one is born with.
Invasive species	Non-native species that causes major ecological, health or economic problems.
Invasiveness	The propensity of an invasive species to invade a recipient ecosystem.
Invasibility	The susceptibility of the recipient ecosystem to the establishment and spread of invasive species.
IUCN	International Union for Conservation of Nature; the global authority on the status of the natural world and the measures needed to safeguard it.
Keratinocyte	A cell in the epidermis that produces keratin.

Neotropic	Biogeographic realm containing the tropical terrestrial ecoregions of the Americas and the entire South American temperate zone.
Niche (ecology)	Functional position and role of an organism within its environment.
Macroecology	Branch of ecology that deals with the study of relationships between organisms and their environment at large spatial scales.
Macrophysiology	Branch of ecology that investigates variation in physiological traits over large geographic and temporal scales.
Mechanistic model (ecology)	Model describing species' requirements in terms of environmental conditions that are based on first principles of biophysics and physiology.
Mesoamerica	Historical and cultural region extending from approximately central Mexico through Belize, Guatemala, El Salvador, Honduras, Nicaragua, and northern Costa Rica.
Mesocosm	Experimental system that examines the natural environment under controlled conditions.
Microbiome	Collection of genomes from all the microorganisms in an environment, including multicellular organisms, but mostly limited to bacteria. See also 'microbiota'.
Microbiota	Collection (community) of commensal, symbiotic and/or pathogenic microorganisms found in or on multicellular organisms.
Observed niche	Representation of the ecological niche restricted by data availability.
Operative temperature (T_e)	Steady-state temperature of an organism in a particular site, in absence of metabolic heating and evaporative cooling.
Ordination	Statistical approach that orders objects characterized by values on multiple variables within a reduced multidimensional space, of which axes represent combinations of the initial variables. Similar objects are located close to each other, dissimilar objects remain distant.
Panzootic	Epizootic that spreads across a large region (for example a continent), or even worldwide.
Pathogenicity	Ability of an organism to cause disease.
Pathogen pollution	Human-mediated introduction of a pathogen to a new host or region.
PCA	Principal Component Analysis; a classical ordination approach.
PCR	Polymerase Chain Reaction; laboratory technique to rapidly amplify pre-determined DNA regions, for instance to detect DNA of a focal organism in a sample.
Perennial (water)	A permanent water body.
Prevalence	Proportion of a particular population found to be affected by a medical condition.
Realized niche	Niche occupied by an organism subject to biotic interactions.
Resistant	Host does not become infected, there is no disease.
Rhizoid	A rootlike structure in fungi and some plants that aids in absorption of nutrients.
Saprobic	Feeding on dead or decaying organic matter.

Set-point range (T_{set})	See ‘thermal preference’.
Taxonomy (biology)	Branch of science that encompasses the description, identification, nomenclature, and classification of organisms.
Thallus	Body of algae, fungi, and other lower organisms.
Thermal inertia	Property of an object or organisms as a function of thermal conductivity and volumetric heat capacity.
Thermal preference (T_{pref})	Range of preferred temperatures chosen by an ectotherm when free of ecological costs.
Thermal time constant	Unit of time required for an object or organism to respond to a change in its ambient temperature.
Thermoconformity	Adopting ambient temperature.
Thermoregulation	Regulation of body temperature through physiological or behavioral means as a response to fluctuations in ambient temperature.
Tolerant	Host becomes infected, but there is no disease or mortality.
TSS	True Skill Statistic; metric to evaluate performance of (among others) ecological niche models.
Virulence	Degree to which an organism can cause damage to a host.
Zoospore	Motile, asexual spore created by various protists, bacteria and fungi.

General introduction

General introduction

I. Preface

About ten years ago, enigmatic declines decimated the few fire salamander *Salamandra salamandra* populations located in the southern Netherlands (Spitzen-van der Sluijs *et al.*, 2013). Research into the cause of this worrisome development led to the discovery of *Batrachochytrium salamandrivorans* (*Bsal*), a pathogenic fungus that preys on salamanders and newts (Martel *et al.*, 2013). The origin of *Bsal* lies in East-Asia, from which it has been vectored by means of the pet trade (Nguyen *et al.*, 2017; Yuan *et al.*, 2018). As such, *Bsal* represents a textbook case of pathogen pollution driven by increasing globalisation, while posing a significant threat to Western Palearctic amphibian biodiversity as most (potential) host species are highly susceptible to infection (Martel *et al.*, 2014). After its discovery *Bsal* was quickly found to cause disease outbreaks in Belgium and Germany and Spanish Catalonia as well (Spitzen-van der Sluijs *et al.*, 2016; Dalbeck *et al.*, 2018; Wagner *et al.*, 2019). While our knowledge on *Bsal* is rapidly increasing, much remains unknown about its disease ecology. In other words, which environmental conditions shape *Bsal* disease dynamics in the wild, and how? Gaining practical understanding of these processes is critical to inform mitigation efforts, to direct further research and prioritize conservation (Bozzuto & Canessa, 2019; Thomas *et al.*, 2019).

Below, I first set the scene by going into detail about why we are in the midst of a global amphibian crisis, while putting particular emphasis on the role that emerging diseases play in declines of anurans, salamanders and caecilians worldwide. Then, I shift focus towards how the environment may influence host-pathogen interactions. Finally, I introduce the *Bsal* case in detail, which lays the basis for the scientific aims of this doctorate.

II. Amphibians face a multitude of threats around the globe

Amphibian diversity worldwide is under threat by environmental change. Habitat loss, chemical pollution, invasive species and disease are among the main factors that drive declines and local extinction in numerous populations (Stuart *et al.*, 2004). Additive threats include over-exploitation (for consumption, or the pet-trade), UV-B radiation, and climate change. These factors often act in synergy, exacerbating negative pressures (Kiesecker *et al.*, 2001; Sodhi *et al.*, 2008; Blaustein *et al.*, 2011; Hof *et al.*, 2011; Cohen *et al.*, 2019a). While early concerns about the welfare of amphibian populations date back to the 1970', the true scale of this crisis only became apparent in 2004 following publication of the first Global Amphibian Assessment (GAA, summarized in Stuart *et al.*, 2004). The results showed that a staggering 32% (1856 species) of amphibians worldwide are threatened, which is considerably more when compared to birds (12%) or mammals (23%). A second GAA is currently underway (2012-2020), which aims to provide a major update including first-time assessments for the more than 1700 species that have been described since then (Tapley *et al.*, 2018). The prospects are bleak, as more and more empirical studies confirm the recent predictions by Hof *et al.* (2011) which suggest that habitat loss, disease emergence and climate change will continue to affect amphibians on a global scale.

Habitat loss

The primary threat to amphibians worldwide is habitat loss (Stuart *et al.*, 2004). As humanity changes the landscape amphibian habitats become affected, or simply disappear, for instance due to urbanization, deforestation or intensive agriculture. The first GAA identified habitat loss to exert a dominant, negative pressure on amphibian populations across large areas of Europe, Australia, West Africa and parts of the Americas, notably the western USA (Fig. 1). Since then, deforestation increased in various other regions such as South-East Asia, likely leading to comparable negative consequences (Rowley *et al.*, 2009). Habitat modification and degradation may similarly affect amphibian persistence, although not all species are equally susceptible to changes in their surroundings. Cushman (2006), Pineda and Halffter (2004) and Nowakowski *et al.* (2017) showed that especially narrowly-distributed species, those whose larvae develop on land or in lotic water (e.g. streams or rivers) and species that have limited dispersal capabilities are most sensitive to habitat change. Habitat modification may furthermore lead to decreased connectivity between or within populations, for instance when an aquatic breeding habitat becomes separated from a terrestrial forest habitat by a road. Such fragmentation can cause increased mortality during dispersal, depauperate genetic diversity and species richness, and finally result in population extirpation (Kolozsvary & Swihart, 1999; Pineda & Halffter, 2004; Cushman, 2006; Parris, 2006; Noël *et al.*, 2007; Dixo *et al.*, 2009).

Chemical pollution

Pollution, in the form of chemical contaminants, has been put forward as another main threat to amphibian biodiversity (Stuart *et al.*, 2004; but see Kerby *et al.*, 2010). Four main types of pollutants can be distinguished; nitrogenous and phosphorous compounds, heavy metals, pesticides, and road deicers (e.g. road salt). Variation in sensitivity to contaminants is generally independent of phylogeny, meaning that pollution causes similar effects in different species (Egea-Serrano *et al.*, 2012). The most commonly encountered effects include decreases in body mass, persistence and increases in body abnormalities, although different chemicals can induce different effects. Nitrogen pollution, caused by for instance agricultural runoff, livestock and effluents from industrial and human waste, may cause reduced feeding and swimming activity in amphibian larvae, paralysis, development of abnormalities and eventually death (Marco *et al.*, 1999; Rouse *et al.*, 1999). Heavy metals can cause similar negative effects on amphibian larvae (Rowe *et al.*, 1996; Rowe *et al.*, 2001), but reports on local population declines as a result remain rare (Sparling, 2003; Kerby *et al.*, 2010). The effect of pesticides is more complex, and strongly dependent on environmental context. For instance, research in a mesocosm environment hosting tadpoles of three species (*Hyla versicolor, Anaxyrus americanus*, and *Lithobates pipiens*), zooplankton, and algae revealed that some pesticides may cause direct harm, while others cause beneficial effects by extirpating predatory insects (Relyea *et al.*, 2005). Finally, deicers such as road salt may affect embryonic and larval survival as well as tadpole behavior, but their effect is mediated by the environment and remains localized to the vicinity of roads (Karraker *et al.*, 2008; Denoël *et al.*, 2010).

Invasive species

Increasing globalization frequently leads to introductions of invasive species to naive environments. Amphibians may be negatively affected by such introductions in different ways, which include direct stressors such as predation or competition, as well as indirect effects that change their habitats or behavior (Kats & Ferrer, 2003; Stuart *et al.*, 2004). A vast body of literature on the impact of invasive species exists, from which I will list a small number of examples. Introduced species that have significantly contributed to amphibian declines by means of predation include alien species of fish, bullfrogs (*Lithobates catesbeianus*) and crayfish, but also turtles and apple snails (*Pomacea canaliculata*) (Kats & Ferrer, 2003; Polo-Cavia *et al.*, 2010; Karraker & Dudgeon, 2014). Competition by invaders such as African clawed frogs (*Xenopus laevis*) and bullfrogs may lead to shifts in amphibian behavior and community composition (D'Amore *et al.*, 2009; Lillo *et al.*, 2011). Similarly, invasive plants may alter amphibian abundance and community structure by changing the landscape (Maerz *et al.*, 2009; Watling *et al.*, 2011). Finally, invasive species may also act as disease reservoirs.

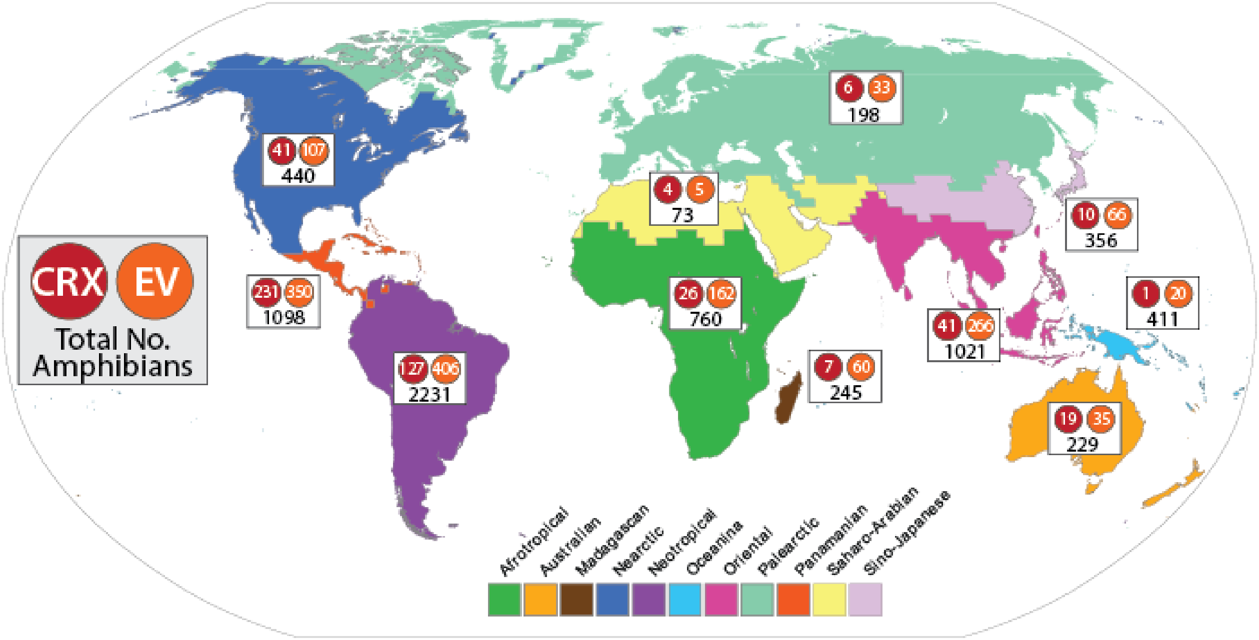

Figure 1: The number of Critically Endangered (CRX) and Vulnerable (EV) amphibian species in respect to the total number, per Wallacean Biome sensu Holt *et al.* (2013). Cartography by Michelle Koo, 2014, courtesy of AmphibiaWeb, 2019 https://amphibiaweb.org. University of California, Berkeley, CA, USA. Accessed 16 Jan 2020.

Other threats

Other threats faced by amphibians that cannot go unmentioned include over-exploitation and climate change. Amphibians are harvested from nature in various areas around the world for traditional medicine, to serve as food or fishing bait, and to serve as exotic pets (Picco & Collins, 2008; Mohneke *et al.*, 2009; Warkentin *et al.*, 2009; Rowley *et al.*, 2016). Such practice can lead to decreases in abundance and local or even regional extirpation when consistently performed over longer time periods, as for instance shown for *Quasipaa* frogs in China (Chan *et al.*, 2014).

Because amphibians are ectotherms (i.e. 'cold-blooded'), they produce little heat through metabolism. Activity of terrestrial amphibians is furthermore largely restricted to periods of rain or high humidity, as water loss rates of most species are high. What follows is that climate change is expected to significantly impact populations worldwide because most amphibians are assumed to possess limited means to buffer against shifting temperature- and precipitation regimes (Blaustein *et al.*, 2010). Nevertheless, while climate change has been shown to locally influence amphibian phenology it remains largely unclear which costs or benefits are associated with such changes (Blaustein *et al.*, 2001; Todd *et al.*, 2011). Moreover, most research that links climate to amphibian declines is correlative, due to which direct causal relationships need further research, and alternative hypotheses need to be considered

(Carey & Alexander 2003; Li *et al.*, 2013). It is however clear that climate change can act as secondary driver of amphibian declines, for instance by predisposing individuals to disease (Kiesecker *et al.*, 2001; Lips *et al.*, 2008; Cohen *et al.*, 2019a). This brings us to the threat that has gone largely unmentioned so far, and is the topic of this doctorate; amphibian disease.

III. The role of disease as driver of amphibian loss

Stuart *et al.* (2004) identified a group of 435 amphibian species during the first GAA that were declining rapidly, of which about half were affected by enigmatic stressors that were suspected or recently confirmed to include disease. Now, about 15 years later, there remains little doubt that pathogens do indeed threaten amphibian populations worldwide (e.g. Daszak *et al.*, 2003; Blaustein *et al.*, 2012; Scheele *et al.*, 2019). These pathogens may be viral, bacterial of fungal in nature, while trematodes and metazoans may parasitize on amphibians as well. The prevalence of some disease agents is staggering – for instance, infections with ranaviruses or *Batrachochytrium* fungi have been detected in hundreds of amphibian species (Skerratt *et al.*, 2007; Gray & Chinchar, 2015). Even while not all infections lead to disease it is assumed that *Batrachochytrium* spp. Alone have contributed to declines of hundreds amphibian species (Berger *et al.*, 1998; Lips *et al.*, 2006; Skerratt *et al.*, 2007). The various factors that fuel this situation can be summarized as follows.

Wide host ranges and establishment in the environment

First, globalisation increasingly facilitates pathogens to emerge beyond their native range (Martel *et al.*, 2014; O'Hanlon *et al.*, 2018). Examples include viral pathogens of the genus *Ranavirus* that have been spread across country borders through the amphibian pet trade, use of infected larval salamanders as fishing bait or by trade in frogs farmed for human consumption (Schloegel *et al.*, 2009; Kolby *et al.*, 2014; Brunner *et al.*, 2015). Similarly, *Batrachochytrium* species have invaded amphibian communities around the world following repeated human-mediated expansion from East-Asian source populations (O'Hanlon *et al.*, 2018; Byrne *et al.*, 2019). Successful pathogen invasions have regularly been shown to go hand-in-hand with the ability to infect a wide range of hosts and persist under an extensive range of environmental conditions (Martel *et al.*, 2014; Gray & Chinchar, 2015; Stegen *et al.*, 2017). Some hosts may become reservoirs that spread disease for weeks or even months. Furthermore, pathogens may be able to persist in the environment (i.e., outside of the host) for extended periods of time, such as *Batrachochytrium salamandrivorans* (Stegen *et al.*, 2017). What follows is that it remains highly difficult to remove amphibian pathogens from the environment once emerged (but see e.g. Bosch *et al.*, 2015), while invasion regularly leads to establishment; an epizootic presence becomes enzootic (Briggs *et al.*, 2010; Voyles *et al.*, 2018).

Lack of a protective immune response and sustained pathogenicity

Secondly, amphibians may not develop a protective immune response following non-lethal pathogen exposure, and pathogenicity of infectious agents may not attenuate over time (Stegen *et al.*, 2017; Voyles *et al.*, 2018). In theory, this means that pathogen re-emergence in a recovering population may be as destructive as the initial outbreak. An increasing amount of studies however show that host community build-up and environmental context influence the degree to which disease affects amphibian populations following an epizootic-enzootic transition. For instance, some populations that initially declined may persist at low host densities or even recover (Briggs *et al.*, 2010; Voyles *et al.*, 2018), in which absence of reservoir hosts or access to refuge habitats detrimental to pathogen persistence may play a role (Spitzen-van der Sluijs *et al.*, 2017; Brannelly *et al.*, 2018; Canessa *et al.*, 2019). Recent studies also suggest that in some cases evolution in host defences does take place (e.g. Voyles *et al.*, 2018). Nevertheless, cases in which an entire amphibian assemblage recovers are not known, and some of the species have reported to recover are present in much lower densities than before. Sustained pathogenicity also means that pathogen dispersal or further spread may lead to epizootic events in other populations (Spitzen-van der Sluijs *et al.*, 2018; Byrne *et al.*, 2019).

Synergy with other stressors

Finally, it is possible that other stressors or environmental conditions exacerbate disease. As already mentioned several times before, the outcome of amphibian host-pathogen interactions depends on their surroundings. This means that for instance climate conditions which weaken the host may tip the balance in favour of the disease agent (Raffel *et al.*, 2013; Cohen *et al.*, 2019a). Pollution can similarly weaken hosts to disease (Gendron *et al.*, 2003; Davidson *et al.*, 2007). However, beneficial environmental conditions may however also shelter amphibians against infection (e.g. Richards-Zawacki, 2010; Rowley & Alford, 2013; Blooi *et al.*, 2017). Gaining understanding on how the environment drives or inhibits amphibian disease is therefore a key to understand disease dynamics in the wild, which may in turn help to inform mitigation efforts and prioritize conservation actions (Bozzuto & Canessa, 2019). The second part of this introduction elaborates on environmental context, which is the focus of this doctorate.

IV. Environmental context matters

The notion that environmental context may influence the outcome of amphibian disease is not new (Blaustein *et al.*, 2012). It has also been acknowledged that spatial scale modulates the strength of different environmental factors; for instance, abiotic parameters such as climate are significant predictors of *Batrachochytrium dendrobatidis* prevalence at the landscape scale, while host richness shows increased importance at local scales (Cohen *et al.*, 2016). Much however remains unclear about how the environment shapes infection outcomes, and to what extent hosts and pathogens may use local environmental variation to their advantage. As an example, I will elaborate on temperature-driven disease dynamics of *Batrachochytrium* fungi, which cause the disease chytridiomycosis.

As amphibians are ectotherms, they produce little heat through metabolism and therefore rely on temporal and spatial variation in their thermal surroundings for overall activity and performance (Brattstrom, 1979; Feder & Lynch, 1982; Bovo *et al.*, 2018). Such thermal variation may govern patterns in microbiome composition, (micro)predation and migration timing, which are well-acknowledged drivers of amphibian disease (Blaustein *et al.*, 2012; Schmeller *et al.*, 2014; Woodhams *et al.*, 2014; Daversa *et al.*, 2018a; Kueneman *et al.*, 2019). Ambient temperature may however also directly determine the outcome of host-pathogen interactions, depending on thermal preferences and behaviour of either organism (Rowley & Alford, 2013; Sonn *et al.*, 2017; Voyles *et al.*, 2017; Sauer *et al.*, 2018; Sauer *et al.*, 2019; Stevenson *et al.*, 2020). For instance, temperatures below or above species thermal optima can strongly compromise the amphibian immune response (Cohen *et al.*, 2017; Greenspan *et al.*, 2017b). Changing climate regimes or conditions that favour pathogen rather than host fitness may further exacerbate this effect, which can result in significant population loss (Raffel *et al.*, 2013; Voyles *et al.*, 2017; Cohen *et al.*, 2019a).

Not all species however respond in a similar manner. Specifically, amphibians employ a range of physiological and behavioral mechanisms to temper climate fluctuations and maintain homeostasis, including evaporative cooling, active body temperature regulation, and microhabitat selection (reviewed by Bovo *et al.*, 2018). Various studies have shown that these mechanisms can be used to combat disease as well. For instance, amphibians infected with viral or bacterial agents may regulate their body temperature to invoke behavioural fever, which boosts the immune response and affects pathogen fitness. Routine body temperature regulation in the wild may similarly mediate infection dynamics, and protect amphibian hosts against colonisation by disease agents (Richards-Zawacki, 2010; Rowley & Alford, 2013).

How local environments shape infection outcomes, with on the one hand ambient temperature as principal driver of disease and on the other amphibian thermal responses as potential inhibitor, conclusively remains unclear (Raffel *et al.*, 2010; Richards-Zawacki, 2010; Becker & Zamudio, 2011; Rowley & Alford, 2013; Stevenson *et al.*, 2020). Such information could nevertheless aid in mitigating disease impacts, and inform conservation action (Scheele *et al.*, 2014; Heard *et al.*, 2018).

V. The emergence of *Batrachochytrium salamandrivorans*

Amphibian disease, pathogen invasion, and the intricacy of hosts-pathogen interactions with their environment recently came together through emergence of *Batrachochytrium salamandrivorans* (*Bsal*). This chytrid fungus, native to East-Asia, has been vectored to Europe at least twice where it was discovered in the wake of enigmatic declines among Dutch salamander populations (Martel *et al.*, 2013; Spitzen-van der Sluijs *et al.*, 2013). Whereas *Bsal* lives in apparent stable co-existence with hosts in its native range (Laking *et al.*, 2017), it drives declines and local extinction among European salamanders by causing chytridiomycosis (Spitzen-van der Sluijs *et al.*, 2016; Dalbeck *et al.*, 2018; Wagner *et al.*, 2019). Many European and American salamanders are lethally susceptible to *Bsal,* creating considerable concern about the potential impact of this pathogen on amphibian biodiversity (Martel *et al.*, 2014; Carter *et al.*, 2020).

Bsal morphology, physiology and pathogenesis

Bsal is a member of the Chytridiomycota, a phylum containing microscopic fungi that are characterized by the presence of motile, flagellated zoospores. Chytrid fungi generally occur in water bodies or moist soil, which permits the zoospores to disperse and in case of *Bsal* actively move towards potential hosts (Martel *et al.*, 2013; Van Rooij *et al.*, 2015). Zoospores are vulnerable to environmental stressors including temperature fluctuations and micropredators (e.g. Schmeller *et al.*, 2014), which *Bsal* counters through production of additional encysted spores (Stegen *et al.*, 2017). This second type of infectious spore shows increased environmental resistance and may float at the water surface where it quickly adheres to salamander skin or scales of the feet of waterfowl (Fig. 2).

Bsal zoospores eventually encyst by absorbing their flagella and secreting a wall to form a germling with fine tread-like rhizoids (Van Rooij *et al.*, 2015). The second part of the *Bsal* lifecycle ensues when the germling matures into a reproductive body or thallus known as a zoosporangium, in which the zoospores are produced. Discharge papillae or tubes, blocked inside by a plug, are formed during the growth of the sporangium. At maturity the plug dissolves after which zoospores are released into the environment. This entire process takes approximately five days at 15°C in the *Bsal* type strain (AMFP13/1) but may delay considerably at lower temperatures. Growth occurs at temperatures slightly above 0°C up to 20°C. *Bsal* does not survive negative temperatures or those that exceed 25°C, although encysted spores may tolerate temperature extremes for limited time periods (Martel *et al.*, 2013; Stegen *et al.*, 2017). Within its native range, *Bsal* has been found to infect *Tylototriton* newts in water temperatures close to or exceeding the critical maximum of the type strain, hinting that increased variation in thermal tolerance may exist throughout its wide East-Asian range (Laking *et al.*, 2017).

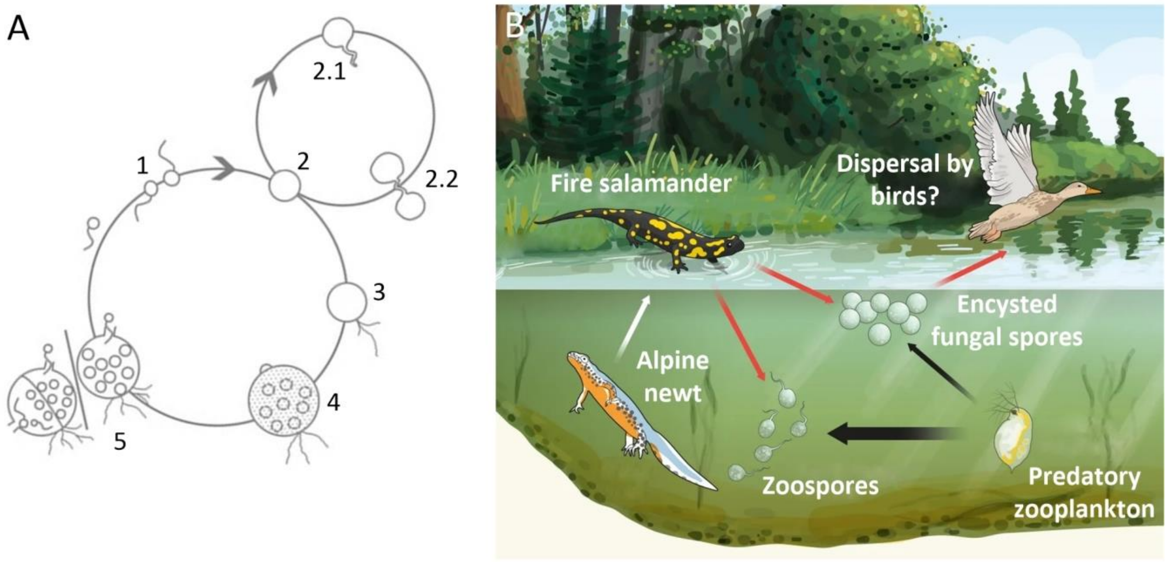

Figure 2: Life cycle (A) and factors that might contribute to spread (B) of *Batrachochytrium salamandrivorans* (*Bsal*). Numbers in panel A refer to flagellated motile zoospores (1); encysted zoospores (2); germling with germtube (2.1); transfer of the cell contents into a newly formed thallus (2.2); zoospore cyst with rhizoids (3); immature sporangium (4); and mature monocentric zoosporangium with discharge tube (at the right), colonial thallus containing several sporangia, each with their own discharge tube (at the left). Infected salamanders such as the fire salamander *Salamandra salamandra* shed motile and encysted zoospores, which may find their way to other individuals and species, including reservoir hosts such as the alpine newt *Ichthyosaura alpestris* (B). Predatory zooplankton and birds are potential drivers that influence *Bsal* dispersal, although their exact roles are in need of confirmation. Adapted from Van Rooij *et al.* (2015) and Fisher (2017).

As most chytrid fungi inhabit water bodies and moist soil where they are saprobic or parasitic on plants, algae or invertebrates, *Bsal* forms a rare exception by parasitizing on amphibians (Barr, 2001). Salamanders may become infected with *Bsal* through colonization of free-living zoospores and by contact with other salamanders, for instance during reproductive behavior (Martel *et al.*, 2013; Stegen *et al.*, 2017). Not all salamanders are equally susceptible to *Bsal*, which leads to the creation of reservoirs hosts that may shed zoospores for weeks or even months before developing clinical disease (Martel *et al.*, 2014). *Bsal* colonization of the amphibian skin is followed by growth of numerous intracellular colonial thalli that spread over the epidermis, and marked necrosis of the adjacent keratinocytes (Van Rooij *et al.*, 2015). The resulting erosions and epidermal ulcerations coincide with clinical signs that include excessive skin shedding, anorexia, apathy, ataxia and death (Fig. 3). Asymptomatic infection has been shown to occur in captive collections as well (Sabino-Pinto *et al.*, 2018). Why *Bsal* affects salamanders but is rarely found in anurans, and whether or not effective immune responses are elicited during infection remain unclear (Van Rooij *et al.*, 2015).

Figure 3: A fire salamander *Salamandra salamandra* that succumbed to chytridiomycosis caused by *Batrachochytrium salamandrivorans*. Note the presence of ulcerations on the head and limbs.

Bsal origin and spread

Bsal is native to East-Asia, where its eastern distribution border is formed by the main Japanese islands, the Ryukyu Archipelago and Taiwan (Martel *et al.*, 2014; Beukema *et al.*, 2018). On the Asian mainland this pathogen occurs throughout southern China, northern Vietnam and in northern Thailand, while presence in Laos is deemed likely (Martel *et al.*, 2014; Laking *et al.*, 2017; Yuan *et al.*, 2018). Little is known about the ecology of *Bsal* in its native range, although the few existing studies suggest that overall prevalence in the wild is low, while salamander hosts do not appear to be (severely) impacted by presence of the fungus (Laking *et al.*, 2017; Yuan *et al.*, 2018). At least fifteen species from seven different genera in three distinct salamander families have nevertheless been reported to carry *Bsal*, including the regularly-traded fire-bellied newts (*Cynops*, *Paramesotriton*) and *Tylototriton* crocodile newts (Rowley *et al.*, 2016; Yuan *et al.*, 2018). *Bsal* has also been detected in commercial Chinese giant salamander *Andrias* spp. Farms (Yuan *et al.*, 2018), which number in the hundreds throughout China and significantly contribute to illegal wildlife trade and spread of disease (Cunningham *et al.*, 2018). As a result, *Bsal* has likely been vectored out of Asia multiple times, resulting for example in establishment and spread within various European captive collections (Sabino-Pinto *et al.*, 2015; Fitzpatrick *et al.*, 2018). Such introductions have led to salamander mortality (Fitzpatrick *et al.*, 2018), but may also cause asymptomatic infection (Sabino-Pinto *et al.*, 2018), which considerably increases risk for further spread. Survey efforts have so far not lead to discovery of this pathogen within US salamander collections (Klocke *et al.*, 2017).

Introduction to Europe

Although it remains unsure when *Bsal* was introduced to Europe, its presence has been confirmed in two disjunct areas. The first comprises Montnegre and El Corredor Nature Reserve, in Catalonia, Spain, where declines in several newt species were observed, of which some were not native to the region. Pathogen spread in this area appears to have been successfully contained due to rapid conservation actions, partially motivated by the close presence of *Calotriton arnoldi*, a vulnerable micro-endemic brook newt. The other area, in which salamander declines led to the discovery and description of *Bsal*, comprises the general border area of Belgium, the Netherlands and Germany (Spitzen-van der Sluijs *et al.*, 2016; Dalbeck *et al.*, 2018; Wagner *et al.*, 2019). Here, *Bsal* affects at least wild *Salamandra salamandra* and *Ichthyosaura alpestris*. Extensive mortality is especially observed in *S. salamandra* populations in easternmost Belgium and the German the Eifel region and Ruhrgebiet. Negative impacts on the newts *Lissotriton vulgaris* and *Triturus cristatus*, also present in this area remain largely unclear. This ambiguity may partially stem from differences in host susceptibility – for instance, *I. alpestris* has been shown to sustain or even clear infection with low *Bsal* doses under laboratory conditions (Martel *et al.*, 2014; Stegen *et al.*, 2017).

In order to halt the advance of *Bsal* and prevent further amphibian population declines, the European Commission (EC) launched the call for tender 'Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity (ENV.B.3/SER/2016/0028)' in March 2017. The aims of the European consortium that has assembled under this banner include creation of an early warning system and emergency action plan, with development of sustainable, long term mitigation measures as long-term goal. Knowledge on the distribution and ecology of *Bsal* is fundamental to these efforts (Thomas *et al.*, 2019). In this thesis, I focus on the role of the environment, and particularly temperature, in shaping *Bsal* disease dynamics in northwestern Europe. Emphasis is furthermore given to improving spatial predictions of *Bsal* spread.

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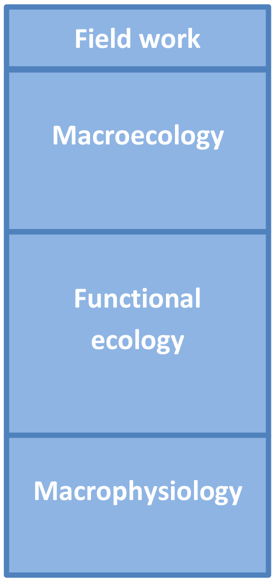
Scientific aims

Batrachochytrium salamandrivorans (*Bsal*) is a salamander-pathogenic chytrid fungus native to East-Asia, which has been introduced to Europe. The invasion of *Bsal* has led to declines and local extinction among especially fire salamander *S. salamandra* populations, but other species are affected as well while *Bsal* expands its geographical distribution and host range. In order to mitigate the impact of this pathogen it is crucial to gain practical understanding of its disease ecology, which may be used to direct further research and prioritize conservation efforts.

The general goal of this PhD was to assess the role of the environment, and particularly temperature, in shaping *Bsal* disease dynamics in north-western Europe. Emphasis was furthermore given to improve spatial predictions of *Bsal* spread. Specific steps to reach this aim were to;

1. Obtain a detailed *Bsal* distribution overview through field surveillance;
2. Use this overview to characterise and compare the invasive and native bioclimatic *Bsal* niches to estimate potential for future colonisation and spread in the Western Palearctic;
3. Explore the influence of selected climatic drivers at local scale by assembling a primer on thermal ecology of a *Bsal* host assemblage during three years, and mapping thermal host behaviour to *Bsal* contact;
4. Improving predictions of landscape invasibility to *Bsal* across Europe by incorporating the obtained data on pathogen-host interactions.



This thesis sets off with two introductory chapters, in which the impact of chytridiomycosis on amphibians worldwide is determined (Chapter 1) and the main model host, fire salamander *Salamandra salamandra* is presented (Chapter 2). Then, the first specific aim is addressed in Chapter 3 which presents an overview of the European *Bsal* distribution obtained through extensive field surveys. Chapter 4 deals with the second aim, which is tackled using ordination-based niche overlap analyses and ecological niche modelling. Potential for further *Bsal* colonisation and spread across the Western Palearctic is estimated, while taking into account if, and how differences between the invasive and native climatic *Bsal* niches may influence predictions. Focus is then shifted to local level in Chapter 5, which combines data from field surveys, a mesocosm experiment and a series of thermal infection experiments to investigate fire salamander thermal behaviour during *Bsal* contact, relative to local environmental context (the fourth specific aim). These experiments lay the groundwork for creation of a biophysical model, also included in Chapter 5 and comprising the final specific aim, which explores if, and to what extent fire salamanders can find refuge in their thermal surroundings in the face of *Bsal* invasion.

Chapter 1

Chapter 1

Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity

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My contribution to this chapter consisted of extracting range size characteristics and bioclimatic data using IUCN distribution data, and additions to the natural history dataset through literature searches.

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Abstract

Anthropogenic trade and development have broken down dispersal barriers, facilitating the spread of diseases that threaten Earth's biodiversity. We present a global, quantitative assessment of the amphibian chytridiomycosis panzootic, one of the most impactful examples of disease spread, and demonstrate its role in the decline of at least 501 amphibian species over the past half-century, including 90 presumed extinctions. The effects of chytridiomycosis have been greatest in large-bodied, range-restricted anurans in wet climates in the Americas and Australia. Declines peaked in the 1980s, and only 12% of declined species show signs of recovery, whereas 39% are experiencing ongoing decline. There is risk of further chytridiomycosis outbreaks in new areas. The chytridiomycosis panzootic represents the greatest recorded loss of biodiversity attributable to a disease.

Highly virulent wildlife diseases are contributing to Earth's sixth mass extinction (Fisher *et al.*, 2012). One of these is chytridiomycosis, which has caused mass amphibian die-offs worldwide (Lips *et al.*, 2006; Skerratt *et al.*, 2007). Chytridiomycosis is caused by two fungal species, *Batrachochytrium dendrobatidis* [discovered in 1998, Berger *et al.* (1998)] and *B. salamandrivorans* [discovered in 2013, Martel *et al.* (2014)]. Both *Batrachochytrium* species likely originated in Asia, and their recent spread has been facilitated by humans (Martel *et al.*, 2014; O'Hanlon *et al.*, 2018). Twenty years after the discovery of chytridiomycosis, substantial research has yielded insights about its epidemiology (Skerratt *et al.*, 2007; Olson *et al.*, 2013; James *et al.*, 2015; Lips, 2016), yet major knowledge gaps remain. First, the global extent of species declines associated with chytridiomycosis is unknown [see (Stuart *et al.*, 2004; Skerratt *et al.*, 2007) for initial assessments]. Second, although some regional declines are well studied, global spatial and temporal patterns of chytridiomycosis impacts remain poorly quantified. Third, ecological and life history traits have been examined only for a portion of declined species (Lips *et al.*, 2003; Bielby *et al.*, 2008). Finally, after initial declines, it is unknown what proportion of declined species exhibit recovery, stabilize at lower abundance, or continue to decline. Here we present a global epidemiological analysis of the spatial and temporal extent of amphibian biodiversity loss caused by chytridiomycosis. We conducted a comprehensive examination of evidence from multiple sources, including the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2018), peer-reviewed literature, and consultation with amphibian experts worldwide (data S1). We classified declined species into five decline-severity categories corresponding to reductions in abundance. Species declines were attributed to chytridiomycosis on the basis of diagnosis of infection causing mortalities in the wild or, if this was unavailable, evidence consistent with key epidemiological characteristics of this disease. Most evidence is retrospective because many species declined before the discovery of chytridiomycosis (data S1).

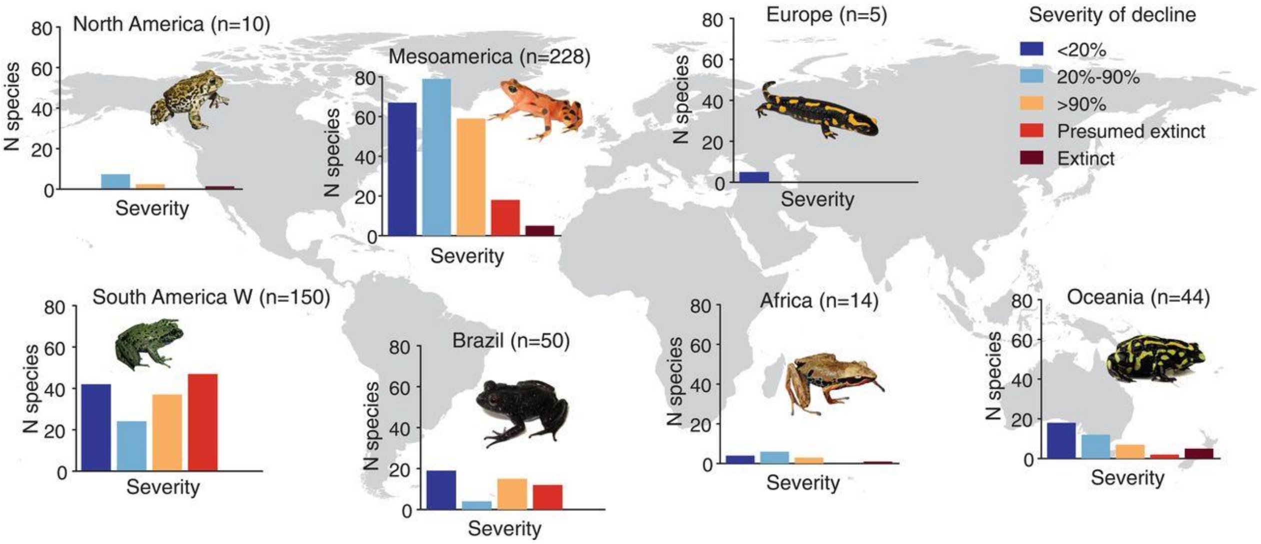

Figure 1: Global distribution of chytridiomycosis-associated amphibian species declines. Bar plots indicate the number (N) of declined species, grouped by continental area and classified by decline severity. Brazilian species are plotted separately from all other South American species (South America W); Mesoamerica includes Central America, Mexico, and the Caribbean Islands; and Oceania includes Australia and New Zealand. No declines have been reported in Asia. N, total number of declines by region. [Photo credits (clockwise from top left): *Anaxyrus boreas*, C. Brown, U.S. Geological Survey; *Atelopus varius*, B.G.; *Salamandra salamandra*, D. Descouens, Wikimedia Commons; *Telmatobius sanborni*, I.D.l.R; *Cycloramphus boraceiensis*, L.F.T.; *Cardioglossa melanogaster*, M.H.; and *Pseudophryne corroboree*, C. Doughty]

We conservatively report that chytridiomycosis has contributed to the decline of at least 501 amphibian species (6.5% of described amphibian species; Figs. 1 and 2). This represents the greatest documented loss of biodiversity attributable to a pathogen and places *B. dendrobatidis* among the most destructive invasive species, comparable to rodents (threatening 420 species) and cats (*Felis catus*) (threatening 430 species) (Doherty *et al.*, 2016). Losses associated with chytridiomycosis are orders of magnitude greater than for other high-profile wildlife pathogens, such as white-nose syndrome (*Pseudogymnoascus destructans*) in bats (six species; Thogmartin *et al.*, 2013) or West Nile virus (*Flavivirus* sp.) in birds (23 species; George *et al.*, 2015). Of the 501 declined amphibian species, 90 (18%) are confirmed or presumed extinct in the wild, with a further 124 (25%) experiencing a >90% reduction in abundance (Figs. 1 and 2). The declines of all species except one (*Salamandra salamandra* affected by *B. salamandrivorans*) were attributed to *B. dendrobatidis*.

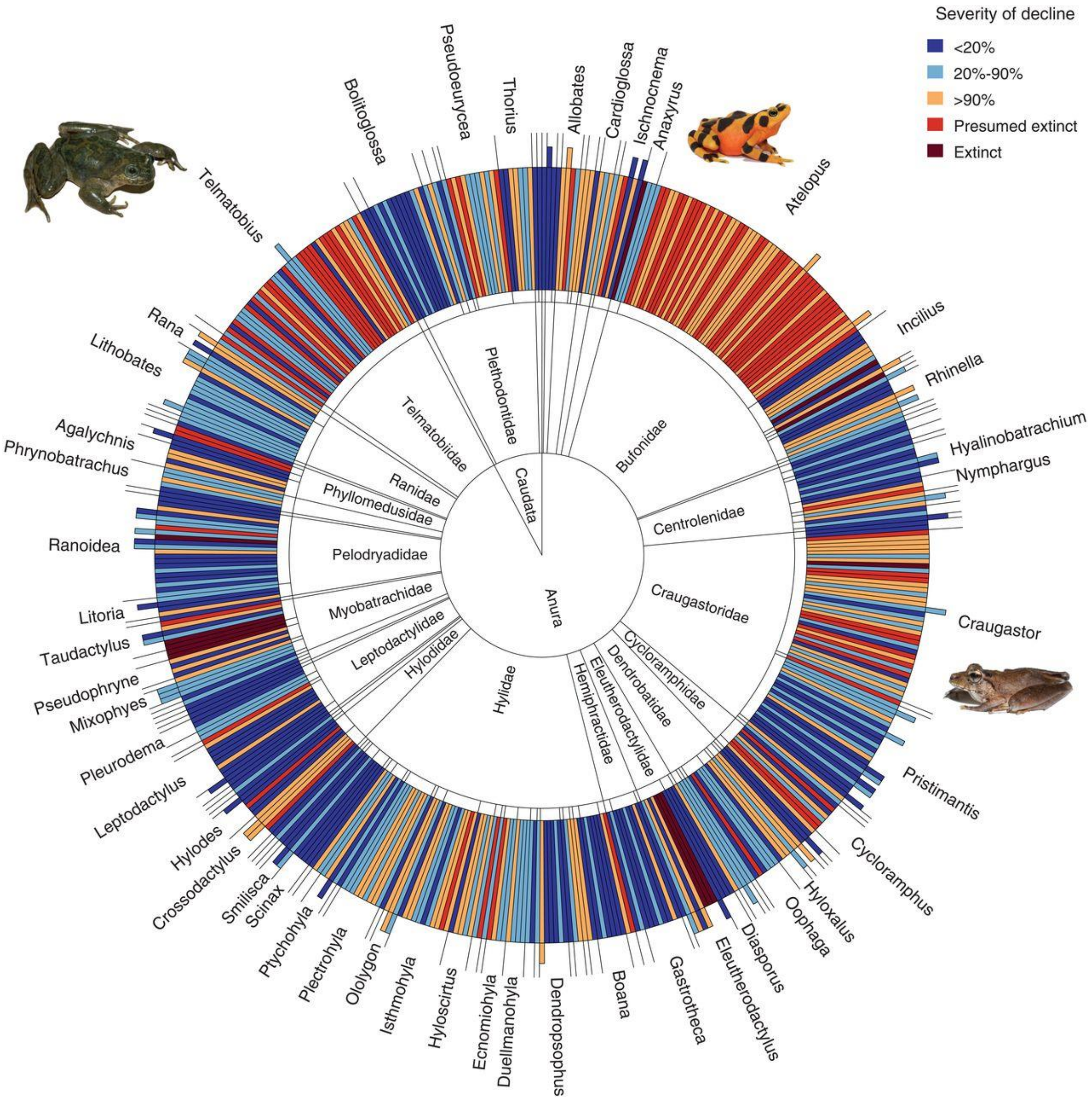

Figure 2: Taxonomic distribution of chytridiomycosis-associated amphibian declines. Each bar represents one species, and color denotes the severity of its decline. Concentric circles indicate, from inner to outer, order (Caudata or Anura), family, and genus. Full names are given only for families and genera that include >5 and >2 species, respectively; details for all taxa are in table S4. Within each taxonomic level, sublevels are ordered alphabetically. Protruding bars indicate species for which there is evidence of recovery. [Photo credits (left to right): *Telmatobius bolivianus*, I.D.l.R.; *Atelopus zeteki*, B.G.; and *Craugastor crassidigitus*, B.G.]

Declines were proportional to taxonomic abundance, with anurans having 93% of severe declines (they comprise 89% of all amphibian species). Within anurans, there has been marked taxonomic clustering of declines, with 45% of severe declines and extinctions occurring in the Neotropical genera *Atelopus, Craugastor*, and *Telmatobius* (Fig. 2) (La Marca *et al.*, 2005). Chytridiomycosis is lethal to caecilians (Gower *et al.*, 2013), but there have been no caecilian declines due to the disease, although data are limited. The capacity for *B. dendrobatidis* to cause major declines is attributable to its maintenance of high pathogenicity (Skerratt *et al.*, 2007; Voyles *et al.*, 2018), broad host range (Olson *et al.*, 2013), high transmission rate within and among host species (Skerratt *et al.*, 2007; James *et al.*, 2015), and persistence in reservoir host species and the environment (Valenzuela-Sánchez *et al.*, 2017). For many species, chytridiomycosis is the principal driver of decline, exemplified by precipitous mass mortalities in undisturbed environments (Skerratt *et al.*, 2007). In other species, chytridiomycosis acts in concert with habitat loss, altered climatic conditions, and invasive species to exacerbate species declines (Wake & Vredenburg, 2008).

Most amphibian declines have occurred in the tropics of Australia, Mesoamerica, and South America (Fig. 1), supporting the hypothesis that *B. dendrobatidis* spread from Asia into the New World (O'Hanlon *et al.*, 2018). Asia, Africa, Europe, and North America have had notably low numbers of declines attributable to chytridiomycosis, despite widespread occurrence of *B. dendrobatidis* (Olson *et al.*, 2013). Relative lack of documented declines could reflect less knowledge of amphibian populations in Asia and Africa (Houlahan *et al.*, 2000; Lips, 2016), early introduction and potential coevolution of amphibians and *B. dendrobatidis* in parts of Africa and the Americas [e.g., (Talley *et al.*, 2015)], the comparatively recent emergence of *B. dendrobatidis* in Western and Northeast Africa (O'Hanlon *et al.*, 2018), or unsuitable conditions for chytridiomycosis. It remains unknown whether chytridiomycosis contributed to widespread amphibian declines reported in North America and Europe in the 1950s to 1960s (Houlahan *et al.*, 2000; Talley *et al.*, 2015; Lips, 2016) or current enigmatic salamander declines in eastern North America. Although the number of new declines has now eased (Fig. 3), additional declines could occur if *B. dendrobatidis* or *B. salamandrivorans* are introduced into new areas, highly virulent lineages are introduced into areas that currently have less-virulent lineages (O'Hanlon *et al.*, 2018), and/or environmental changes alter previously stable pathogen-host dynamics (Lips, 2016).

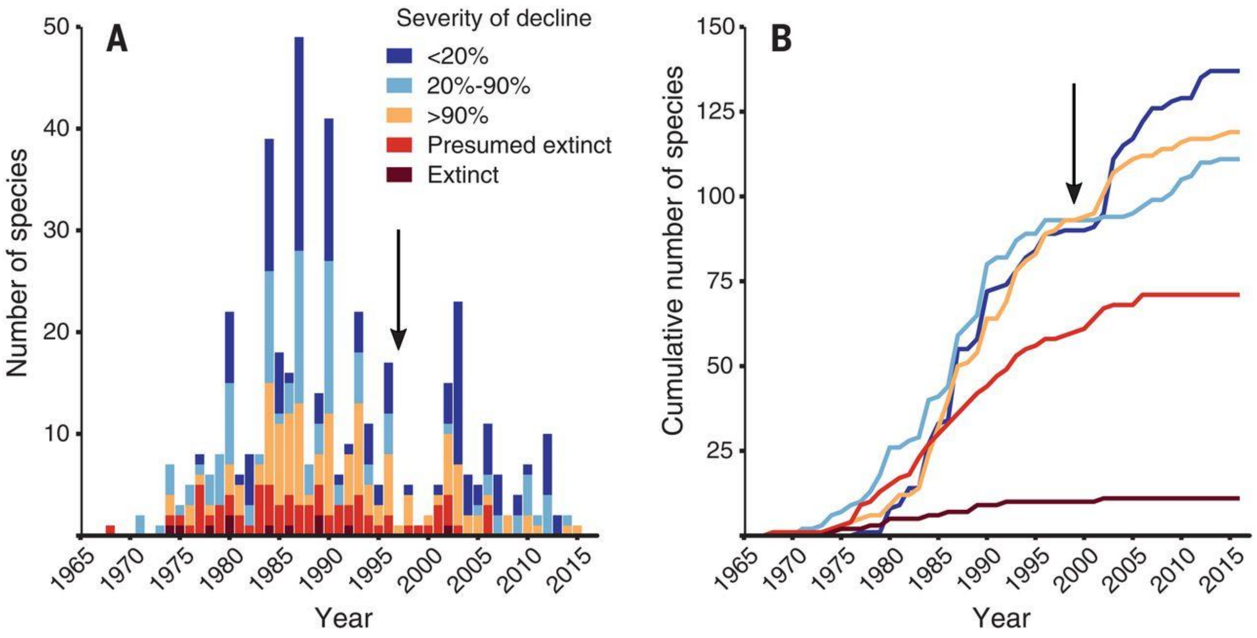

Figure 3: Timing of chytridiomycosis-associated amphibian declines. (A) Declines by year. Bars indicate the number of declines in a given year, stacked by decline severity. For species for which the exact year of decline is uncertain, the figure shows the middle year of the interval of uncertainty, as stated by experts or inferred from available data. (B) Cumulative declines. Curves indicate the cumulative number of declines in each decline-severity category over time. In (A) and (B), the arrows mark the discovery of chytridiomycosis in 1998.

Chytridiomycosis-associated declines peaked globally in the 1980s, between one and two decades before the discovery of the disease (Fig. 3 and table S1), and coincident with anecdotal recognition of amphibian declines in the 1990s (Wake, 1991). A second, smaller peak occurred in the early 2000s, associated with an increase in declines in western South America (Fig. 3 and fig. S1). Regionally, temporal patterns of decline are variable (fig. S1). For example, in some areas of South America and Australia, declines commenced in the late 1970s (Skerratt *et al.*, 2007; Lips *et al.*, 2008), whereas in other areas, declines started in the 2000s (Catenazzi *et al.*, 2011). *B. dendrobatidis* is associated with ongoing declines in 197 assessed species. Ongoing declines after a transition to enzootic disease dynamics (Valenzuela-Sánchez *et al.*, 2017) might be driven by a lack of effective host defenses, maintenance of high pathogenicity (Voyles *et al.*, 2018), and presence of *B. dendrobatidis* in amphibian and nonamphibian reservoirs (James *et al.*, 2015; Valenzuela-Sánchez *et al.*, 2017).

We examined host life history traits and environmental conditions to understand why some species declined more severely than others, using multinomial logistic regression and accounting for the degree of evidence that chytridiomycosis was implicated in each species's decline (Fig. S2 and Table S2). Decline severity was greatest for larger-bodied species, those occurring in consistently wet regions, and those strongly associated with perennial aquatic habitats. These patterns are likely due to favorable environmental conditions for *B. dendrobatidis* in wet regions (James *et al.*, 2015), because the fungus dies when desiccated, as well as the general pattern of increased time to maturity in large-bodied amphibians resulting in less reproductive potential to offset mortality due to chytridiomycosis (Morrison & Hero, 2003). Declines were less severe for species with large geographic and elevational ranges (Fig. 4), potentially owing to the greater chance of their range encompassing environmental conditions unfavorable for *B. dendrobatidis* (Lips, 2016) and/or information bias, because population extinctions can be assessed with more certainty in restricted-range species. Our results are consistent with previous studies that show that the risk of chytridiomycosis is associated with host aquatic habitat use, large body size, and narrow elevational range (Lips *et al.*, 2003; Bielby *et al.*, 2008).

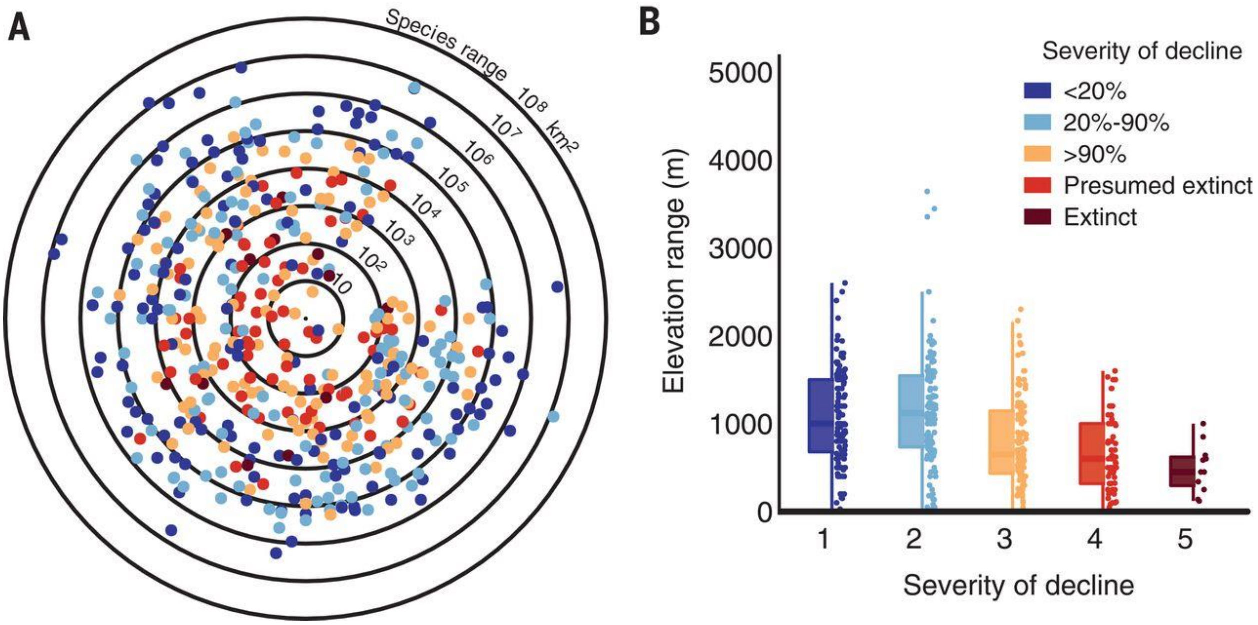

Figure 4: Severity of chytridiomycosis-associated amphibian declines in relation to the geographic and elevational ranges of species. (A) Declines in relation to geographic range. Each dot indicates a species, located randomly along the perimeter of a circle with radius equal to the log10 of the species's geographic range in kilometers squared. (B) Declines in relation to elevational range. Horizontal bars, boxes, and vertical bars indicate, respectively, mean, first and second quartiles, and 95% quantiles of elevation ranges within each category of decline severity.

Encouragingly, of the 292 surviving species for which population trends are known, 60 (20%) have shown initial signs of recovery. However, recoveries generally represent small increases in abundance of individual populations, not complete recovery at the species level. Logistic regression showed the probability of recovery was lower for species that experienced more recent or more severe declines, for large-bodied or nocturnal species, and for species occurring at higher elevations (fig. S2 and table S3). When holding those predictors of recovery at their mean value, the chance of a species recovering from a severe (>90%) decline was less than 1 in 10. Low probability of recovery for high-elevation species might be related to suitable climatic conditions for fungal persistence as well as limited connectivity to source populations and/or longer host generation time (Morrison & Hero, 2003). Some recoveries may be underpinned by selection for increased host resistance (Voyles *et al.*, 2018), whereas management of concurrent threats may have facilitated other recoveries (a promising avenue for conservation interventions) (Knapp *et al.*, 2016). Unfortunately, the remaining 232 species have shown no signs of recovery.

The unprecedented lethality of a single disease affecting an entire vertebrate class highlights the threat from the spread of pathogens in a globalized world. Global trade has recreated a functional Pangaea for infectious diseases in wildlife, with far-reaching impacts on biodiversity (this study), livestock (Wiethoelter *et al.*, 2015), and human health (Olival *et al.*, 2017). Effective biosecurity and an immediate reduction in wildlife trade are urgently needed to reduce the risk of pathogen spread. As mitigation of chytridiomycosis in nature remains unproven (Garner *et al.*, 2016), new research and intensive monitoring that utilizes emerging technologies are needed to identify mechanisms of species recovery and develop new mitigation actions for declining species.

Supplementary methods, figures and data associated with this publication can be found at https://science.sciencemag.org/content/suppl/2019/03/27/363.6434.1459.DC1.

Our methods used to assign evidence categories linking chytridiomycosis to species declines were criticized by Lambert *et al.* (2020). Their technical comment can be found at https://doi.org/10.1126/science.aay1838. Our response to this comment has been added to this chapter as supplementary material.

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Supporting Information for

Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity

Response to Comment on "Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity"

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Lambert et al. question our retrospective and holistic epidemiological assessment of the role of chytridiomycosis in amphibian declines. Their alternative assessment is narrow and provides an incomplete evaluation of evidence. Adopting this approach limits understanding of infectious disease impacts and hampers conservation efforts. We reaffirm that our study provides unambiguous evidence that chytridiomycosis has affected at least 501 amphibian species.

In Scheele *et al.* (2019), we quantified the impact of chytridiomycosis—a disease first described in 1998—on the world's amphibians. Our contribution builds on previous work that inextricably links chytridiomycosis and global amphibian declines (Skerratt *et al.*, 2007) and is underpinned by extensive research on two fungal species that cause chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). Our assessment concluded that the disease has contributed to the decline of at least 501 species, including 90 presumed extinctions. Our estimate is likely conservative, as the disease has caused declines in undescribed species (Crawford *et al.*, 2010). Lambert *et al.* (2020) challenge our methodology, focusing on the evidence we used to assign "strength of evidence scores" that implicate chytridiomycosis in declines.

Lambert et al. claim that our strength of evidence scores were not adequately justified, and reassigned species from higher scores to the lowest score of "expert opinion of the assessor only" (Scheele *et al.*, 2019). The fundamental difference between our approach and that of Lambert et al. is that we incorporated expert knowledge of amphibian species and integrated a comprehensive epidemiological framework to evaluate all available evidence. In contrast, the Lambert et al. approach lacked species expertise, applied a limited evidence framework, and used only easily accessible sources. They claim that their approach is more replicable and hence more rigorous. However, on the contrary, their exclusion of expertise, evidence, and data is neither comprehensive, nor is it best practice. For example, using a less comprehensive assessment led Lambert et al. to question some of the most well-documented chytridiomycosis-driven declines [e.g., *Bsal*-driven salamander collapses in northern Europe and *Atelopus chiriquiensis* declines in Mesoamerica (Lips, 1998; Martel *et al.*, 2013)]. Thus, adopting a restricted approach may seriously limit understanding of disease impacts and hamper conservation efforts in the face of the current mass extinction crisis (Johnson *et al.*, 2017).

The narrow approach adopted by Lambert et al. ignores information we provided justifying species categorization as either "single line of correlative evidence" or "multiple lines of correlative evidence." Lambert et al. assumed the data provided in columns J to M of data S1 (Scheele *et al.*, 2019) to be the only information justifying the assigned strength of evidence scores, and hence misrepresent our methodology. However, we used multiple lines of evidence that were not all captured in these four columns [supplementary materials of (Scheele *et al.*, 2019)]. We included columns J to M to show what published reports were available for these common and easy-to-categorize lines of evidence. However, our strength of evidence scores were informed by integrating evidence from 454 cited references, which was supplemented in some cases with expert judgment (see below). This holistic assessment, considering all available evidence, was only possible by drawing together information regarding specific species and locations, amphibian biology, disease ecology, epidemiology, and pathology.

Lambert et al. critique our use of expert opinion. Expert judgment is commonly used in many scientific fields and is necessary to critically evaluate multiple lines of evidence. Following best practices in expert elicitation (Sutherland & Burgman, 2015), we assembled a large, diverse, and global group that combined disease and amphibian expertise with a first-hand understanding of the broader amphibian conservation challenges in the assessed countries. Furthermore, our assessments were accomplished via iterative group processes, using well-defined, structured, quantitative questions, with assessments cross-checked by the group of experts (Sutherland & Burgman, 2015). For transparency, and to help readers understand our methodology, we included eight “worked examples” in our original paper [see data S1 (Scheele *et al.*, 2019)], but these were overlooked by Lambert et al. Thus, our study provides the best-vetted and comprehensive dataset on amphibian declines at a global scale, and is underpinned by a substantial body of empirical research.

Lambert et al. make an unsubstantiated claim that our referencing is inaccurate. Our assessment included evidence of species declines that occurred before *Bd* was described but are now linked to chytridiomycosis [figure 3, A and B, in (Scheele *et al.*, 2019)]. The fact that many declines subsequently attributed to chytridiomycosis are described in publications predating the description of *Bd*, and that many of those publications tentatively suggested other drivers of decline, neither invalidates data contained in those sources nor contradicts more recent findings implicating chytridiomycosis. In fact, pre-Bd sources provide invaluable information regarding the spatiotemporal patterns and processes of species declines that are fundamental components of holistic epidemiological assessments (O’Hanlon *et al.*, 2018). For example, research published in 1989 documented the decline of Australia’s iconic corroboree frogs (*Pseudophryne corroboree*) and noted that declines occurred during a drought (Osborne, 1989). However, subsequent research has clearly demonstrated the role of chytridiomycosis in corroboree frog declines (Scheele *et al.*, 2017).

Lambert et al.'s critique raises several deeper issues for the study of infectious disease and conservation. Lambert et al. treat expert knowledge and retrospective evidence as unreliable (at best) and suspicious (at worst) sources of information. Used rigorously, such forms of evidence are fundamental epidemiological tools routinely applied to diagnose the role of pathogens in disease outbreaks (Dohoo *et al.*, 2009), particularly for declines that occurred before a causative agent is identified, as with chytridiomycosis. More broadly, omitting evidence compromises the ability to achieve a global overview by biasing assessments to regions that have more resources, infrastructure, and funding for wildlife research. Heterogeneity of evidence is a common challenge in conservation (Wilson *et al.*, 2016; Christie *et al.*, 2019). Such challenges are amplified when integrating data at a global scale. For conservation information to be applicable to as many regions as possible, we need to rigorously consider all evidence available.

We are in an era of unprecedented biodiversity loss (Johnson *et al.*, 2017). The scientific community has a great responsibility to be both rigorous and holistic in providing data that are unbiased and inclusive of all the information available. Our research demonstrates that chytridiomycosis has irrefutably devastated amphibians and remains a global threat. Our timely assessment documents the current state of knowledge, which researchers can augment as new information becomes available. Under the scenario of ongoing amphibian declines, the scientific community has five important tasks: (i) to describe and quantify threats to biodiversity, (ii) to help prevent further declines and extinctions due to chytridiomycosis, (iii) to facilitate the recovery of affected species, (iv) to build on the lessons learned from chytridiomycosis to prevent further wildlife panzootics, and (v) to document current species distributions and abundances (with appropriate metadata) to provide baseline data against which to assess the impacts of future emerging diseases (Morrison *et al.*, 2017). In accomplishing these goals, we can confront the global conservation challenge.

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Chapter 2

Chapter 2

Quick Guide: *Salamandra*

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What is* Salamandra*? The best known salamander genus of Europe and adjoining regions, most of whose members display extensive phenotypic polymorphism in terms of body sizes, color patterns and reproductive modes. *Salamandra* comprises six species. Two of these are live-bearing (viviparous) mountain specialists (the 'Alpine salamanders') which are completely black. The other four species (the 'fire salamanders') generally give birth to aquatic larvae (larviparous) and are characterized by additional yellow and sometimes red and grey spots, stripes or blotches, giving these amphibians a rather exotic appearance (Figure 1). A select number of fire salamander subspecies have independently viviparity.

Figure 1: (A) Male *S. salamandra fastuosa*; (B) Iberian *S. salamandra gallaica* may display extensive red coloration; (C) Lanza's salamander, *S. lanzai*; (D) Toxins excreted from dorsal and lateral glands by *S. salamandra*; (E) Pattern variation in a single population of *S. salamandra bernardezi*; (F) Viviparous *S. salamandra bernardezi* with neonate; (G) Amplexus of male (bottom) and female (top) *S. salamandra terrestris*. (Photos: Wouter Beukema (A–C, E), Guillermo Velo-Antón (D, F), Jeroen Speybroeck (G).)

'Fire' salamanders? About two millennia ago, Pliny the Elder threw a salamander into the fire to see whether its icy body temperature would allow the animal to survive, and even extinguish the flames, as had initially been claimed by Aristotle. Whereas the salamander failed to do so, Pliny confirmed Aristotle's claims in his Naturalis Historia. On the side, Pliny ascribed an extremely high degree of toxicity to the beast. These myths persisted for hundreds of years, after which medieval observations of hibernating fire salamanders escaping from burning firewood might have added fuel to the fire. Although actual body temperatures of *Salamandra* species fall within the range of those deemed characteristic for amphibians, both fire and Alpine salamanders are indeed poisonous. When threatened, these salamanders may release skin secretions (Figure 1D) containing steroidal alkaloids called 'samandarines'.

These alkaloids are not derived from food items, which is a rare exception among amphibians. Although generally harmless to the human skin, samandarin can be highly detrimental when ingested.

Why are Salamandra species interesting? Like most Eurasian salamanders, fire and Alpine salamanders do not deposit eggs. Instead, they deposit fully-developed juveniles, or aquatic larvae. *Salamandra* species generally occur close to streams in forests, often in hilly or mountainous terrain. However, a high degree of humidity also allows fire salamanders to persist on cliffs along the Atlantic Coast, or in Mediterranean cork-oak forests. Taking a wide variety of small invertebrates as food, *Salamandra*s can live for several decades. Owing to their high degree of phenotypic diversity (Figure 1), *Salamandra* species are ideal study subjects for evolutionary biologists. While ice ages and island formation promoted historical, allopatric divergence, recent ecological (i.e. sympatric) differentiation has occurred. Specifically, larval adaptation to either still or flowing water in a Central European population of *S. salamandra* eventually drives females to select males originating from the same habitat, regardless of genetic distance. Furthermore, larval presence in Mediterranean temporary ponds affects the food web of this fragile ecosystem, leading to an increase in the amount of periphyton and bacteria. Fire salamanders play a significant role in the global amphibian crisis, with various populations suffering or disappearing due to chytridiomycosis, a fungal disease that is decimating amphibian populations worldwide.

What drives morphological variation in Salamandra? It was long thought that the color pattern in the fire salamanders is a warning coloration. However, the uniformly black Alpine salamanders possess similar toxins to their patterned cousins. As the two similar looking Alpine salamanders are not sister species, scientists presume that external drivers (such as climate, or predators) influence variation in color pattern. This assumption is supported by the recent discovery of color polymorphism among several north Spanish populations of *S. salamandra* (Figure 1E). Climate is known to influence body shape as species in hotter and drier regions of the genus's range have adopted a digging lifestyle. In addition, viviparous individuals, comprising the Alpine salamanders but also several fire salamander populations, are generally smaller than their larviparous relatives, owing to differences in embryonic growth rates.

What else is known about differences in reproductive modes? Salamanders deposit eggs above or under water. However*, Salamandra* species reproduce in a slightly different way, as eggs are retained in the body until birth can be given to aquatic larvae, or even fully developed young. Most fire salamander populations deposit larvae (they are larviparous). For instance, *Salamandra infraimmaculata* females spread their larvae over several water bodies that provide some degree of shelter. Local behavioral adaptation to conditions like the presence of predators may similarly induce *S. salamandra* females to breed in underground streams or ponds. Conversely, Alpine salamanders, but also several populations of the fire salamanders *S. salamandra* and *S. algira* have independently evolved viviparity. Greater independence from water bodies, which facilitates the colonization of new environments, is thought to underlie this transition. In Alpine salamanders, a single egg is fertilized per oviduct, where the larvae feed on undeveloped eggs for several years. The gestation period ends with the birth of two fully developed, terrestrial salamanders. Viviparity in fire salamander populations is less sophisticated: numerous eggs are fertilized, from which multiple larvae hatch that consume each other. Up to 20 fully developed terrestrial juveniles emerge, much smaller than Alpine salamander neonates.

So, do Salamandra have decent family values? Fire salamander larvae frequently display aggression to each other, but often spare their siblings. Monogamy is rejected by both sexes, with males spending prolonged periods surveying their surroundings with stretched front legs in search of females (Figure 1A), during which encounters with other males occasionally may lead to fairly vigorous combat. Females often display polyandry, in which up to four males can act as sires, potentially increasing reproductive success by betting on more than one horse. Their ability to store sperm for several years nevertheless permits females to avoid mating (amplexus; Figure 1G) while continuing to reproduce.

Are these salamanders threatened? In their range, *Salamandra* species may be very abundant (up to several hundred animals per hectare). Nevertheless, habitat destruction threatens lowland populations of several species. Especially worrying are the recently documented declines and extinctions among Western European populations of *S. salamandra*, which are thought to be due to an invasive chytrid fungus, *Batrachochytrium salamandrivorans*. Most European salamander species are highly susceptible to this fungus, and fire salamanders show particularly high mortality.

Where can I find out more?

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Chapter 3

Chapter 3

Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe

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My contribution to this chapter consisted of population surveys and collection of skin swabs at localities 8 and 15-24.

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Amphibians provide an iconic example of disease-driven global loss in biodiversity. The recently described chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) is an emerging pathogen that is driving amphibian populations to local extinction (Martel *et al.*, 2013; Martel *et al.*, 2014). This highly pathogenic fungus causes a lethal skin disease that has so far been restricted to urodele amphibians (newts and salamanders); the fungus was most likely introduced from East Asia into Europe via the pet trade (Martel *et al.*, 2014). In Europe, *Bsal* infection has led to dramatic declines of fire salamander (*Salamandra salamandra*) populations in the Netherlands and Belgium (Martel *et al.*, 2014). Within 7 years after the supposed introduction of the fungus, a population in the Netherlands declined by 99.9% (Spitzen-van der Sluijs *et al.*, 2013; Goverse & de Zeeuw, 2015). In the United Kingdom and Germany, *Bsal* has been detected in captive salamanders and newts (Cunningham *et al.*, 2015; Sabino-Pinto *et al.*, 2015). Infection trials suggest that *Bsal* represents an unprecedented threat to diversity of Western Palearctic urodeles (Martel *et al.*, 2014); nevertheless, reports of deaths among salamanders and newts in their naturalized ranges have been restricted to a few populations of a single salamander species in the southern Netherlands and adjacent Belgium (Martel *et al.*, 2013; Spitzen-van der Sluijs *et al.*, 2013). Using data from field surveillance, we examined the hosts and the geographic range of *Bsal* in Europe.

The Study

During 2010–2016, we collected samples of free-living populations of newts and salamanders from 48 sites in the Netherlands, Belgium, and adjacent regions of the Eifel region in Germany (near the border with the Netherlands and Belgium) (Figure 1; Technical Appendix Table 1). Site selection was based on reported amphibian deaths, apparent negative amphibian population trends, preventive *Bsal* surveillance in susceptible populations, or geographic proximity to known outbreak sites. Samples were also collected at 6 additional sites in Germany and 1 in the Netherlands, which were located >100 km from the nearest known outbreak (Technical Appendix Table 2). Sampling was conducted by swabbing skin (Hyatt *et al.*, 2007; Van Rooij *et al.*, 2011) of live animals and collecting skin samples from dead animals. All samples were kept frozen at -20°C until they were analyzed for the presence of *Bsal* DNA via real-time PCR, as described (Blooi *et al.*, 2013).

Across all 55 sites, we tested 1,019 fire salamanders (43 dead, 976 skin swab samples); at site 14, skin swab samples instead of tissue samples were collected from 16 dead salamanders. We also collected samples from 474 alpine newts (*Ichthyosaura alpestris*; 18 dead, 456 skin swab samples), 239 smooth newts (*Lissotriton vulgaris*; 2 dead, 237 skin swab samples), 80 palmate newts (*Lissotriton helveticus*; all skin swab samples), 79 crested newts (*Triturus cristatus*; all skin swab samples), and 30

Italian crested newts (*Triturus carnifex*; all skin swab samples). To obtain a Bayesian 95% credible interval for prevalence (Technical Appendix), we used the computational methods of Lötters *et al.* (2012). We ran 3 parallel Markov chains with 20,000 iterations each and discarded the first 5,000 iterations as burn-in; chains were not thinned.

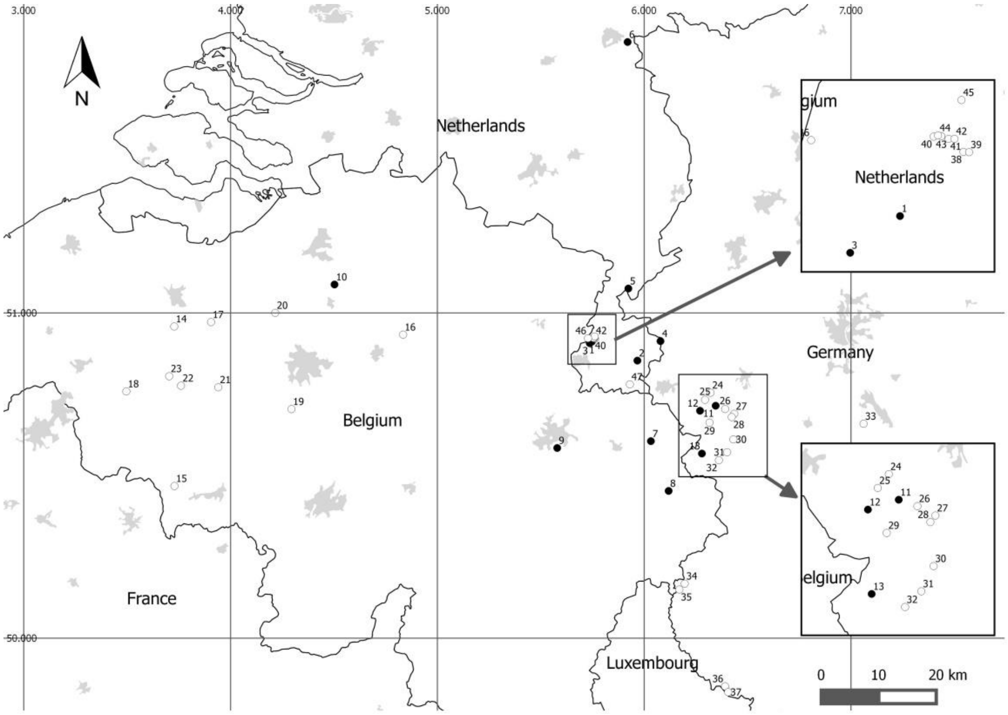

Figure 1. Study sites for collection of amphibians in Western Europe, 2010–2016. Numbers correspond to field sites at which amphibians were collected and examined for *Batrachochytrium salamandrivorans* (*Bsal*) (Technical Appendix). Solid circles, *Bsal* detected; open circles, *Bsal* not detected. Larger cities are indicated in light gray. Note that there are additional sites where the fungus remained undetected (not shown).

Bsal was found at 14 of the 55 sites; infected amphibians were fire salamanders, alpine newts, and smooth newts. Our results demonstrate that the range of *Bsal* distribution may be up to ≈10,000 km^2 (measured as the surface of a minimum convex polygon encompassing the outermost points) across Germany, Belgium, and the Netherlands (Figure 1). The presence of *Bsal* in wild alpine newts and smooth newts shows distinct expansion of the known host range in the wild (Technical Appendix Table 1). Furthermore, we document that *Bsal* is present in natural fire salamander populations in Germany (confined to the Eifel region). At some sites, because of our sample sizes, the upper limit of the 95% credible interval for *Bsal* prevalence was as high as 0.7; therefore, we may have failed to detect *Bsal* at these sites (Technical Appendix Table 1). In addition, the fungus may have been present at several sites before first detection. For example, *Bsal* was detected at site 4, where population-monitoring efforts in the years before detection (2000–2013) showed declines in 4 newt species (www.ravon.nl/EID_SI_Spitzen_et_al_2016). However, because no samples were collected before 2015, we have no evidence for a causal relationship between the presence of *Bsal* and the declines. We have also recorded the presence of *Bsal* in populations with no evidence of population change so far, such as the incidental findings of dead *Bsal*-positive newts in fyke nets at sites 5 and 11, and the incidental findings of dead *Bsal*-positive fire salamanders at sites 12 and 14. Clinical signs of mycosis, such as lethargy and skin shedding (Martel *et al.*, 2013), were observed at some *Bsal*-positive sites (1, 2, 7, 8, 14) but not at others.

Conclusions

Our study provides evidence that *Bsal* among wild amphibians in Europe is more widely distributed and affects a wider host range than previously known, which can either indicate recent spread of the fungus or point to historically infected sites that hitherto remained undetected. The presence of *Bsal* in wild populations can easily remain unnoticed because the lesions develop only near the final stage of the disease (Martel *et al.*, 2013). This information is crucial for the design of field surveys for *Bsal* surveillance. Our data might be used to inform a management strategy and to implement the recommendation of the Bern Convention (Standing Committee of the Convention on the Conservation of European Wildlife and Natural Habitats, 2015) to halt the spread of *Bsal* in Europe. Research to search for molecular evidence that the outbreak locations are connected is under way. Chytrid disease dynamics are affected by multiple factors (e.g., temperature regimes (Martel *et al.*, 2013)), and yet undetermined environmental determinants might be essential for disease outbreaks (Van Rooij *et al.*, 2015). Untangling these factors, as well as the modes of *Bsal* spread and its geographic distribution, are points for further research to fully map the problem and identify populations and species at risk.

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Ms. Spitzen-van der Sluijs is a senior project manager at Reptile, Amphibian & Fish Conservation Netherlands and a PhD candidate at Ghent University. Her work focuses on the effects of emerging infectious diseases on native amphibian population dynamics.

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Technical Appendix Table 1. Field sites where *Bsal* was detected, sampled species, numbers of *Bsal*-positive and total sampled specimens*

Site no., location, and amphibian collected	**No. Bsal-positive/total tested (year)**	**Observed prevalence (Bayesian 95% credible intervals)**	**Remarks**
The Netherlands			
1, Bunderbos, deciduous forest			
Fire salamander	3/3 (2010)	1.00 (0.42–1.00)	Past mass deaths; 99.9% population decline (1997–2014)
	1/1 (2011)	1.00	
	1/1 (2012)	1.00	
	0/3 (2014)	0 (0–0.61)	
	2/14 (2015)	0.14 (0.04–0.40	
	0/1 (2016)	0	
Alpine newt	1/1 (2013)	1.00	Possibly declining (monitoring started in 2013)†
	1/39 (2014)	0.03 (0.01–0.13)	
	1/10 (2015)	0.10 (0.02–0.43)	
	0/6 (2016)	0 (0–0.43)	
2, Putberg, deciduous forest			
Smooth newt	0/2 (2014)	0 (0–0.70)	Possibly declining†
Alpine newt	0/10 (2014)	0 (0–0.31)	Possibly declining†
	1/1‡ (2014)	1.00	
	1/1‡ (2015)	1.00	
3, Meerssen, garden pond			
Fire salamander	0/1 (2015)	0	No evidence of decline§
Smooth newt	4/43 (2015)	0.09 (0.04–0.21)	No evidence of decline§
Alpine newt	0/9 (2015)	0 (0–0.30)	No evidence of decline§
4, Wormdal, clusters of natural ponds in nature conservation area¶			
Smooth newt	1/22 (2015)	0.05 (0.01–0.21)	87% decline(2000–2013)†#
Alpine newt	0/12 (2015)	0 (0–0.26)	96% decline (2000–2013)†#
5, Pepinusbeekdal, extensive agriculture			
Smooth newt	½‡ (2014)	0.50 (0.09–0.88)	No evidence of decline†
6, Berg en Dal, garden pond			
Alpine newt	12/12‡ (2015)	1.00 (0.74–1.00)	Yearly mass deaths; species still present§
7, Vijlenerbosch, deciduous forest			
Alpine newt	0/1 (2013)	0	No evidence of decline§
	0/30 (2014)	0 (0–0.11)	
	1/18 (2015)	0.05 (0.02–0.24)	
Smooth newt	0/8 (2014)	0 (0–0.31)	No evidence of decline§
	0/11 (2015)	0 (0–0.26)	

Palmate newt	0/1 (2014) 0/9 (2015)	0 0 (0–0.30)	No evidence of decline§
Belgium 8, Eupen, deciduous forest			
Fire salamander	½ (2013)	0.50 (0.09–0.88)	Deaths, probably fire salamanders severely declining, no monitoring trend available
9, Robertville, deciduous forest			
Fire salamander	16/30 (2014)	0.53 (0.36–0.69)	Deaths, severe decline, monitoring ongoing
10, Liège, deciduous forest			
Fire salamander	5/5 (2014)	1.00 (0.55–.00)	Deaths
11, Duffel, garden pond			
Alpine newt	2/30‡ (2015)	0.07 (0.02–0.22)	2 dead in fyke; no evidence of decline
Smooth newt	0/16 (2015)	0 (0–0.20)	No deaths; no evidence of decline
Germany 12, Weisse Wehe, deciduous forest			
Fire salamander	4/11‡ (2015)	0.36 (0.15–0.65)	No evidence of decline†
13, Solchbachtal, mixed forest			
Fire salamander	0/2 (2014) 1/51 (2015)	0 (0–0.70) 0.02 (0.01–0.10)	Decreased newts and salamanders§
Palmate newt	0/19(2014)	0 (0–0.18)	Decreased newts and salamanders§
Alpine newt	0/5(2014)	0 (0–0.44)	Decreased newts and salamanders§
14, Belgenbachtal, mixed forest			
Fire salamander	21/22‡ (2015)	0.96 (0.79–0.99)	Remarkable deaths (16 dead), noted only since Nov 2015†

†Population monitored.

‡Includes individual(s) found dead by chance.

§Anecdotal reports.

¶At this site, crested newts and smooth newts decreased with similar percentages over the same period (96%; 94%, respectively).

#http://www.ravon.nl/EID_SI_Spitzen_et_al_2016.

Technical Appendix Table 2. Field sites studied where *Bsal* was not detected, number of sampled species and specimens*

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Belgium			
15, Nerenbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
16, Heilig Geestgoed, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
17, Kasteel van Horst, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
18, Smetledebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
19, Kluisbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
20, Hallerbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
21, Buggenhoutbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
22, Raspaillebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
23, Haeyesbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
24, t Burreken, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
Germany			
25, Lamersiefen, deciduous forest			
Fire salamander	17 (2014) 32 (2015)	0 (0–0.19) 0 (0–0.11)	No evidence of decline†
26, Fischbach, deciduous forest			
Fire salamander	36 (2014) 51 (2015)	0 (0–0.09) 0 (0–0.07)	No evidence of decline; 3 dead-found Specimens tested negative for Bsal via histology (2014)†
Alpine newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline
Palmate newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline
27, Kallerbach, deciduous forest			
Fire salamander	24(2015)	0 (0–0.15)	No evidence of decline†
28, Rosbach, deciduous forest			
Fire salamander	47 (2015)	0 (0–0.07)	No evidence of decline†

29, Zweifallshammer, deciduous forest			
Fire salamander	41 (2015)	0 (0–0.08)	No evidence of decline†
30, Peterbach, mixed forest			
Palmate newt	12 (2014)	0 (0–0.26)	No evidence of decline
Alpine newt	4 (2014)	0 (0–0.52)	No evidence of decline
31, Haftenbach, deciduous forest			
Fire salamander	46 (2015)	0 (0–0.08)	No evidence of decline†
32, Sauerbach, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline†
Alpine newt	1 (2015)	0,00	No evidence of decline
33, Härtgessief, deciduous forest			
Fire salamander	15 (2014)	0 (0–0.19)	Strong evidence of decline†
34, Kottenforst, deciduous forest			
Fire salamander	51 (2015)	(0–0.07)	No evidence of decline
35, Großkampenberg, mixed forest			
Alpine newt	4 (2015)	0 (0–0.52)	No evidence of decline
Palmate newt	1 (2015)	0	No evidence of decline
36, Lützkampen –mixed forest			
Alpine newt	8 (2015)	0 (0–0.31)	No evidence of decline
37, Ferschweiler- mixed forest			
Alpine newt	2 (2015)	0 (0–0.70)	No evidence of decline
Palmate newt	8 (2015)	0 (0–0.31)	No evidence of decline
38, Ernzen, mixed forest			
Fire salamander	4 (2015)	0 (0–0.52)	No evidence of decline†
The Netherlands 39, Moerveld surroundings (A), Bunderbos vicinity			
Alpine newt	13 (2015)	0 (0–0.22)	No evidence of decline‡
40, Moerveld surroundings (B), Bunderbos vicinity			
Alpine newt	34 (2015)	0 (0–0.11)	No evidence of decline‡
41, Snijdersbergweg 21, pond			
Alpine newt	60 (2015)	0 (0–0.06)	No evidence of decline‡
42, Mevr van der Meijstraat 12, pond			
Alpine newt	19 (2015)	0 (0–0.18)	No evidence of decline‡
43, Mevr van der Meijstraat 20, pond			
Alpine newt	17 (2015)	0 (0–0.19)	No evidence of decline‡
44, Snijdersbergweg 20, 2 garden ponds			
Alpine newt	30 (2015)	0 (0–0.11)	No evidence of decline‡
45, Snijdersbergweg 23b, garden pond			
Alpine newt	15 (2015)	0 (0–0.19)	No evidence of decline‡
46, Broekhoven, garden pond			
Fire salamander	2 (2015)	0 (0–0.70)	No evidence of decline‡

47, Meerssen, deciduous forest			
Fire salamander	57 (2013)	0 (0–0.06)	No deaths; no evidence of decline†
	43 (2014)	0 (0–0.08)	
	29 (2015)	0 (0–0.11)	
	2 (2016)	0 (0–0.70)	
48, Carisberg, deciduous forest			
Alpine newt	8 (2014)	0 (0–0.31)	No information available
Palmate newt	23 (2014)	0 (0–0.14)	No information available
Smooth newt	2 (2014)	0 (0–0.70)	No information available
Additional far-out sites (Germany)			
N.S., Solling, deciduous forest			
Fire salamander	23 (2015)	0 (0–0.14)	No evidence of decline‡
N.S., Ilsenburg, deciduous forest			
Fire salamander	8 (2015)	0 (0–0.31)	No evidence of decline‡
N.S., Lelm, deciduous forest			
Alpine newt	57 (2015)	0 (0–0.06)	No evidence of decline‡
Palmate newt	6 (2015)	0 (0–0.43)	No evidence of decline‡
Smooth newt	16 (2015)	0 (0–0.20)	No evidence of decline‡
Crested newt	29 (2015)	0 (0–0.11)	No evidence of decline‡
N.S., Kleiwiesen, exposed ponds surrounded by deciduous forest			
Alpine newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
Smooth newt	117 (2015)	0 (0–0.03)	No evidence of decline‡
Crested newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
N.S., Waldecker Schlossgrund, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline‡
N.S., Closewitz, exposed ponds surrounded by deciduous forest			
Crested newt	23 (2015)	0 (0–0.14)	No evidence of decline‡
Additional far-out site (the Netherlands)			
N.S., Veluwe, deciduous forest			
Italian crested newt	0 (2015)	0 (0–0.11)	No evidence of decline‡

**Bsal*, *Batrachochytrium salamandrivorans*; N.S., not shown on map (Figure 1). Data provide an overview of novel information and previously published

Chapter 4

Chapter 4

Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palearctic

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Abstract

Aim Identifying hosts and regions susceptible to invasion by an emerged pathogen is vital to inform early risk assessments. We here show how differences between a pathogen's native and invasive observed niche and their underlying environments affect this process, using the recent emergence of *Batrachochytrium salamandrivorans* (*Bsal*) as a critical, empirical example.

Location Palearctic.

Methods To quantify observed niches we first gathered occurrences from the native Asian and invasive European distribution of *Bsal*. Through ordination in PCA-bound environmental space we then applied overlap tests to compare native and invasive *Bsal* niches with those of 56 putative Western Palearctic host species. Finally, we construct bivariate niche models (ESMs) for each *Bsal* niche to assess how differences in observed niches influenced suitability predictions.

Results The observed invasive *Bsal* niche is a conservative, partially filled subset of its wider native niche. Pathogen-host overlap measured using the narrow invasive niche was nevertheless found to be significantly higher than that of the native niche, which is partly situated in subtropical conditions absent from the Western Palearctic. ESMs created using the native niche predict high suitability for *Bsal* throughout Europe. Conversely, a more restricted range was predicted using the invasive niche, which coincides with presence of oceanic climates in north-western Europe and several Mediterranean mountain ranges.

Main conclusions Unequal relative availability of environments in native and invasive ranges may lead to underestimation of observed niche overlap between native pathogen populations and putative hosts in the invasive range. The existence of partially filled niches may add uncertainty to overlap measurements, and predictions of potential further spread. Results based on the invasive niche therefore provide a conservative estimate; yet demonstrate, in our case, that *Bsal* is already present in conditions shared by numerous host species. Further niche filling may accordingly increase risk for salamander diversity across the Western Palearctic.

Keywords

Biological invasion, chytrid, emerging infectious disease, partial niche filling, salamander

Introduction

Emerging infectious diseases are increasingly causing species declines and extinction in natural and agricultural systems (Jones *et al.*, 2008; Fisher *et al.*, 2012; Hatcher *et al.*, 2012; Johnson *et al.*, 2015; Fisher *et al.*, 2016). Among the pathogens that drive these diseases are fungi, which are responsible for several iconic epidemics including chytridiomycosis in amphibians, white-nose syndrome in bats, and rice blast disease in grasses and sedges (reviewed by Fisher *et al.*, 2012; Fisher *et al.*, 2016). Key traits that promote emergence and spread of such epidemics have been repeatedly shown to include rapid dispersal, and the ability to infect a wide range of hosts (e.g. Langwig *et al.*, 2016; Stegen *et al.*, 2017). Accordingly, development of spatial predictions on how, or where infection may spread has become a crucial step in early risk assessments following pathogen emergence (Ostfeld *et al.*, 2005; Purse & Golding, 2015). Niche models have become popular tools to this aim, because prevalence and occurrence of fungal pathogens have repeatedly been shown to vary as a function of environmental conditions such as climate, predator pressure, and (host)community build-up (e.g. Raffel *et al.*, 2013; Schmeller *et al.*, 2014; Johnson *et al.*, 2015; Cohen *et al.*, 2016; Langwig *et al.*, 2016). Hence, identification of hosts or regions susceptible to colonization can be performed by measuring niche overlap between a disease agent and its host, or by generating predictions of a pathogen's potential occurrence (reviewed by Peterson, 2006; Hay *et al.*, 2013; Purse & Golding, 2015). Ultimately, such assessments may provide support for directed conservation actions, including mitigation prioritization (Peterson, 2006).

Niche-based analyses in disease ecology are however affected by two pervasive, not mutually-exclusive issues. First, information about the ecology of a pathogen is generally limited prior to emergence of the infectious disease it causes (Peterson, 2006; Desprez-Loustau *et al.*, 2007; Hay *et al.*, 2013; Tompkins *et al.*, 2015). How, and to what extent biotic interactions and dispersal shape a fungal pathogen's realized niche may consequently remain unknown, which hampers the use of mechanistic models that depend on biophysical data (Kearney & Porter, 2009). Ecological niche models are therefore often used instead, which quantify an approximation of the realized niche by correlating distribution observations with widely-available macroecological data (Guisan & Zimmermann, 2000). We here refer to this approximation as the 'observed niche' (Warren *et al.*, 2008; Soberón & Nakamura, 2009; Broennimann *et al.*, 2012), given that natural factors including species interactions and habitat availability, but also uneven sampling effort may shape the distribution as observed by us (see also Lobo *et al.*, 2010; Kramer-Schadt *et al.*, 2013). Following niche quantification, an ecological niche model may be projected across a landscape or time period to, among other goals, assess risk of pathogen colonization or spread (Peterson & Shaw, 2003; Rödder *et al.*, 2009; Kriticos *et al.*, 2013; Escobar *et al.*, 2014; Becker *et al.*, 2015; Yap *et al.*, 2015; Feldmeier *et al.*, 2016; Katz & Zellmer, 2018). To permit such predictions, a species is assumed to inhabit all available areas that host suitable environmental conditions, and to retain its habitat preference over time – in other words, ecological niche models assume that a niche is fully filled, and remains conserved (Guisan & Zimmermann, 2000; Soberón & Nakamura, 2009). Recent studies have however argued that these assumptions might not always be met during pathogen emergence, especially when a disease agent is invasive (Hatcher *et al.*, 2012; Purse & Golding, 2015).

Emergence of a fungal pathogen in a novel host, environment or geographical region is in essence an invasion event (Woolhouse *et al.*, 2005; Desprez-Loustau *et al.*, 2007; Hatcher *et al.*, 2012; Purse & Golding, 2015). Both extrinsic and intrinsic factors can drive a pathogenic invasion; for instance, non-biological factors including globalization and increased trade in plants and animals have introduced numerous fungal pathogens to naïve ecosystems (Fisher *et al.*, 2012; Fisher *et al.*, 2016). Evolutionary processes may lead to changes such as increased virulence or thermal preference, allowing colonization of novel hosts or habitats (Woolhouse *et al.*, 2005). The main issue when using ecological niche models is that these invasion events may go hand in hand with a change in the observed niche, and thereby breach model assumptions of niche stability (Soberón & Nakamura, 2009; Guisan *et al.*, 2014; Purse & Golding, 2015). Indeed, even when no evolutionary change takes place, the observed niche of a pathogen may differ from its native counterpart during the early invasion process when not all available environments have become occupied. The niche accordingly remains conserved, but is not entirely filled (Elith *et al.*, 2010; Broennimann *et al.*, 2012; Václavík & Meentemeyer, 2012; Qiao *et al.*, 2017). Hence, it is vital to disentangle the relative roles of partially niche filling and environmental differences between native and invasive pathogen distributions to facilitate early invasion assessments, for instance using ordination (Guisan *et al.*, 2014). Failure to do so may lead to a high degree of model uncertainty, and likely affect identification of hosts and regions susceptible to pathogen colonization and spread (Peterson, 2006). Nevertheless, few studies have considered the extent to which a pathogens' observed niche change may impact early risk assessments in spatial disease ecology (but see Peterson & Shaw, 2003), perhaps because ecological information from both native and invasive ranges is rarely available (Hay *et al.*, 2013; Purse & Golding, 2015).

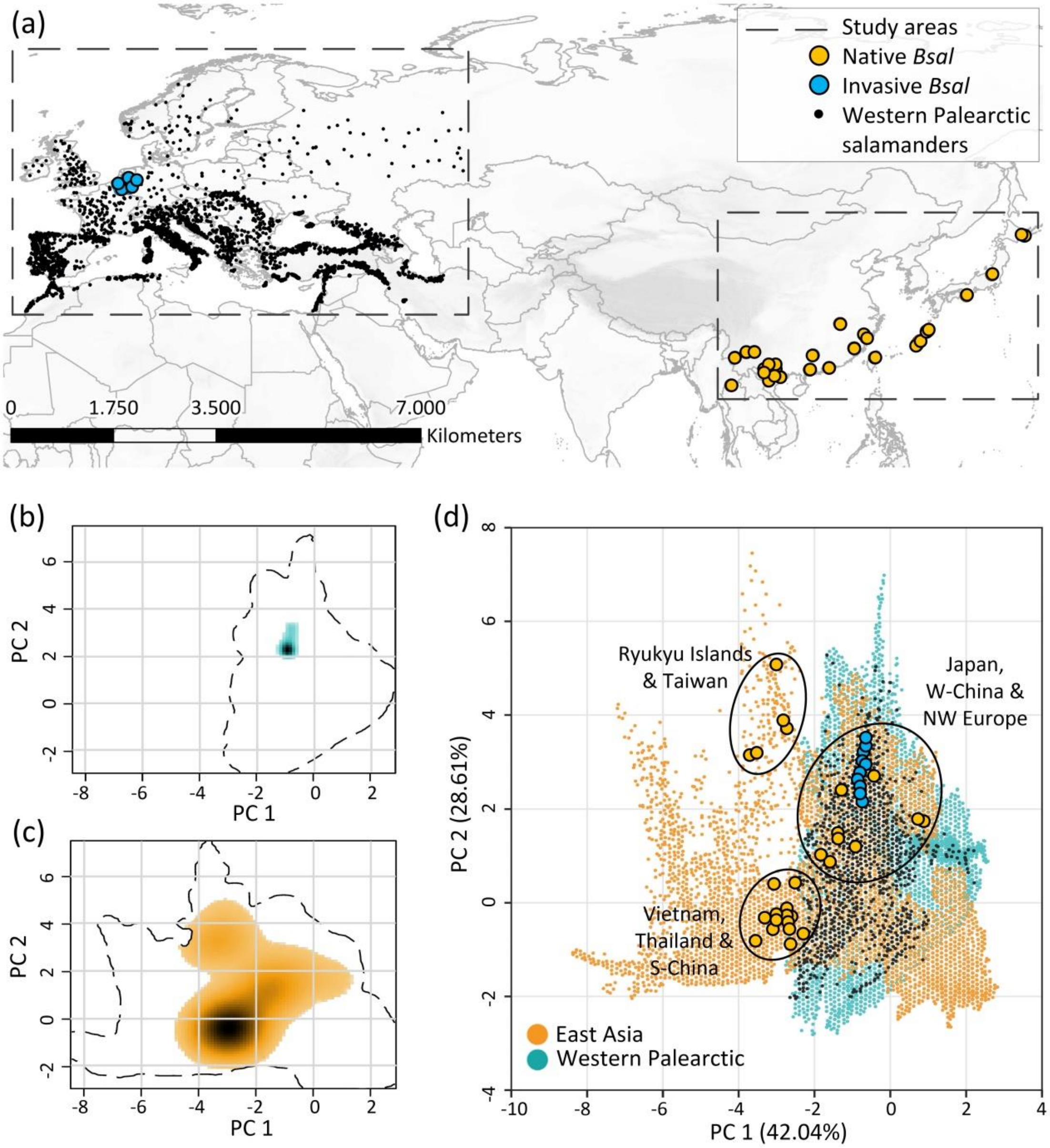

Figure 1: Occurrence of *Batrachochytrium salamandrivorans* (*Bsal*) and its Western Palearctic salamander hosts in geographical and environmental space. Climatic data extracted from the study areas (panel a, delineated by dashed lines) was used to create two dimensional, PCA-bound environmental space, on which observed niches of invasive (b) and native (c) *Bsal* and its hosts were calibrated. Panel (c) summarizes the findings, revealing that environmental conditions in the Western Palearctic and East Asia only partially overlap; several native *Bsal* populations occur in conditions absent from the Western Palearctic, which reduces possibilities for niche overlap with Western Palearctic salamander hosts and affects model transferability.

Here, we explore how differences between a pathogen's native and invasive observed niche and the environmental conditions which characterize their distribution affect pathogen-host niche overlap, and identification of areas susceptible to colonization. To this aim, we use the recently emerged chytrid *Batrachochytrium salamandrivorans* (*Bsal*) as a case study. *Bsal* originates from East Asia, where this fungus is present on the skin of various salamander species (Martel *et al.*, 2014; Laking *et al.*, 2017; Yuan *et al.*, 2018). Human-mediated dispersal, probably through the pet trade, has moved *Bsal* into north-western Europe (Martel *et al.*, 2013). Within its invasive range, *Bsal* drives declines and extinction among an increasing number of salamander populations by causing the lethal skin disease chytridiomycosis (Martel *et al.*, 2013; Spitzen-van der Sluijs *et al.*, 2016; Stegen *et al.*, 2017). Large-scale infection experiments have furthermore revealed that *Bsal* is lethal to the vast majority of European salamander species beyond its small, but expanding invasive range (Martel *et al.*, 2014; Spitzen-van der Sluijs *et al.*, 2016). A pressing need exists to identify regions, along with hosts, that occur in conditions favourable to *Bsal*. Although several early risk assessments were recently generated (e.g. Yap *et al.*, 2015; Feldmeier *et al.*, 2016; Schmidt *et al.*, 2017; Katz & Zellmer, 2018), *Bsal* natural history data was hitherto insufficient to characterize the breadth of environmental conditions in which this pathogen occurs, especially within its native range. Here, we aim to set the scene for spatial assessments of *Bsal* invasion risk by answering; i) if the observed niche of *Bsal* changed during its invasion into Europe; ii) whether observed niche change is the result of partial filling, or expansion; iii) if, and how differences between native and invasive niches and their environmental context influence calculations of host-pathogen niche overlap, and; iv) if these differences affect identification of host and areas susceptible to *Bsal*.

Methods

Study areas and species

Bsal is native to East Asia, where its distribution is geographically extensive (Fig. 1; Martel *et al.*, 2014; Laking *et al.*, 2017; Yuan *et al.*, 2018). In north-western Europe, where *Bsal* is invasive, this pathogen occupies a small range that is currently expanding (Spitzen-van der Sluijs *et al.*, 2016). As its salamander hosts are largely absent from Central Asia (Sparreboom, 2014), we delimited two rectangular study areas instead of one, which include the native (72.22 to 29.72 and -14.28 to 58.22, WGS84) and invasive (46.16 to 16.16 and 95.03 to 146.78) ranges of *Bsal*, but also encompass the distributions of all putative Western Palearctic host species (Fig. 1a). To describe environmental variation within the study areas we chose the commonly-used Bioclim dataset at 2.5 arc minutes (± 5x5 km), which features annual trends, seasonality and extremes in precipitation and temperature (Hijmans *et al.*, 2005). Although potential correlations between these parameters would not affect our initial ordination-based niche overlap measurements, it may negatively influence subsequent niche model calibration (e.g. Guisan & Zimmermann, 2000; Broennimann *et al.*, 2012). Accordingly, we assembled a dataset for each study area, containing the same set of parameters of which Pearson's correlation coefficients (*r*) remained <0.7. The final parameters in both datasets comprised isothermality (Bio03), temperature seasonality (Bio04), maximum temperature of the warmest month (Bio05), minimum temperature of the coldest month (Bio06), precipitation seasonality (Bio15), precipitation of the wettest quarter (Bio16) and precipitation of the driest quarter (Bio17). These water- and temperature related parameters have been proven biologically relevant to characterize amphibian distributions, also at coarse geographical scale (Laking *et al.*, 2017). The final datasets were used to measure niche overlap, and to build niche models in geographical space.

Bsal occurrence records were obtained from Martel *et al.* (2014), Spitzen-van der Sluijs *et al.* (2016), Laking *et al.* (2017) and Yuan *et al.* (2018). Additionally, we found *Bsal* to be present on three previously unidentified salamander hosts in Taiwan (See Appendix S1 in Supporting Information). We thereby provide the first records from central East Asia, which bridge the gap between earlier identified populations from Japan and those in Vietnam and Thailand (Martel *et al.*, 2014; Laking *et al.*, 2017). Finally, we added two further records to the invasive occurrence dataset following discovery of *Bsal* in southern Belgium near Dinant, and western Germany, near Essen. *Bsal* presence was established at each of these localities by means of quantitative PCR (Blooi *et al.*, 2013), based on DNA extracted from dry swabs that were rubbed across the underside, throat, and cloacal region of salamanders. The final dataset contained a total of 59 occurrences, of which 43 records originated from the native East-Asian *Bsal* range, while the remaining 16 records define the invasive range, comprising the southern and eastern Netherlands, Belgium, and north-western Germany (Fig. 1a, c). Susceptibility to *Bsal* has been confirmed in all hitherto tested Western Palearctic salamander species, although sensitivity of *Lissotriton helveticus* remains ambiguous (EFSA Panel on Animal Health and Welfare *et al.*, 2017). Hence, each salamander taxon occurring in this region was regarded as a putative host. Host species taxonomy follows AmphibiaWeb (AmphibiaWeb, 2017). In order to describe host niches we randomly placed 100 occurrence records within their IUCN-defined geographical ranges, which were subsequently filtered based on the species-specific altitudinal extent of occurrence (IUCN, 2015; also see Appendix S2.1 for the underlying rationale; Yap *et al.*, 2015). The final host occurrence dataset consisted of 5092 records divided over 61 species (Appendix S1, average 83.48 records per species). Susceptibility to *Bsal* has been confirmed in twelve of these, which originate from two distinct families, divided over nine different genera (Table S3.2).

Pre-modelling niche quantification and overlap

Observed niches may be compared by using ordination to measure overlap between occurrence records in multivariate environmental space, or through comparison of predicted distributions generated by ecological niche models in geographical space (reviewed in Guisan *et al.*, 2014). The main advantage of the latter approach lies in its statistical or machine-learning nature, which includes optimized weighting of the environmental parameters to provide (often valuable) information on relative parameter importance in shaping a distribution (Guisan & Zimmermann, 2000; Phillips & Dudík, 2008; Elith *et al.*, 2010). However, partial niche filling, sampling bias, or unequal availability of environments in the native and invasive range could lead to errors while fitting species-environment response curves, which may negatively affect calculations of parameter importance and significantly bias the predicted distributions used for calculating overlap (Broennimann *et al.*, 2012; Václavík & Meentemeyer, 2012; Guisan *et al.*, 2014; Qiao *et al.*, 2017). Given potential non-equilibrium of the invasive *Bsal* niche, we therefore chose to measure overlap using the ordination framework presented by Di Cola *et al.* (2017). Use of this framework permitted us to test whether differences between the two observed *Bsal* niches are the result of partial niche filling or expansion, as relative environmental availability across native and invasive ranges is accounted for during ordination (see also Broennimann *et al.*, 2012). To assess the degree of pathogen-host overlap we subsequently employed equivalency and similarity tests (Warren *et al.*, 2008). The equivalency test measures whether two niches are identical or not. Similarity tests assess if the two niches are more, or less similar than expected by chance, based on their environmental background (see Appendix S2.2 for a complete description). Equivalency- and similarity tests have only occasionally been applied to assess observed pathogen-host niche overlap (but see Rödder *et al.*, 2013; Purse & Golding, 2015). Here, we use these tests to assess which putative host species occur in environmental conditions significantly similar to those inhabited by native and invasive *Bsal*, with the aim of identifying potential key hosts (Rödder *et al.*, 2013). We deem this comparative approach to be more appropriate at this (early) stage of invasion than measuring overlap between predicted distributions in geographical space, especially given previously mentioned uncertainties relating to non-equilibrium of the invasive *Bsal* niche.

In order to quantify observed niches we used three ordination approaches; PCA-env, WITHIN-env and ENFA, which were implemented using the R (3.3.2) package ‘ecospat’ (Di Cola *et al.*, 2017). We chose these approaches because simulations using virtual species recently revealed that when measuring niche overlap, PCA-env, WITHIN-env and ENFA outperform other ordination techniques and ecological niche models in terms of accuracy (Broennimann *et al.*, 2012). Niches were quantified by applying a Gaussian kernel density function with standard bandwidth to the occurrence records of each species, after which the resulting smoothed occurrence densities were mapped within environmental space. Absolute overlap between two given niches was calculated based on the position of the occurrence densities using the *D* metric of Schoener (1968), which ranges from 0 (no overlap) and 1 (complete overlap).

Ecological niche modelling

Despite not using ecological niche models to measure niche overlap, we did build ensembles of small models (ESMs, Breiner *et al.*, 2015) to illustrate the effects of observed niche differences on model predictions. We chose to use ESMs because the number of *Bsal* occurrences in the native and invasive ranges remains limited, which may cause ecological niche models to overfit, and lose explanatory power (Guisan & Zimmermann, 2000; Wisz *et al.*, 2008; Yackulic *et al.*, 2013; Katz & Zellmer, 2018). ESMs fit numerous small bivariate models, which are subsequently ensembled based on submodel performance. Because the parameter-to-occurrence ratio remains balanced using this approach, performance estimations remain robust while minimizing overfitting (Breiner *et al.*, 2015). Nevertheless, the large extent of our study areas used for niche overlap calculations could have resulted in overprediction of the native *Bsal* models (e.g. Lobo *et al.*, 2010), and may have caused invasive *Bsal* models to underpredict owing to potential non-equilibrium (Elith *et al.*, 2010; Václavík & Meentemeyer, 2012). The latter issue, and differences in availability of environmental backgrounds underlying native and invasive *Bsal* populations may furthermore drive model predictions into novel conditions (i.e., extrapolation), which sharply increases predictive uncertainty (Elith *et al.*, 2010; Mesgaran *et al.*, 2014). We therefore restricted backgrounds used for model calibration to Köppen-Geiger climate regions (Peel *et al.*, 2007) already inhabited by *Bsal* within the study area (Fig. S2.1; see also Phillips & Dudík, 2008; Elith *et al.*, 2010; Kramer-Schadt *et al.*, 2013). Because the dispersal range of *Bsal* remains ambiguous (e.g. Schmidt *et al.*, 2017; Stegen *et al.*, 2017), we regard that limiting the potentially accessible area for this pathogen to conditions which are already part of the observed niche as the most conservative approach. To identify regions which are subject to extrapolation despite the precautions described above, we used EXDET (Mesgaran *et al.*, 2014). EXDET is able to detect two types of novel conditions. Type 1 novelty is found when a given area on which calibrated models are transferred features at least one environmental parameter outside the range of the calibration data. Type 2 novelty occurs when novel combinations of environmental parameters are identified (Mesgaran *et al.*, 2014). Data preparation, niche modelling, ESM creation and model evaluation were performed in R 3.3.2, using the 'biomod2' and 'ecospat' packages, respectively (Thuiller *et al.*, 2016; Di Cola *et al.*, 2017). We chose Generalized Linear Models (GLM), and MAXENT (Phillips & Dudík, 2008; MAXENT.Phillips in biomod2) to quantify native and invasive *Bsal* niches, because of their ability to outdo other niche modelling techniques in terms of general performance and transferability (e.g. Wisz *et al.*, 2008; Broennimann *et al.*, 2012; Breiner *et al.*, 2015; see Appendix S2.3 for a detailed explanation of our ESM approach). In order to evaluate performance of submodels and ESMs created throughout the modelling process we used five different metrics; model sensitivity, specificity, the True Skill Statistic (TSS; Allouche *et al.*, 2006), Boyce index (Breiner *et al.*, 2015, and references therein) and the AUC. Pairwise Pearson correlations were used to assess conformity amongst these metrics.

Results

Niche overlap

The first and second axes of the Principal Component Analyses explained respectively 42.04% and 28.61% of the variation when composed by PCA-env, and 47.16% and 27.83% using WITHIN-env. Explained variation in PCAs constructed for ENFA varied as different pseudo-absences were selected for each separate run. In PCAs generated by PCA-env and WITHIN-env, temperature-related parameters were generally grouped on the first axis (BIO03-06, but also BIO16), while precipitation parameters formed the second axis (BIO15 and BIO17; see also Fig. S3.1). Average climate conditions at native and invasive *Bsal* sites significantly differed, with native populations showing broader occurrence (i.e. a wider range) in each of the considered parameters (Table S4.1). Overlap scores between the native East-Asian and invasive European *Bsal* niches were low (PCA-env $D = 0.004$; WITHIN-env $D = 0.003$; ENFA $D = 0$), due to which niche equivalency but also similarity (either higher or lower than expected by chance) were rejected for the comparisons made by each ordination approach. Visual inspection of these niches revealed that this low degree of overlap is caused by niche conservatism and partial niche filling, as the invasive *Bsal* niche represents a comparatively small subset of the native niche (Fig. 1b). Calculations of niche stability, filling and expansion between the native and invasive niche using PCA-env and WITHIN-env support this notion, which show little (0.092 for PCA-env, on a scale of 0 to 1) to no (0 for WITHIN-env) expansion of the invasive niche, and therefore high niche stability (0.905 and 1, respectively), and a large potential for further niche filling (0.977 for PCA-env and 0.945 for WITHIN-env). ENFA results differed strongly, suggesting that only niche expansion (1) took place, leading to absence of stability (0), and high potential for further niche filling (1). In addition, climatic conditions characterizing Europe and Asia were found to be only partially dissimilar, with a relatively large section of the native *Bsal* niche situated in conditions that fall beyond the environmental background of the Western Palearctic (Fig. 1b, c).

Niche overlap scores obtained by comparing native and invasive *Bsal* to their Western Palearctic salamander hosts did not significantly differ between algorithms (Kruskal-Wallis H=5.83, df=5, *p*=0.37), with mean rank overlap scores for PCA-env, WITHIN-env and ENFA of respectively 154.38, 160.84 and 178.19 regarding native *Bsal*, and 163.71, 163.66 and 190.21 for its invasive counterpart (Fig. 2). Niche equivalency was rejected in all cases. Significant higher-than-expected niche similarity between native *Bsal* and a host species was recovered during only six comparisons, comprising one confirmed and five putative host species (Table 1, S3.2). When the invasive *Bsal* niche was compared those of its salamander hosts, significant higher-than-expected niche similarity was however found during 42 comparisons. These cases include all confirmed host species apart from *Lissotriton vulgaris*, *Neurergus crocatus*, *Pleurodeles waltl* and *Salamandrella keyserlingii*, and an additional 14 putative hosts (Table 1, S3.2). Accordingly, niche overlap scores calculated using the larger, native niche were found to be significantly lower (D = 0.02 ($\bar{X}$) ± 0.03 (sd)) compared to those measured using the smaller invasive niche (D = 0.06 ± 0.10; $t(334)= -5.210$, $p < 0.00$; see also Table S3.2).

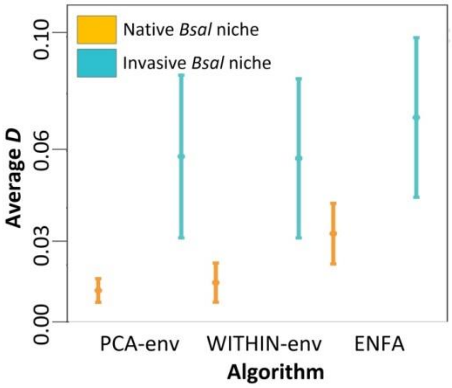

Figure 2: Observed niche overlap scores (average Schoener's *D*) between *Batrachochytrium salamandrivorans* (*Bsal*) and its Western Palearctic salamander hosts produced by three different ordination algorithms. Niche overlap was measured using the native and invasive *Bsal* niches separately.

Table 1: Western Palearctic salamander species of which the ecological niche shows significant similarity to the native or invasive niche of *Batrachochytrium salamandrivorans* (*Bsal*) and/or vice versa, according to at least one of three used ordination approaches. Niche overlap was measured using Schoener's *D*, which ranges between 0 (no overlap) and 1 (complete overlap). Comparisons between the host and *Bsal* are shown before the /, vice versa after. *represents significant similarity (also marked in **bold**), ns means 'not significant'.

Ecological niche overlap (*D*)

Host species	**Native niche**			**Invasive niche**		
	PCA-env	**WITHIN-env**	**ENFA**	**PCA-env**	**WITHIN-env**	**ENFA**
Calotriton arnoldi	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,104**$^{*/*}$	**0,104**$^{*/*}$	0,000$^{ns/ns}$
Calotriton asper	0,001$^{ns/ns}$	0,024$^{ns/ns}$	0,002$^{ns/ns}$	**0,178**$^{*/ns}$	**0,178**$^{*/ns}$	**0,093**$^{*/ns}$
Euproctus montanus	0,003$^{ns/ns}$	0,004$^{ns/ns}$	0,023$^{ns/ns}$	0,019$^{ns/ns}$	0,019$^{ns/ns}$	**0,430**$^{*/*}$
Euproctus platycephalus	0,000$^{ns/ns}$	0,006$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,052**$^{*/*}$
Hydromantes ambrosii	0,001$^{ns/ns}$	0,002$^{ns/ns}$	0,004$^{ns/ns}$	**0,082**$^{ns/*}$	**0,082**$^{ns/*}$	**0,046**$^{*/*}$
Hydromantes genei	0,000$^{ns/ns}$	**0,007**$^{*/*}$	0,006$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Hydromantes imperialis	0,000$^{ns/ns}$	0,003$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,001**$^{ns/*}$
Hydromantes italicus	0,004$^{ns/ns}$	0,002$^{ns/ns}$	0,060$^{ns/ns}$	0,164$^{ns/ns}$	0,164$^{ns/ns}$	**0,146**$^{ns/*}$
Hydromantes sarrabusensis	0,000$^{ns/ns}$	**0,003**$^{*/*}$	0,017$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Hydromantes strinatii	0,007$^{ns/ns}$	0,006$^{ns/ns}$	0,005$^{ns/ns}$	**0,528**$^{*/*}$	**0,528**$^{*/*}$	**0,208**$^{*/ns}$
Ichthyosaura alpestris	0,023$^{ns/ns}$	0,018$^{ns/ns}$	0,048$^{ns/ns}$	**0,213**$^{*/*}$	**0,213**$^{*/*}$	**0,083**$^{*/*}$
Lissotriton helveticus	0,019$^{ns/ns}$	0,027$^{ns/ns}$	0,037$^{ns/ns}$	**0,154**$^{ns/*}$	0,154$^{ns/ns}$	**0,299**$^{*/*}$
Lissotriton lantzi	0,032$^{ns/ns}$	0,013$^{ns/ns}$	0,047$^{ns/ns}$	**0,169**$^{*/*}$	**0,169**$^{ns/*}$	0,121$^{ns/ns}$
Lissotriton montandoni	0,018$^{ns/ns}$	0,002$^{ns/ns}$	0,134$^{ns/ns}$	0,007$^{ns/ns}$	0,007$^{ns/ns}$	**0,333**$^{*/*}$
Lissotriton vulgaris	0,090$^{ns/ns}$	**0,168**$^{*/*}$	0,145$^{ns/ns}$	0,031$^{ns/ns}$	0,031$^{ns/ns}$	0,025$^{ns/ns}$
Lyciasalamandra fazilae	0,006$^{ns/ns}$	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,094**$^{ns/*}$
Mertensiella caucasica	0,019$^{ns/ns}$	0,023$^{ns/ns}$	0,052$^{ns/ns}$	**0,118**$^{ns/*}$	0,118$^{ns/ns}$	0,113$^{ns/ns}$
Ommatotriton ophryticus	0,026$^{ns/ns}$	0,014$^{ns/ns}$	0,025$^{ns/ns}$	**0,117**$^{*/*}$	0,117$^{ns/ns}$	**0,051**$^{*/ns}$
Paradactylodon persicus	0,001$^{ns/ns}$	0,009$^{ns/ns}$	0,012$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,205**$^{*/*}$
Pleurodeles nebulosus	0,002$^{ns/ns}$	**0,013**$^{*/*}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Pleurodeles poireti	0,001$^{ns/ns}$	**0,006**$^{*/*}$	0,011$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Proteus anguinus	0,008$^{ns/ns}$	0,009$^{ns/ns}$	0,085$^{ns/ns}$	0,464$^{ns/ns}$	**0,429**$^{*/*}$	**0,440**$^{*/*}$
Salamandra atra	0,015$^{ns/ns}$	0,020$^{ns/ns}$	0,096$^{ns/ns}$	**0,129**$^{ns/*}$	0,129$^{ns/ns}$	**0,145**$^{*/*}$
Salamandra salamandra	0,037$^{ns/ns}$	0,049$^{ns/ns}$	0,056$^{ns/ns}$	**0,078**$^{ns/*}$	0,078$^{ns/ns}$	**0,056**$^{*/ns}$
Salamandrina perspicillata	0,008$^{ns/ns}$	0,007$^{ns/ns}$	0,041$^{ns/ns}$	0,150$^{ns/ns}$	**0,150**$^{*/*}$	0,150$^{ns/ns}$
Salamandrina terdigitata	0,003$^{ns/ns}$	0,008$^{ns/ns}$	0,020$^{ns/ns}$	0,005$^{ns/ns}$	0,005$^{ns/ns}$	**0,041**$^{*/ns}$
Triturus carnifex	0,018$^{ns/ns}$	0,018$^{ns/ns}$	0,032$^{ns/ns}$	**0,230**$^{ns/*}$	**0,230**$^{ns/*}$	**0,196**$^{*/*}$
Triturus karelinii	0,021$^{ns/ns}$	0,013$^{ns/ns}$	0,041$^{ns/ns}$	0,047$^{ns/ns}$	0,047$^{ns/ns}$	**0,022**$^{ns/*}$
Triturus macedonicus	0,012$^{ns/ns}$	0,010$^{ns/ns}$	0,035$^{ns/ns}$	**0,105**$^{*/ns}$	0,105$^{ns/ns}$	0,073$^{ns/ns}$
Triturus marmoratus	0,014$^{ns/ns}$	0,026$^{ns/ns}$	0,011$^{ns/ns}$	0,010$^{ns/ns}$	0,010$^{ns/ns}$	**0,127**$^{*/*}$
Triturus pygmaeus	0,004$^{ns/ns}$	0,020$^{ns/ns}$	**0,129**$^{*/*}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$

Table 2: Average evaluation scores of Generalized Linear Models (GLM), Maxent models, and their ensembles, calibrated using either native or invasive occurrences of *Batrachochytrium salamandrivorans* (*Bsal*). The scores are divided into four runs, in which different *Bsal* occurrences were used for training and testing. Abbreviations are as follows; TSS: True Skill Statistic, Boyce; Boyce index, AUC; Area Under the Curve.

Niche	Run	Technique	Sensitivity	Specificity	TSS	Boyce	AUC	Somers' D
Native *Batrachochytrium salamandrivorans*	1	GLM	0.778	0.856	0.634	0.752	0.824	0.649
		Maxent	0.889	0.787	0.676	0.868	0.862	0.724
		Ensemble	0.778	0.826	0.603	0.814	0.838	0.675
	2	GLM	1	0.644	0.644	0.067	0.858	0.716
		Maxent	0.889	0.774	0.663	0.931	0.901	0.802
		Ensemble	1	0.693	0.693	0.373	0.88	0.759
	3	GLM	0.778	0.751	0.529	0.459	0.790	0.580
		Maxent	1	0.628	0.628	0.745	0.867	0.734
		Ensemble	0.889	0.734	0.623	0.830	0.846	0.693
	4	GLM	0.889	0.806	0.695	0.903	0.908	0.815
		Maxent	0.778	0.952	0.730	0.771	0.908	0.817
		Ensemble	0.778	0.938	0.716	0.881	0.914	0.828
Invasive *Batrachochytrium salamandrivorans*	1	GLM	1	0.766	0.766	0.652	0.919	0.838
		Maxent	1	0.769	0.769	0.875	0.913	0.826
		Ensemble	1	0.760	0.760	0.766	0.920	0.839
	2	GLM	1	0.846	0.846	0.941	0.954	0.907
		Maxent	1	0.900	0.900	0.819	0.948	0.897
		Ensemble	1	0.908	0.908	0.911	0.961	0.923
	3	GLM	1	0.937	0.937	0.806	0.979	0.958
		Maxent	1	0.897	0.897	0.898	0.952	0.904
		Ensemble	1	0.935	0.935	0.895	0.971	0.941
	4	GLM	1	0.934	0.934	0.821	0.984	0.968
		Maxent	1	0.894	0.894	0.837	0.955	0.910
		Ensemble	1	0.920	0.920	0.964	0.980	0.959

Core niche modelling

A total of 168 bivariate GLM and Maxent models were created for each *Bsal* niche, spread across four runs characterized by differently partitioned training and testing occurrences. Maxent models were successfully generated during all runs, whereas twenty-three of the native *Bsal* GLM models and eight of the invasive *Bsal* GLM models (distributed across runs) failed. Model evaluation scores obtained through sensitivity analyses, specificity analyses, TSS and the AUC were highly correlated (Pearson $r > 0.7$) for the native as well as invasive *Bsal* niches, while correlations between these metrics and the Boyce index were lower for both niches (Appendix S4.2, S4.3). Mean evaluation scores of the combined ESMs produced per replicate were overall high; native *Bsal* niche models averaged 0.861±0.106 based on sensitivity analyses, 0.798±0.109 for specificity analyses, 0.659±0.054 for TSS, 0.725±0.236 for the Boyce index and 0.870±0.035 for the AUC. Invasive *Bsal* models averaged 1.000±0.000 when using sensitivity analyses, 0.881±0.081 for specificity analyses, 0.881±0.081 for TSS, 0.884±0.084 for the Boyce index and 0.958±0.026 for the AUC. Evaluation scores per algorithm (GLM, MAXENT, and their ensembles) are displayed per run, and per *Bsal* niche, in Table 2. The native model appeared to perform well in the invasive range, given that invasive *Bsal* records were without exception located in 'highly suitable' grid cells (Fig. 3).

The spatial predictions displaying suitability for native and invasive *Bsal* were found to differ considerably (Fig. 3). Predictions generated using native *Bsal* records suggest the possibility for an extensive distribution across Europe, in addition to parts of the North African Mediterranean shore and Anatolia (Fig. 3a, c, e). GLM and MAXENT results differ, with the former suggesting occurrence to be overall more restricted in comparison to the latter. Novel climates (type 1 novelty) in respect to the native *Bsal* distribution were found to occur in Western Europe and the British Isles, as well as various desert regions. Examination of EXDET-generated maps (Fig. S4.5) revealed that this novelty is driven by precipitation of the wettest quarter (bio16), which is much higher in the native *Bsal* range when compared to the Western Palearctic (see also Table S4.1). Novelty in desert regions is driven by higher precipitation seasonality (bio15) in respect to East Asia. The predicted occurrence obtained by using invasive *Bsal* records was found to be more restricted compared to that of the native niche (Fig. 3b, d, f). High consensus between GLM and MAXENT was however found, due to which these models, as well as the final ESM suggest that north-western Europe and various mountain ranges (Pyrenees, northern Apennines, Western- and Dinaric Alps, as well as parts of the Caucasus) may be climatically suitable for *Bsal* colonization. Geographical regions subject to Mediterranean, continental (most of Eastern, but also Northern Europe) and subarctic (Northern Europe) climates are suggested to be largely unsuitable for further *Bsal* spread, being characterized by climate conditions (Fig. S4.6) not found in the invasive range.

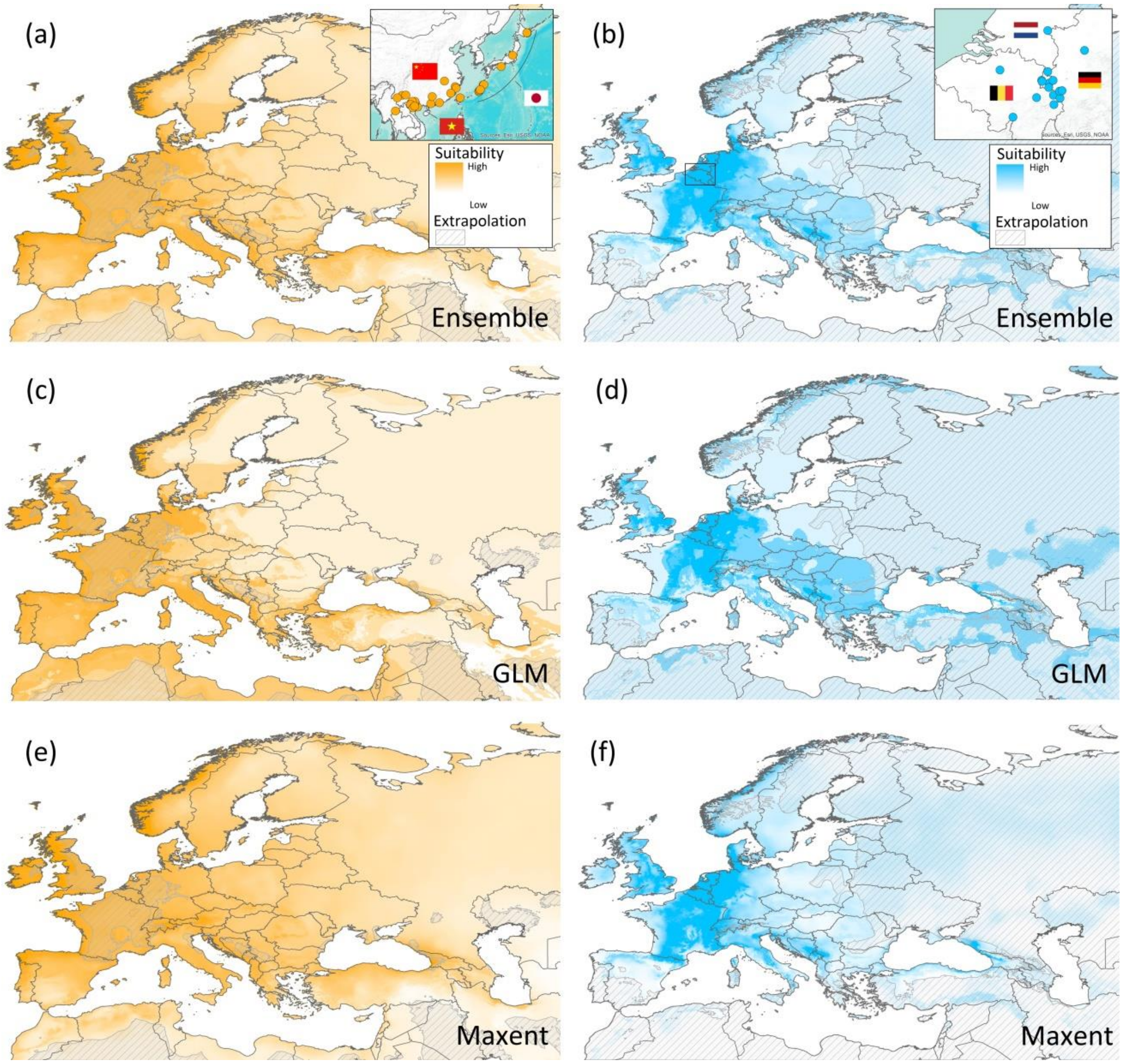

Figure 3: Relative habitat suitability for *Batrachochytrium salamandrivorans* (*Bsal*) across part of the Western Palearctic, generated through ensembles of small niche models (ESMs), using either native East Asian (a, c, e) or invasive European (b, d, f) occurrences (insets). Hatched areas indicate regions with novel climate conditions (extrapolation) in respect to those characterizing the native or invasive *Bsal* range. The upper panels (a, b) display weighted ensembles composed of performance-evaluated GLM (c, d) and MAXENT (e, f) niche models. Insets show the *Bsal* distribution, with flags denoting countries of main occurrence.

Discussion

By first measuring ecological niche overlap between native and invasive *Bsal* niches, we revealed that the niche of this pathogen has so far stayed conserved during its European invasion, and is only partially filled. The presence of niche conservatism is not unexpected, given that the majority of putative salamander hosts occur in climate conditions found in East Asia as well (Fig. 1). Surprisingly, we found that overlaps between these hosts and the wide, native observed *Bsal* niche were overall significantly lower compared to overlaps calculated using the narrow, invasive niche. A pervasive underestimation of niche overlap between native *Bsal* and putative hosts in the invasive range (see below) seems to partly explain this difference. Nevertheless, relative higher overlap between invasive *Bsal* and its Western Palearctic hosts also suggests that the former (already) occurs in climates shared by a multitude of salamanders. Indeed, observed niches of 25 host species from north-western Europe, Italy, and several (circum-European) mountain ranges show significant similarity to the invasive *Bsal* niche (or vice versa, Table 1). These hosts represent more than a third of Western Palearctic salamander diversity (Sparreboom, 2014; AmphibiaWeb, 2017).

Pathogen-host overlap depends on environmental context

Guisan *et al.* (2014) argued that invasive species models which fail to take differences between environmental conditions in a species' native and invasive ranges into account may falsely detect niche shifts, and create inaccurate predictions of invasion risk (see also Warren *et al.*, 2008; Broennimann *et al.*, 2012; Early & Sax, 2014). Several empirical studies confirmed this notion (e.g. Escobar *et al.*, 2014; Qiao *et al.*, 2017), which reinforced the need for ongoing development of approaches that correct for environmental dissimilarity (Di Cola *et al.*, 2017; Hattab *et al.*, 2017). Despite the obvious importance of this step in invasive species modelling, a caveat seems to exist; when using ordination-based niche overlap analyses, lack of similarity between two observed niches can be driven by actual differences, but also by environmental context. Specifically, a recent study suggested that similarity between two niches may be underestimated when invasive populations which inhabit homogenous environmental conditions are compared to native counterparts that occupy an environmentally heterogeneous distribution (Qiao *et al.*, 2017). We here confirm occurrence of this issue, given that overlaps calculated between the native *Bsal* niche and putative Western Palearctic host species were overall extremely low and usually non-significant (Table 1, Fig. 2), despite the fact that the former regularly encompassed salamander niches in environmental space. A similar result was found when comparing native and invasive *Bsal* niches (Fig. 1). Large differences in niche breadth, either due to partial niche filling or because a species is simply characterized by a narrow observed niche, seem to exacerbate this situation. What follows is that early invasion risk analyses based on ordination may fail to detect possible host species when their environmental background and observed niche breadth differ considerably from those of the pathogen in its native range. In turn, this could affect conservation prioritization efforts. Potential solutions include restricting analyses to environments shared between the two species in question, however, this also decreases statistical power (Qiao *et al.*, 2017). A more suitable alternative could be to compare pathogen suitability derived from an ecological niche model based on native range data with the distribution of possible hosts in geographical space (Warren *et al.*, 2008). In conclusion, we advise caution when using ordination to measure overlap between pathogens and host that differ in environmental context, while noting that the issue raised above has implications for invasive species modelling generally (Qiao *et al.*, 2017).

While our overlap calculations between native *Bsal* and putative Western Palearctic salamander hosts may have been affected by differences in environmental context, we stress that this is not the case for overlaps measured between invasive *Bsal* and these hosts. The invasive observed niche of *Bsal* is only partially filled, but already shows significant similarity to 25 confirmed and putative host species. Our identification of potential key hosts (Table 1) is therefore tentative and conservative, yet, increases concerns on the potential of *Bsal* to cause loss of salamander diversity across the Western Palearctic.

Disentangling additional patterns of pathogen-host overlap

The relatively high overlap scores (Table 1, Fig. 2) recovered by ENFA were not unexpected, as this algorithm is known to overestimate observed niche overlap (Broennimann *et al.*, 2012). ENFA however also suggested that extensive niche expansion took place during the *Bsal* invasion, which contradicts results produced by PCA-env and WITHIN-env. We assume that this result should be interpreted in the light of ENFA being forced to combine all information from what would be multiple specialization factors (Hirzel *et al.*, 2002) into a single tolerance factor, which together with the marginality axis forms environmental space (Broennimann *et al.*, 2012). Changes in niche position therefore cause differences in the distance of the niche from the mean environmental conditions of the study area, but do not necessarily represent occupation of novel conditions. In our case, incomplete niche filling could for instance have been interpreted as a positional change (i.e., niche expansion). Visual evaluation of ENFA-generated comparisons between the native and invasive *Bsal* niches (unpublished results, available upon request) revealed that the latter is characterized by significantly lower marginality compared to the native *Bsal* niche. As this outcome agrees with the peripheral position of the narrow invasive *Bsal* niche produced by PCA-env and WITHIN-env analyses we interpret ENFA results as indicating partial niche filling, rather than demonstrating true niche expansion.

Four previously confirmed hosts which occur in Italy, beyond the current *Bsal* range, were found to significantly overlap with the invasive *Bsal* niche (Table 1). These species co-occur with two additional putative and three confirmed hosts, of which the latter are subject to *Bsal*-related declines in northern

Europe (Spitzen-van der Sluijs *et al.*, 2016; Stegen *et al.*, 2017). Accordingly, we regard north-western Italy, and particularly the northernmost Apennines to rank among the main regions in risk of *Bsal* colonization (see also Appendix S3 for a detailed discussion). Conversely, we did not find significant similarity between either *Bsal* niche and three other confirmed hosts (Table 1), which occur in relatively dry, Mediterranean (Csa) or subarctic (Dfc) conditions not found in East Asia (Peel *et al.*, 2007; Appendix S3). Whether *Bsal* is able to colonize such environments by means of niche expansion consequently remains an interesting topic for future studies.

Bsal *habitat suitability across the Western Palearctic*

Doubts have recently been raised on the usefulness of presence-only niche models in estimating invasion risk or prioritizing species conservation (Guillera-Arroita *et al.*, 2015). Specifically, there is concern because model predictions do not display an actual occurrence probability, as often assumed, but show relative habitat suitability (Phillips & Dudík, 2008; Yackulic *et al.*, 2013). The predictive niche models generated here are therefore restricted to illustrating where risk of *Bsal* spread is high or low, but cannot infer absolute risk. This limitation does not obstruct our aim to show how differences in observed native and invasive *Bsal* niches and their environmental context may affect suitability predictions. Nevertheless, our niche model predictions differ considerably from those obtained by two other studies that recently estimated habitat suitability for invasive *Bsal* (Feldmeier *et al.*, 2016; Katz & Zellmer, 2018), which prompts us to put these into perspective. Using MAXENT and invasive occurrence records, Feldmeier *et al.* (2016) predicted that suitability for *Bsal* in the Belgian-French-German border region would be mostly restricted to hilly regions. Katz and Zellmer (2018) used a model selection approach, from which the best-supported MAXENT model of five candidates suggested *Bsal* habitat suitability to be concentrated in hills and mountains of west- and south Germany. Different study area extents and cell sizes of the environmental parameters seem to first and foremost drive the differences between these studies and ours (Soberón & Nakamura, 2009). Specifically, MAXENT is sensitive to background size, which may cause a model to underpredict when using restricted environmental backgrounds (Lobo *et al.*, 2010), especially when calibration is based on a partially filled niche (Elith *et al.*, 2010; Václavík & Meentemeyer, 2012; Kramer-Schadt *et al.*, 2013). The restricted available amount of invasive *Bsal* occurrence sites may exacerbate this situation (Wisz *et al.*, 2008), as acknowledged in both studies. While the results of Feldmeier *et al.* (2016) and Katz and Zellmer (2018) may therefore be prone to underprediction, ours may overpredict the potential distribution of *Bsal* as we did not take potential dispersal limitations (e.g. biogeographical barriers) into account. Despite these methodological concerns, we nevertheless do not deem it likely that suitable habitat for *Bsal* in especially north-western Europe would be so restricted as suggested by Feldmeier *et al.* (2016) and Katz and Zellmer (2018), especially given the fact that no major biogeographical barriers for salamander dispersal exists in this region, while the distribution of confirmed host species is near-continuous (Sparreboom, 2014). Future studies that incorporate vital information on spatial variation in pathogen prevalence and mechanistic data are therefore badly needed to improve predictions of potential spread.

Surprisingly, ExDET results revealed conditions in north-western Europe (where *Bsal* now occurs) to be novel in respect to the native *Bsal* distribution, thereby suggesting considerable extrapolation to take place. An overall higher amount of rainfall during the wettest quarter in East Asia, likely due to the East Asian monsoon, was found to drive this pattern (Table S4.1; Fig. S4.5). Whereas the invasive *Bsal* range is located within north-western Europe, we are hesitant to conclude that a niche shift would have occurred during invasion. Ordination analyses do not support the presence of such a shift (Fig. 1). Furthermore, the wettest quarters in the Western Palearctic and East Asia correspond respectively to winter and summer, while in both regions salamander activity mainly takes place during spring and autumn (Sparreboom, 2014). We therefore assume that presence of a period with high rainfall preceding activity, rather than the exact season and amount of precipitation will be of importance for *Bsal*.

Moving from macro to micro

Looking beyond the scope of this study, our findings suggest the need for local, mechanistic assessments as we failed to recover niche similarity between *Bsal* and *Lissotriton vulgaris*, a confirmed host species (Martel *et al.*, 2014; Spitzen-van der Sluijs *et al.*, 2016). As discussed above, methodological issues related to environmental differences between the Western Palearctic and East Asia could be responsible for this result, but incongruence of ecological conditions at large- and local spatial levels might be responsible as well (Cohen *et al.*, 2016). Specifically, *Bsal* niches build at broad spatial scales might not necessarily be representative of the conditions in which this pathogen occurs. Climate variation across relatively small landscape features that contain considerable environmental heterogeneity, such as river valleys or narrow mountain ranges, becomes dissolved at the spatial level of our analyses due to which local microhabitat buffering is not accounted for. Furthermore, also biotic interactions, which were not considered by us, are known to influence distribution patterns of species (Guisan & Zimmermann, 2000; Peterson, 2006; Soberón & Nakamura, 2009), perhaps even to a greater extent than generally assumed (Early & Sax, 2014). Studies focusing on invasive, fungal pathogens similar to *Bsal* have confirmed that predation or competition can influence local pathogen prevalence (e.g. Raffel *et al.*, 2013; Schmeller *et* *al.*, 2014; Cohen *et al.*, 2016; Langwig *et al.*, 2016), which could potentially influence distribution patterns (Ostfeld *et al.*, 2005; Purse & Golding, 2015; but see Cohen *et al.*, 2016). We therefore expect that a focus on the mechanisms driving or restricting *Bsal* prevalence, including community or assemblage-level processes, might pose fruitful next steps to further disentangle the invasion ecology of this emerging pathogen.

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Supporting Information for

Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palearctic

Appendix S1 Species occurrence records

Please refer to https://onlinelibrary.wiley.com/doi/full/10.1111/ddi.12795.

Appendix S2 Detailed methodology

S2.1 Data preparation

Rationale for approach used to describe host species ecological niches

Distribution data for many salamander host species is incomplete, because systematic changes involving species-splitting and the discovery of new taxa continue to occur regularly (Sparreboom, 2014), while high-resolution point occurrence data is not available for the majority of Mediterranean and Near-Asian species. Fortunately, Ficetola *et al.* (2014) recently showed that expert-drawn maps of European amphibians created during the Global Amphibian Assessment represent the known distribution of these species well. Accuracy of these maps is greater than that of point data available through the Global Biodiversity Facility (GBIF), at least in case of the region and taxa considered by us. Hence, Ficetola *et al.* (2014) recommended using these maps which were provided in shapefile format by IUCN (2015) for macroecological analyses. Five micro endemic species (*Lyciasalamandra arikani*, *L. billae*, *L. irfani, L. yehudahi* and *Paradactylodon gorganensis*) were omitted because their restricted range size in combination with resolution of our environmental data (2.5 arc minutes) did not permit description of their niche. To describe ecological niches of salamanders we therefore followed the approach of Yap *et al.* (2015); 100 occurrence records per species were randomly placed inside the boundaries of their distribution limits, and subsequently filtered based on the species-specific altitudinal extent of occurrence. In the unexpected event that erroneous occurrence records would be randomly drawn due to considerable mistakes in the used IUCN range maps, the concerning records would be dwarfed in environmental space by the large amount of accurate records, and therefore not have a significant impact on niche overlap calculations. Among other reasons, this advantage was one of the reasons why we chose to measure overlap in environmental space using kernel smoothers (Broennimann *et al.*, 2012; Di Cola *et al.*, 2017).

S2.2 Pre-modelling niche quantification and overlap

Description of niche overlap algorithms

The basis of each ordination approach consists of a Principal Component Analysis (PCA) constructed using the seven environmental parameters described above. The first two axes of this PCA were used to create a 2D representation of gridded environmental space (Broennimann *et al.*, 2012; Di Cola *et al.*, 2017). As explained above, PCA-env and WITHIN-env are calibrated on the entire environmental space of the study area rather than using only climatic values corresponding to occurrence records. However, WITHIN-env uses *a priori* selected groups (in this case the two different study areas) to optimize the combinations of environmental parameters in the first two components of the PCA. While niche overlap measurements using Ecological Niche Factor Analysis (ENFA, Hirzel *et al.*, 2002) are also performed in 2D environmental space, the PCA is constructed differently. Accordingly, the first component explains 100% of the ‘marginality factor’, which is defined as the difference between the average conditions in the occurrence density (see below) of a focal entity and the conditions in the centroid of environmental space. In other words, marginality indicates to what degree a niche is dissimilar from the average conditions in the study area, with higher values indicating higher marginality. The subsequent components consist of ‘specialization factors’: axes which maximize the ratio of variance between the occurrence density and the study area (Hirzel *et al.*, 2002). Marginality can also be seen as an indication for specialization, higher marginality meaning higher specialization. A varying part of the specialization can therefore already be explained within the marginality factor, with the remainder being described in the specialization factors. Due to the fact that specialization values can range from 1 to infinity, ‘tolerance’ was used as an alternative to limit the extent of environmental space (Broennimann *et al.*, 2012); tolerance is calculated as the inverse of specialization (1/S), ranging from 0 (specialist species) to 1 (generalist species).

Explanation of equivalency and similarity tests (sensu Warren et al., 2008)

Equivalency tests assess whether niches of two focal entities are identical by pooling occurrences of both, followed by the creation of two random sets of occurrences featuring the original sample sizes, with which overlap scores are calculated. This procedure is repeated 100 times in order to create a null distribution of overlap scores, which is compared to the actual overlap. When the actual overlap value falls beyond 95% of the simulated values the hypothesis of niche equivalency is rejected. Similarity tests are used to assess whether niches of two focal entities are significantly less or more similar than expected by chance, based on the environmental values that characterise the study area(s). Again, 100 randomizations are created by placing the occurrence density at random within the background of entity A, which was compared to the background of entity B and vice versa. When the actual overlap value is significantly (p <0.05) higher, or lower, than expected from the null distribution based on a two-tailed test, the null hypothesis that the two focal entities are not more or less similar to each other than expected by chance is rejected.

S2.3 Core niche modelling

First, we created four replicate occurrence datasets for each *Bsal* entity by using cross-validation to partition records into training (70%) and testing (30%) data, which enabled performance evaluation of submodels and ESMs. Secondly, we produced 21 bivariate environmental datasets, which together contain all possible combinations of two parameters originating from the original uncorrelated set of seven. The occurrence- and environmental datasets were combined to quantify native and invasive *Bsal* niches using two algorithms; Generalized Linear Models (GLM), and MAXENT (Phillips & Dudík, 2008; MAXENT.Phillips in biomod2). We chose these algorithms based on their ability to outdo other niche modelling techniques in terms of general performance and transferability (e.g. Wisz *et al.*, 2008; Broennimann *et al.*, 2012; Breiner *et al.*, 2015). The GLMs applied here allow linear and quadratic relationships between the response (occurrence) and explanatory (environmental) parameters, and use forward and backward stepwise regression based on the Bayesian Information Criterion (BIC, following Breiner *et al.*, 2015) for parameter selection (Thuiller *et al.*, 2016). MAXENT is a machine-learning algorithm that uses an iterative approach to quantify a niche based on maximum entropy (i.e. that is most spread out, or closest to uniform) subject to the constraint that the expected value for each environmental parameter underlying the niche matches its empirical average (Phillips *et al.*, 2006). Both algorithms were employed based on their default parameters as included in biomod2, apart from the use of BIC in GLM (Thuiller *et al.*, 2016). All bivariate submodels were subsequently evaluated using the Area Under the Curve (AUC; e.g. Lobo *et al.*, 2008; Phillips & Dudík, 2008; Wisz *et al.*, 2008; Rödder *et al.*, 2009; Barve *et al.*, 2011; Di Cola *et al.*, 2017). In turn, the AUC was used to calculate Somers' D (D=2*(AUC-0.5)), which gives more weight to submodels that perform well, and less to those that perform poorly (Breiner *et al.*, 2015). ESMs were created for each of the four runs, comprising a weighted average of GLM and MAXENT outputs characterized by a Somers' D higher than 0 (i.e. AUC > 0.5, so, better than a random model), and again evaluated using the test data reserved for that particular run. The final ESM, and final GLM and MAXENT models were ensembled by combining weighted (i.e., Somer's D evaluated) averages produced by each of the 21 bivariate datasets.

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Appendix S3 Detailed niche overlap results

Table S3.1 Confirmed and putative salamander hosts to *Batrachochytrium salamandrivorans (Bsal)*, their altitudinal range, and pathogen-host niche overlap scores based on three different ordination techniques. Niche overlap was measured using Schoener's *D*, which ranges between 0 (no overlap) and 1 (complete overlap). Comparisons between the host and *Bsal* are shown before the /, vice versa after. *represents significant similarity, ns means 'not significant'. Species that overlap significantly with any *Bsal* niche and/or vice versa are written in **bold**.

Species	**Susceptible**	**Altitude**	***N* occurrences**		**Native niche***			**Invasive niche***	
				PCA-env	**WITHIN-env**	**ENFA**	**PCA-env**	**WITHIN-env**	**ENFA**
Calotriton arnoldi	PUTATIVE	700 - 1200m	92	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,131**$^{*/*}$	**0,131**$^{*/*}$	0,000$^{ns/ns}$
Calotriton asper	PUTATIVE	700 - 2500m	84	0,007$^{ns/ns}$	0,014$^{ns/ns}$	0,035$^{ns/ns}$	**0,201**$^{*/ns}$	**0,201**$^{*/ns}$	**0,119**$^{*/ns}$
Chioglossa lusitanica	PUTATIVE	100-1000m	87	0,007$^{ns/ns}$	0,005$^{ns/ns}$	0,040$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,098$^{ns/ns}$
Euproctus montanus	PUTATIVE	0 - 2260m	100	0,002$^{ns/ns}$	0,002$^{ns/ns}$	0,028$^{ns/ns}$	0,020$^{ns/ns}$	0,020$^{ns/ns}$	**0,479**$^{*/*}$
Euproctus platycephalus	CONFIRMED	50 - 1800m	100	0,000$^{ns/ns}$	0,003$^{ns/ns}$	0,005$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,047**$^{*/ns}$
Hydromantes ambrosii	PUTATIVE	0 - 2300m	100	0,001$^{ns/ns}$	0,001$^{ns/ns}$	0,010$^{ns/ns}$	**0,090**$^{ns/*}$	**0,090**$^{ns/*}$	**0,077**$^{*/ns}$
Hydromantes flavus	PUTATIVE	40 - 1050m	100	0,000$^{ns/ns}$	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,003$^{ns/ns}$
Hydromantes genei	PUTATIVE	8 - 650m	100	0,000$^{ns/ns}$	0,004$^{ns/ns}$	0,021$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Hydromantes imperialis	PUTATIVE	7 - 1170m	100	0,000$^{ns/ns}$	0,002$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,024$^{ns/ns}$
Hydromantes italicus	PUTATIVE	80 - 1600m	99	0,003$^{ns/ns}$	0,001$^{ns/ns}$	0,091$^{ns/ns}$	**0,151**$^{*/ns}$	**0,151**$^{*/ns}$	**0,143**$^{*/ns}$
Hydromantes sarrabusensis	PUTATIVE	200 - 850m	100	0,000$^{ns/ns}$	0,001$^{ns/ns}$	0,005$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Hydromantes strinatii	CONFIRMED	80 - 2400 m	100	0,005$^{ns/ns}$	0,003$^{ns/ns}$	**0,060**$^{ns/*}$	**0,521**$^{*/*}$	**0,521**$^{*/*}$	**0,205**$^{*/ns}$
Hydromantes supramontis	PUTATIVE	100 - 1400m	99	0,000$^{ns/ns}$	0,002$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Ichthyosaura alpestris	CONFIRMED	0 - 2500m	100	0,014$^{ns/ns}$	0,008$^{ns/ns}$	0,054$^{ns/ns}$	**0,214**$^{*/*}$	**0,214**$^{*/*}$	**0,094**$^{*/*}$
Lissotriton boscai	PUTATIVE	0 - 1870m	100	0,009$^{ns/ns}$	0,017$^{ns/ns}$	0,083$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,024$^{ns/ns}$
Lissotriton helveticus	CONFIRMED	0 - 2400m	100	0,017$^{ns/ns}$	0,015$^{ns/ns}$	0,043$^{ns/ns}$	0,165$^{ns/ns}$	0,165$^{ns/ns}$	**0,305**$^{*/*}$
Lissotriton italicus	CONFIRMED	< 2000m	100	0,004$^{ns/ns}$	0,008$^{ns/ns}$	0,080$^{ns/ns}$	0,017$^{ns/ns}$	0,017$^{ns/ns}$	**0,011**$^{*/ns}$
Lissotriton kosswigi	PUTATIVE	> 0m	100	0,001$^{ns/ns}$	0,001$^{ns/ns}$	0,004$^{ns/ns}$	0,044$^{ns/ns}$	0,044$^{ns/ns}$	0,023$^{ns/ns}$
Lissotriton lantzi	PUTATIVE	> 0m	100	0,021$^{ns/ns}$	0,007$^{ns/ns}$	0,047$^{ns/ns}$	0,176$^{ns/ns}$	**0,176**$^{*/ns}$	0,106$^{ns/ns}$
Lissotriton montandoni	PUTATIVE	120 - 2000m	100	0,010$^{ns/ns}$	0,001$^{ns/ns}$	0,140$^{ns/ns}$	0,006$^{ns/ns}$	0,006$^{ns/ns}$	0,317$^{ns/ns}$
Lissotriton vulgaris	CONFIRMED	0 - 2150m	100	0,073$^{ns/ns}$	**0,110**$^{*/*}$	0,079$^{ns/ns}$	0,034$^{ns/ns}$	0,034$^{ns/ns}$	0,036$^{ns/ns}$
Lyciasalamandra antalyana	PUTATIVE	100 - 650m	50	0,003$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,065$^{ns/ns}$
Lyciasalamandra atifi	PUTATIVE	190 - 1500m	99	0,001$^{ns/ns}$	0,002$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Lyciasalamandra fazilae	PUTATIVE	0 - 2000m	100	0,006$^{ns/ns}$	0,001$^{ns/ns}$	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,081$^{ns/ns}$
Lyciasalamandra flavimembris	PUTATIVE	0 - 600m	94	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Lyciasalamandra helverseni	PUTATIVE	> 0m	100	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Lyciasalamandra luschani	PUTATIVE	60 - 840m	52	0,013$^{ns/ns}$	0,000$^{ns/ns}$	0,002$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$

Mertensiella caucasica	PUTATIVE	0 - 1800m	75	0,014$^{ns/ns}$	0,013$^{ns/ns}$	0,040$^{ns/ns}$	**0,127**$^{*/*}$	0,127$^{ns/ns}$	0,120$^{ns/ns}$
Neurergus crocatus	CONFIRMED	1500 - 2000m	18	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Neurergus derjugini	PUTATIVE	1300 - 1400m	16	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,025$^{ns/ns}$
Neurergus kaiseri	PUTATIVE	1500 - 2000m	26	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,001$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,072$^{ns/ns}$
Neurergus strauchii	PUTATIVE	1000 - 2000m	100	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,057$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Ommatotriton ophryticus	PUTATIVE	0 - 2750m	100	0,016$^{ns/ns}$	0,007$^{ns/ns}$	0,032$^{ns/ns}$	0,124$^{ns/ns}$	**0,124**$^{*/ns}$	**0,061**$^{ns/*}$
Ommatotriton vittatus	PUTATIVE	0 - 2750m	100	0,024$^{ns/ns}$	0,006$^{ns/ns}$	0,028$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Paradactylodon persicus	PUTATIVE	0 - 1500m	55	0,000$^{ns/ns}$	0,004$^{ns/ns}$	0,043$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	**0,263**$^{*/*}$
Pleurodeles nebulosus	PUTATIVE	0 - 457m	56	0,002$^{ns/ns}$	0,006$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Pleurodeles poireti	PUTATIVE	> 0m	100	0,000$^{ns/ns}$	**0,003**$^{*/*}$	0,011$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Pleurodeles waltl	CONFIRMED	< 1565m	100	0,008$^{ns/ns}$	0,010$^{ns/ns}$	0,079$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Proteus anguinus	PUTATIVE	Entire range	100	0,006$^{ns/ns}$	0,004$^{ns/ns}$	0,085$^{ns/ns}$	**0,464**$^{*/*}$	**0,464**$^{*/*}$	**0,490**$^{*/ns}$
Salamandra algira	PUTATIVE	80 - 2450m	100	0,003$^{ns/ns}$	0,009$^{ns/ns}$	0,007$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Salamandra atra	PUTATIVE	400 - 2800m	100	0,011$^{ns/ns}$	0,011$^{ns/ns}$	0,094$^{ns/ns}$	**0,141**$^{ns/*}$	0,141$^{ns/ns}$	**0,169**$^{ns/*}$
Salamandra corsica	PUTATIVE	50 - 1750m	100	0,002$^{ns/ns}$	0,001$^{ns/ns}$	0,022$^{ns/ns}$	0,048$^{ns/ns}$	0,048$^{ns/ns}$	0,095$^{ns/ns}$
Salamandra infraimmaculata	PUTATIVE	180 - 2000m	100	0,027$^{ns/ns}$	0,005$^{ns/ns}$	0,019$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Salamandra lanzai	PUTATIVE	1200m - 2600m	100	0,002$^{ns/ns}$	0,004$^{ns/ns}$	0,001$^{ns/ns}$	0,020$^{ns/ns}$	0,020$^{ns/ns}$	0,000$^{ns/ns}$
Salamandra salamandra	CONFIRMED	< 2500m	100	0,023$^{ns/ns}$	0,025$^{ns/ns}$	0,047$^{ns/ns}$	**0,082**$^{ns/*}$	0,082$^{ns/ns}$	**0,062**$^{ns/*}$
Salamandrella keyserlingii	CONFIRMED	> 0m	100	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,014$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$
Salamandrina perspicillata	CONFIRMED	50 - 1500m	94	0,004$^{ns/ns}$	0,003$^{ns/ns}$	0,085$^{ns/ns}$	**0,147**$^{*/ns}$	**0,147**$^{*/ns}$	**0,129**$^{*/ns}$
Salamandrina terdigitata	PUTATIVE	50 - 1500m	96	0,002$^{ns/ns}$	0,006$^{ns/ns}$	0,030$^{ns/ns}$	0,006$^{ns/ns}$	0,006$^{ns/ns}$	0,051$^{ns/ns}$
Triturus carnifex	PUTATIVE	0 - 2000m	100	0,012$^{ns/ns}$	0,008$^{ns/ns}$	0,093$^{ns/ns}$	**0,240**$^{*/*}$	**0,240**$^{*/ns}$	**0,200**$^{*/ns}$
Triturus cristatus	CONFIRMED	0 - 1750m	100	0,029$^{ns/ns}$	**0,100**$^{ns/*}$	0,088$^{ns/ns}$	0,017$^{ns/ns}$	0,017$^{ns/ns}$	**0,095**$^{*/ns}$
Triturus dobrogicus	PUTATIVE	< 300m	100	0,008$^{ns/ns}$	0,000$^{ns/ns}$	0,075$^{ns/ns}$	0,003$^{ns/ns}$	0,003$^{ns/ns}$	0,000$^{ns/ns}$
Triturus ivanbureschi	PUTATIVE	< 1700m	100	0,007$^{ns/ns}$	0,007$^{ns/ns}$	0,016$^{ns/ns}$	0,007$^{ns/ns}$	0,007$^{ns/ns}$	0,036$^{ns/ns}$
Triturus karelinii	PUTATIVE	0 - 2100m	100	0,012$^{ns/ns}$	0,005$^{ns/ns}$	0,070$^{ns/ns}$	0,047$^{ns/ns}$	0,047$^{ns/ns}$	0,030$^{ns/ns}$
Triturus macedonicus	PUTATIVE	< 1725m	100	0,007$^{ns/ns}$	0,005$^{ns/ns}$	0,031$^{ns/ns}$	0,109$^{ns/ns}$	**0,109**$^{*/ns}$	0,084$^{ns/ns}$
Triturus marmoratus	PUTATIVE	0 - 2100m	100	0,007$^{ns/ns}$	0,017$^{ns/ns}$	0,061$^{ns/ns}$	0,010$^{ns/ns}$	0,010$^{ns/ns}$	**0,145**$^{*/*}$
Triturus pygmaeus	PUTATIVE	0 - 1450m	100	0,002$^{ns/ns}$	0,010$^{ns/ns}$	0,101$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$	0,000$^{ns/ns}$

Four host species of which susceptibility to *Bsal* was confirmed during recent infection experiments (Martel *et al.*, 2014), but occur beyond the current invasive range of this disease agent, were found by us to overlap significantly with the invasive *Bsal* niche; *Euproctus platycephalus*, *Hydromantes strinatii*, *Lissotriton italicus* and *Salamandrina perspicillata*. These species are Italian endemics, apart from *H. strinatii* which also occurs in a restricted area of south-eastern France. *Hydromantes strinatii* and *S. perspicillata* share their range in north-western Italy with *Ichthyosaura alpestris*, *L. vulgaris,* and *Salamandra salamandra*, of which north European populations are currently subject to *Bsal*-related declines (Spitzen-van der Sluijs *et al.*, 2016; Stegen *et al.*, 2017). Furthermore, *Hydromantes ambrosii* and *Triturus carnifex*, which are putative hosts but also show high niche similarity to invasive *Bsal*, occur in this particularly salamander-rich Italian region as well. Due to the combination of multiple susceptible salamander species, high endemism and suitable climatic conditions for *Bsal* (see also Fig. 3) we regard the northernmost Apennines to rank among the main regions in risk of *Bsal* colonisation.

Conversely, we did not find significant similarity between either *Bsal* niche and *N. crocatus*, *P. waltl* and *S. keyserlingii*, which have been confirmed as susceptible hosts during infection trials (Martel *et al.*, 2014). Both *N. crocatus* and *P. waltl* occur in relatively dry, Mediterranean conditions (Csa; Peel *et al.*, 2007), that are absent from East Asia. Accordingly, the niche of these species (as those of most other Mediterranean, putative host species including the genus *Lyciasalamandra*) show little to no possibility for overlap with that of *Bsal*. Whether *Bsal* is able to colonize Mediterranean environments consequently remains an interesting topic for future studies. Similar reasoning may explain the lack of niche overlap between *Bsal* and *S. keyserlingii*; the latter largely inhabits subarctic climates (Dfc; Peel *et al.*, 2007) in which invasive or native *Bsal* have not been found to occur. Nevertheless, *Bsal*-infection has been confirmed to occur among S. *keyserlingii* in northern Japan (Martel *et al.*, 2014), which is characterized by a humid, continental climate (Peel *et al.*, 2007).

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Appendix S4 Environmental parameters, evaluation metric comparison and extrapolation

Table S4.1 Environmental parameters used in the current study, including average values ± standard deviation and range for the native and invasive niches of *Batrachochytrium salamandrivorans* (*Bsal*).

	Environmental parameter	**Native *Bsal***	**Invasive *Bsal***
Bio03	Isothermality (c°)	35.45 ± 8.03 (23-52)	32.73 ± 1.39 (30-34)
Bio04	Temperature seasonality (c°, standard deviation)	50.83 ± 18.50 (25.65-93.11)	55.96 ± 5.46 (5.51-5.69)
Bio05	Max temp of the warmest month (c°)	26.57 ± 5.11 (15.6-31.3)	21.29 ± 1.28 (18.6-22.5)
Bio06	Min temp of coldest month (c°)	4.51 ± 7.9 (-13.5-13.6)	-1.07 ± 1.01 (-3.1--0.1)
Bio15	Precipitation seasonality (coefficient of variation)	63.59 ± 23.67 (30-93)	11.87 ± 1.60 (9-15)
Bio16	Precipitation of wettest quarter (mm)	911.32 ± 266.34 (400-1500)	257.13 ± 41.50 (213-353)
Bio17	Precipitation of driest quarter (mm)	167.95 ± 131.02 (36-483)	195.07 ± 34.18 (150-260)

Table S4.2 Pairwise Pearson correlations between evaluation metrics based on models built using the native niche of *Batrachochytrium salamandrivorans*. Abbreviations are as follows; TSS: True Skill Statistic, Boyce; Boyce index, AUC; Area Under the Curve. *represents significant correlation.

		Sensitivity	**Specificity**	**TSS**	**Boyce**	**AUC**
	Pearson Correlation	1	**-0.793***	**0.785***	-0.193	**0.602***
Sensitivity	*Sig. (2-tailed)*		0.002	0.002	0.547	0.038
	N	12	12	12	12	12
	Pearson Correlation	**-0.793***	1	-0.244	0.308	-0.287
Specificity	*Sig. (2-tailed)*	0.002		0.445	0.331	0.367
	N	12	12	12	12	12
	Pearson Correlation	**0.785***	-0.244	1	0.008	**0.667***
TSS	*Sig. (2-tailed)*	0.002	0.445		0.980	0.018
	N	12	12	12	12	12
	Pearson Correlation	-0.193	0.308	0.008	1	0.178
Boyce	*Sig. (2-tailed)*	0.547	0.331	0.980		0.581
	N	12	12	12	12	12
AUC	*Pearson Correlation*	**0.602***	-0.287	**0.667***	0.178	1
	Sig. (2-tailed)	0.038	0.367	0.018	0.581	
	N	12	12	12	12	12

Table S4.3 Pairwise Pearson correlations between evaluation metrics based on models built using the invasive niche of Batrachochytrium salamandrivorans. Abbreviations are as follows; TSS: True Skill Statistic, Boyce; Boyce index, AUC; Area Under the Curve. *represents significant correlation.

		Sensitivity	**Specificity**	**TSS**	**Boyce**	**AUC**
Sensitivity	*Pearson Correlation*	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)		.	.	.	.
	N	12	12	12	12	12
Specificity	*Pearson Correlation*	.[a]	1	**1.000***	0.404	**0.961***
	Sig. (2-tailed)	.		0.000	0.192	0.000
	N	12	12	12	12	12
TSS	*Pearson Correlation*	.[a]	**1.000***	1	0.404	**0.961***
	Sig. (2-tailed)	.	0.000		0.192	0.000
	N	12	12	12	12	12
Boyce	*Pearson Correlation*	.[a]	0.404	0.404	1	0.314
	Sig. (2-tailed)	.	0.192	0.192		0.320
	N	12	12	12	12	12
AUC	*Pearson Correlation*	.[a]	**0.961***	**0.961***	0.314	1
	Sig. (2-tailed)	.	0.000	0.000	0.320	
	N	12	12	12	12	12

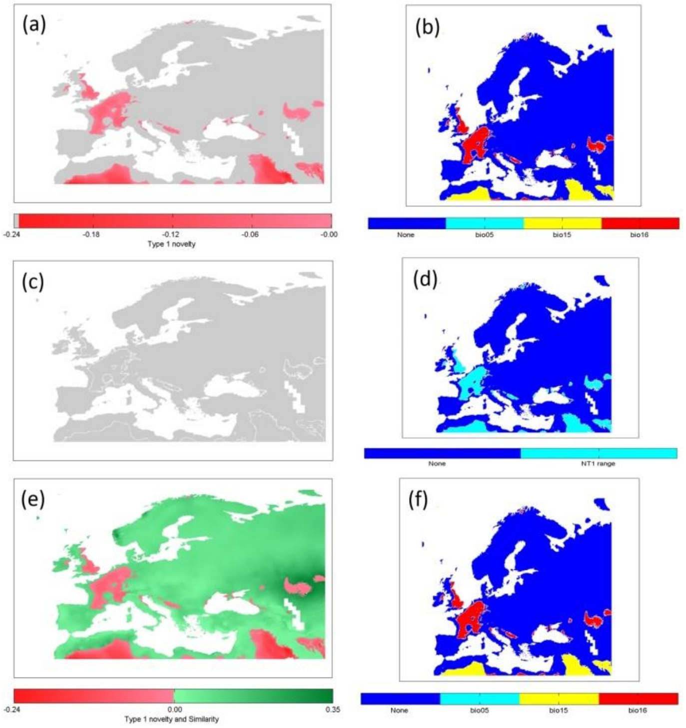


Figure S4.4 Overview of areas and drivers of non-analog climate conditions across the Western Palearctic, based on restricted background sampling of the native, East Asian *Bsal* niche. Type 1 novelty (a) indicates areas with at least one parameter (shown in panel (b)) outside the range of the reference data. Type 2 novelty (c) indicates areas which show non-analog parameter combinations ((d); not applicable here, as type 2 novel conditions were not found). Both novelty types are combined in panels (e) and (f).

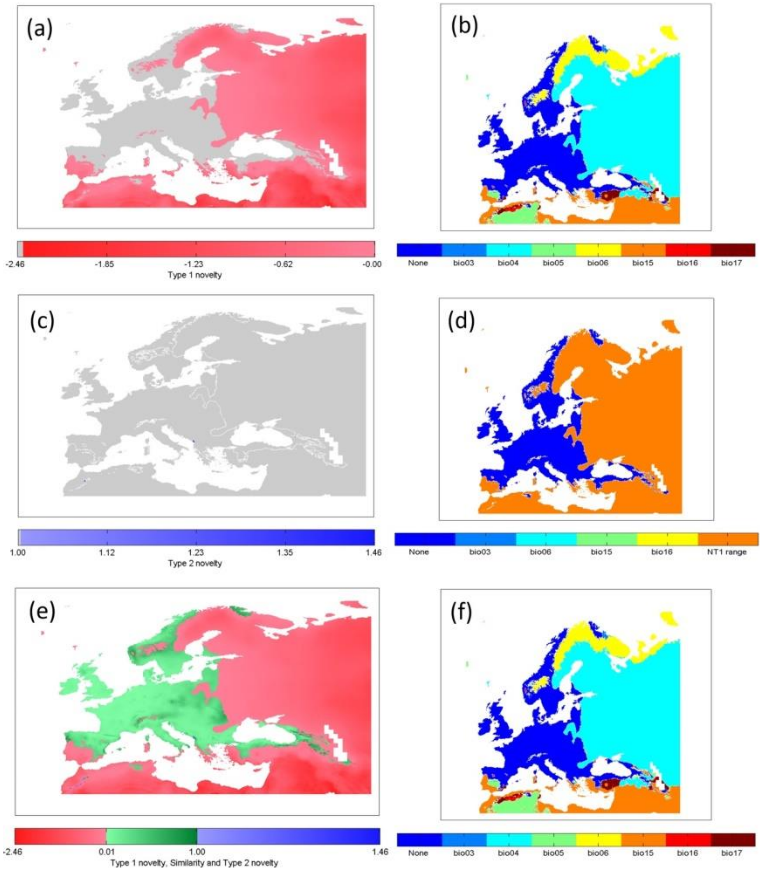
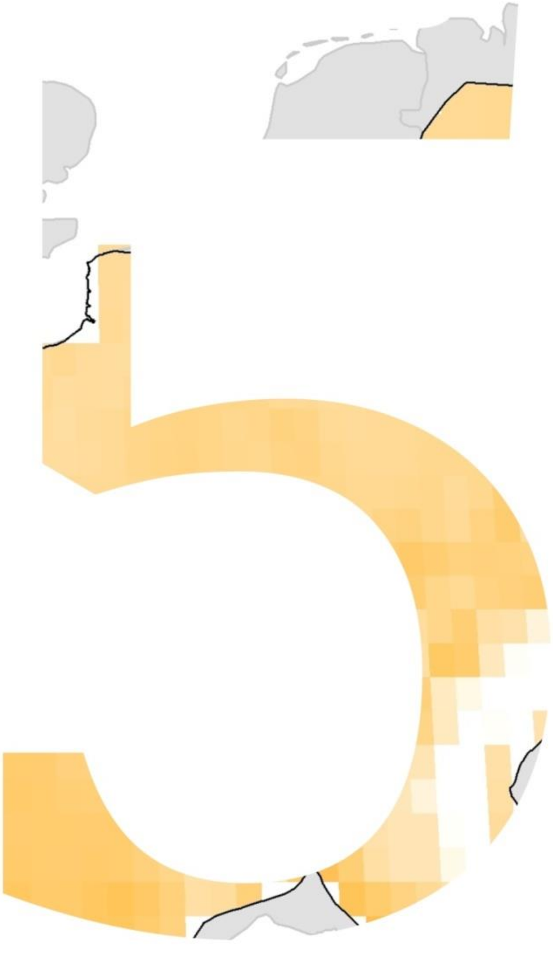


Figure S4.5 Overview of regions and drivers of non-analog climate conditions across the Western Palearctic, based on background sampling of the invasive, northwest European *Bsal* niche. Type 1 novelty (a) indicates areas with at least one parameter (shown in panel (b)) outside the range of the reference data. Type 2 novelty (c) indicates areas which show non-analog parameter combinations (d). Both types are combined in panels (e) and (f).

Chapter 5



Chapter 5

Amphibian thermal behaviour beneficial in mitigating disease may remain fully supressed in the wild

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Abstract

Ambient temperature shapes the disease outbreaks that resulted in global amphibian declines. Whereas thermal behaviour is considered a key characteristic of ectotherm innate defenses, it remains ambiguous to what extent amphibians exploit surrounding temperature variation to combat disease. Here, we study how local microclimates steer the ability of fire salamanders (*Salamandra salamandra)* to fight the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) through thermal behaviour. Laboratory trials reveal that not behavioural fever, but salamander thermal preference can inhibit *Bsal* infection and facilitate recovery, but only under humidity-saturated conditions. Yet, a three-year field study and mesocosm experiment demonstrate absence of such antifungal thermal behaviour under natural conditions. A mechanistic model estimating fire salamander body temperatures year-round predicts equally low opportunities for antifungal behaviour throughout the species' wide European range. Our results show that amphibian innate defences against epizootics may remain constrained in the wild, which predisposes to range-wide disease outbreaks and population declines.

Introduction

When confronted with disease, amphibians profit from a rich line of defence that includes release of bioactive secretions, pathogen-inhibiting skin microbiota, and behavioural responses such as active body temperature regulation (Blaustein *et al.*, 2012; Daskin & Alford, 2012). Why then do they experience higher pathogen-driven declines worldwide than any other animal group (Scheele *et al.*, 2019)? Answers as to why the innate capability to counter infection may not always translate into a beneficial outcome for wild amphibians are complex, and have only recently begun to form (e.g. Rowley & Alford, 2007; Becker & Zamudio, 2011; Raffel *et al.*, 2013; Cohen *et al.*, 2019a). Key to solving this paradox is that amphibian-pathogen interactions are strongly dependent on local environmental context, which leads to variation in infection outcome across the disease landscape.

One of the principal factors that shape amphibian disease dynamics in the wild is ambient temperature. As amphibians are ectotherms, they produce little heat through metabolism, and therefore rely on temporal and spatial variation in their thermal surroundings for activity and performance (Brattstrom, 1979; Feder & Lynch, 1982; Bovo *et al.*, 2018). Such thermal variation may govern patterns in microbiome composition, (micro)predation and seasonal migration timing, which are well-acknowledged drivers of amphibian disease (Blaustein *et al.*, 2012; Schmeller *et al.*, 2014; Woodhams *et al.*, 2014; Daversa *et al.*, 2018a; Kueneman *et al.*, 2019). Ambient temperature may however also directly determine the outcome of host-pathogen interactions, depending on thermal preferences and behaviour of either of both organisms (Rowley & Alford, 2013; Stevenson *et al.*, 2013; Greenspan *et al.*, 2017c; Sonn *et al.*, 2017; Voyles *et al.*, 2017; Sauer *et al.*, 2018; Sauer *et al.*, 2019; Stevenson *et al.*, 2020). For instance, temperatures below or above species thermal optima can strongly compromise the amphibian immune response (Cohen *et al.*, 2017; Greenspan *et al.*, 2017a). Changing climate regimes or conditions that favour pathogen fitness rather than that of the host may further exacerbate this effect, which can result in significant population loss (Raffel *et al.*, 2013; Voyles *et al.*, 2017; Cohen *et al.*, 2019a).

Whereas the aforecited work has provided critical understanding of drivers involved in several iconic anuran declines, not all species respond in a similar manner. Specifically, different amphibians employ different physiological and behavioural mechanisms to temper climate fluctuations and maintain homeostasis, including evaporative cooling, active body temperature regulation, and microhabitat selection (reviewed by Bovo *et al.*, 2018). Various studies have shown that these mechanisms can be used to combat disease as well. For example, amphibians infected with viral or bacterial agents may regulate their body temperature to invoke behavioural fever (see Box 1), which boosts the immune response and affects pathogen fitness. Routine body temperature regulation in the wild may similarly mediate infection dynamics, and protect amphibian hosts against colonisation by disease agents (Richards-Zawacki, 2010; Rowley & Alford, 2013).

With on the one hand ambient temperature as a principal disease driver, and on the other hand amphibian thermal behaviour as its potential inhibitor, how do infections vary across hosts and space (Raffel *et al.*, 2010; Richards-Zawacki, 2010; Becker & Zamudio, 2011; Rowley & Alford, 2013; Stevenson *et al.*, 2020)? Long-term field studies could form a basis to answer this question, especially when focusing on thermal variation in assemblages of hosts and reservoirs, but these remain surprisingly rare (but see Daversa *et al.*, 2018a; Sonn *et al.*, 2019; Stevenson *et al.*, 2020). Amphibian thermal biology is nevertheless assumed to harbour significant potential in mitigating disease impacts, especially in combination with habitat manipulation (Scheele *et al.*, 2014; Heard *et al.*, 2018; Hettyey *et al.*, 2019). A fundamental as well as practical need therefore exists to improve our understanding on the role of thermal behaviour in disease dynamics.

Here, we demonstrate how amphibian thermal behaviour beneficial in countering disease may become constrained, or even fully suppressed in the wild. We do so by studying how local microclimate steers the ability of *Salamandra salamandra* (fire salamander) to fight the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*), a pathogenic East-Asian chytrid that recently emerged in Europe (Martel *et al.*, 2013). *Bsal* is a microscopic fungus which produces motile zoospores and environmentally-resistant encysted spores, and affects a wide range of salamander species by inducing chytridiomycosis (Martel *et al.*, 2014; Stegen *et al.*, 2017). Ongoing outbreaks of this disease have already resulted in host mortality and population declines in at least four European countries (Spitzen-van der Sluijs *et al.*, 2016; Wagner *et al.*, 2019). While knowledge on *Bsal* pathogenesis has steadily increased during recent years, much remains unknown about its disease ecology.

Our overarching aim is to assess environmental conduciveness to host thermal defences of a temperate amphibian. *Bsal* epizootics are often rapid and cryptic, which may shroud gaining understanding of the host's functional response repertoire in the wild. We therefore focus on solving three complementary questions. First, we use thermal infection experiments to test if *S. salamandra* thermal behaviour, either implicitly or through behavioural fever, can mitigate *Bsal* infection. Focus is then shifted to thermal ecology of a *S. salamandra* population located close to the invasive *Bsal* range. Through controlled thermal preference experiments and nocturnal field surveys we secondly answer if wild salamanders exert similar thermal behaviour as their experimental conspecifics, and quantify how effectively this is expressed under natural conditions. Insight into temperatures experienced during inactivity is gained through an additional mesocosm trial. Thirdly, we expand our view across the wide

European distribution of *S. salamandra* using mechanistic modelling. We combine *Bsal* growth data at different temperatures with our earlier findings to inform a model of *S. salamandra* body temperature across the species' range. The results provide insight into how opportunities for antifungal thermal behaviour against *Bsal* vary across the landscape. Our results show that amphibian innate defences against epizootics may remain constrained in the wild, which predisposes to range-wide disease outbreaks and population declines.

Box 1: Behavioural fever in amphibians

When exogenous pyrogens invade the body of an ectothermic vertebrate, the animal may react by raising its body temperature above thermal preference limits through behavioural thermoregulation. This reaction, known as behavioural fever, increases survival by boosting the immune response or directly affecting pathogen fitness (reviewed by Rakus *et al.*, 2017). Behavioural fever has been repeatedly shown to occur in reptiles and fish, but has only occasionally been reported in amphibians. Specifically, although bacterial and viral agents have been shown to induce fever in amphibians (e.g. Kluger, 1977; Sherman *et al.*, 1991; Sauer *et al.*, 2019), there is no conclusive evidence that fungal pathogens such as chytrids do so as well (Sauer *et al.*, 2018, current study). Previous reports of behavioural fever in context to chytridiomycosis outbreaks (e.g. Richards-Zawacki, 2010) may be better explained by the existence of variation in amphibian individual thermal preferenda, rather than by that of an effective response to infection (Sauer *et al.*, 2018).

Methods

Ethics statement

Experiments were conducted under BSL2 conditions in accordance with European guidelines and Belgian legislation on animal housing and experimentation (KB 29/05/2013), following approval from the Ghent University Faculty of Veterinary Medicine ethical committee (permits no. EC2015/83, EC2016/55&73 and EC2017/66). Field surveys and amphibian collection were granted through permits ANB/BL/FF-V15-00131 and ANB/BL/FF-V1800013 from the Agentschap voor Natuur en Bos (ANB), Belgium.

Host thermal behavior in the face of infection

Captive-bred, recently metamorphosed *S. salamandra* (ssp. *terrestris*) were from a private breeder, which were confirmed to be free of *Bsal* and *Batrachochytrium dendrobatidis* (*Bd*) through duplex real-time PCR (Blooi *et al.*, 2013). All animals were kept in climate-controlled (15°C) chambers for four months following acquisition to avoid thermal acclimation effects. We then conducted three experiments, using a humid thermal gradient ranging between 0–35°C (Fig. 1), to compare temperature selection between salamanders exposed to the *Bsal* type strain AMFP13/1 (Martel *et al.*, 2013) with sham-exposed conspecifics. Body temperature was measured twice per day using an Omega HHC210 infrared thermometer (Omega Engineering Inc., 0.1°C precision). Please refer to Appendix S1 for a detailed description of the different experiments and analytical procedures summarized below.

First, we tested if hosts actively increase their body temperature upon *Bsal* contact. Temperature selection was compared during a 31-day experiment between twenty individually housed salamanders. Ten of these were inoculated with a low *Bsal* dose (10^4 zoospores) on day 9 of the experiment, while the other 10 were sham-inoculated. Five additional *Bsal*-inoculated individuals were housed in a separate container at 15°C and used as positive control.

This procedure was repeated during a second, 15-day experiment, in which 10 different salamanders were inoculated with a high *Bsal* dose (10^6 zoospores for 2h) on day 10 and compared to 10 sham-inoculated conspecifics. Additionally, to assess the effect of water availability on temperature selection, we dehydrated 5 randomly-chosen gradient enclosures per treatment on day 7. Dehydration led to an average of 27.7% body weight (water) loss during the first 16 hours, prompting us to hydrate enclosures again on day 8.

Finally, host thermal behaviour between 10 already-infected salamanders and 10 non-infected conspecifics was compared during a 20-day period in a third experiment. The infected individuals were inoculated simultaneously with those of the first, low-dose experiment, and left to develop infection for four weeks at 15°C.

Salamanders were checked daily for clinical signs, while skin swabs collected for qPCR analysis were taken every 7 days and/or at the time of death, until 4 weeks after the experiment ended. All individuals were treated as described by Blooi *et al.* (2015) following experimental procedures. Salamanders belonging to control groups were treated after qPCR analysis demonstrated an increase in *Bsal* load during 2 consecutive weeks (humane endpoint of the trial).

Amphibian thermal responses to pathogen exposure may not be linear, but instead peak for a short time (Sauer *et al.*, 2019). Furthermore, considerable in individual variation thermal preferences may exist (Sauer *et al.*, 2018). To account for uncertainty in the timing and duration of a potential response to *Bsal* we therefore compared salamander body temperatures between treatments over time using Generalized Additive Mixed Models (GAMMs, Appendix S1). Subject (individual) was incorporated as random effect in each model. GAMMs were built in R using the *mgcv::bam()* function, while the *itsadug* package was used for model interpretation. To test if treatment groups differed significantly in body temperature we converted treatment to an ordered factor while using the sham-inoculated/infected group in each experiment as reference (van Rij *et al.*, 2017). Difference plots were used to visualise the estimated differences and determine when significant differences occurred.

Thermal ecology

We first assessed whether wild *S. salamandra* exert similar thermal preference as their experimental conspecifics. To this end, 10 individuals were temporarily collected from a forest fragment (50.95°, 3.72°) close to the invasive *Bsal* range near Ghent, Belgium. This fragment consists of a mature *Fagus sylvatica* forest (50.95°, 3.72°) interspersed by various perennial streams and small ephemeral water bodies, where elevational variation remains below 2 meters. Body temperature was measured in the thermal gradient described above using an infrared thermometer (Omega HHC210) twice per day for 6 days, following an initial two-day acclimation period. Vitamin-powdered crickets were provided *ad libitum* to avoid that satiation level affected preferred temperature selection. Each individual was returned to its original place of capture during nights with favourable, humid weather. For comparative purposes, preferred temperatures of four co-occurring amphibian species, *Bufo bufo* (common toad), *Ichthyosaura alpestris* (alpine newt), *Lissotriton helveticus* (palmate newt) and *Rana temporaria* (common frog) were recorded as well, following the same procedures. Measurements for each species were summarized by calculating the set-point range (T_{set}), which ranges between the average lower (25% quartile) and upper (75% quartile) thermal bounds (Hertz *et al.*, 1993). Linear Mixed-Effects Models (LMM) implemented through *lme4::lmer* in R were used to compare between species and groups (salamanders vs. anurans). Individual was added as random effect to account for non-independence of repeated measures.

Active field body temperatures (T_{b}) of *S. salamandra* and the other amphibian species were measured during a series of 34 nocturnal surveys across a three-year period. A standardized 1300m transect was followed during each survey, of which the first half runs along a forest edge, while the second half was located in mature forest. An Omega HHC210 infrared thermometer was used to measure mid-dorsal T_{b} of each encountered amphibian, which was recorded using a Garmin GPSmap60Cx (Garmin Ltd., Connecticut, USA) along with the corresponding date, time and geographical position. Insight into the availability of environmental (operative, T_{e}) temperatures was gained by measuring 10 agar (10g/l) models during each session (Navas & Araujo, 2000). New agar models were created before each visit, which were placed along the transect at fixed locations shortly before sunset preceding each session.

Following the popular protocols of Hertz *et al.* (1993), we finally combined T_{set}, T_{b} and T_{e} measurements to quantify efficacy of thermal behaviour under natural conditions. Before doing so, we conducted a pilot study to assess if the different species in our assemblage showed different thermal inertia (Appendix 2.2). As this was not found to be the case, we subsequently explored patterns of active T_{b} variation by fitting a LMM with the fixed effects of species, humidity, time since sundown, and location along the transect. Sampling event was nested in season and introduced as random effect (Appendix 2.3). Thermoregulation effectiveness (*E*) was determined separately, which is an index that measures the extent to which organisms maintain T_{b} within T_{set}, given the range of T_{e} available in their habitat. *E* values around 0 suggest thermoconformity, while higher numbers represent increasingly effective regulation. We calculated *E* using the *ectotemp* R package, but only for the year and meteorological season during which T_{set} was measured to avoid seasonal acclimation effects. Please refer to Appendix 2.3 for details.

As field surveys only reveal which temperatures are experienced during activity, we conducted an additional mesocosm trial to measure *S. salamandra* body temperatures during the often long periods of inactivity, which is generally spent underground. This trial took place in the nearby Aelmoeseneiebos experimental forest of Ghent University (50.97°, 3.80°), because regional environmental legislation prohibited invasive procedures on wild salamanders, or their habitats. A 470x240cm forest mesocosm was constructed around a mature 240cm circumference *F. sylvatica* tree, outlined by 70cm high mesh fencing fixed 30cm deep into the ground. The forest soil was left to recover for a year after construction. A thick leaf litter layer, several logs, and cavities between the tree roots provided ample opportunity for shelter. To obtain temperature measurements we used DST nano-T data loggers (http://www.unobv.com), which were surgically fitted in 3 captive bred, adult *S. salamandra* following the protocol of Dervo *et al.* (2010). After a 3-week recovery period the salamanders were placed in the mesocosm, where hourly T_{b} measurements were recorded for a four-month period. Hourly ambient temperature and humidity data were obtained from an on-site climate station. Individual Linear Models (LM) were fitted to determine to what extent variance in T_{b} was explained by ambient temperature (°C), humidity (%), and rainfall (mm). A time-series GAM was used to compare temperature selection between individuals over time; model interpretation followed that of the infection experiments described above.

Mechanistic estimates of host body temperature across Europe

Part of our earlier obtained results were used to inform a mechanistic model that estimated where, and when across the wide European fire salamander range i) *Bsal* growth may occur, and ii) host thermal behaviour may inhibit infection. Because details on the *Bsal* thermal niche are lacking (but see Martel *et al.*, 2013; Stegen *et al.*, 2017), we first conducted a 10-day *in vitro* growth experiment by exposing *Bsal* AMFP13/1 to 6 different temperatures ranging between 0 and 30°C. The resulting data were used to fit a range of polynomial thermal growth curves to sporangia counts using *growthcurver* in R, of which the best-fitting curve was identified using *stats::anova* (Fig. 4b). Please refer to Appendix S3.1 for details.

The *Bsal* growth curve was subsequently applied to a mechanistic model that estimated fire salamander steady-state body temperature across its distribution. Steady-state temperatures essentially equal T_e, as these represent the temperature an organism would stabilize at in a particular microclimate in absence of metabolic heating and evaporative cooling (Kearney & Porter, 2009). We hence first built a microclimate model using the *NicheMapR* R package, which estimates hourly ground-level conditions available to our focal host (Kearney & Porter, 2017). Parameterization was achieved by computing fourteen topographic and climatic variables (Table S3.1) derived from a Digital Elevation Model (SRTM v4.1) and the ERA5 climate reanalysis dataset (Copernicus Climate Change Service, 2017). The microclimate model was then used as input for the *NicheMapR* ectotherm module (Kearney & Porter, 2020) set to iteratively calculate hourly steady-state body temperature throughout the year for an organism of 40g with a lizard-like shape. Evaporative water loss was taken into account by considering the surface of the organism as a free-water exchanger. Nocturnal and crepuscular activity was assumed, and the percentage of shaded conditions was set between 90 and 100%. Finally, we assumed that the organism would retreat up to 20cm underground when relative humidity fell below 85%, after which its body temperature adopted that of the soil. For further settings, please refer to Table S3.2. Climate data and associated fire salamander T_b measurements taken during the mesocosm trial were used for model validation.

We queried our model in R to estimate average fire salamander body temperature across its range, and identify areas where *Bsal* infection may be inhibited by T_b exceeding 20°C for at least 5 days (see also below). We then inferred where, and when *Bsal* growth may occur across the fire salamander distribution. No relationship between temperature and pathogenicity was assumed (Stegen *et al.*, 2017).

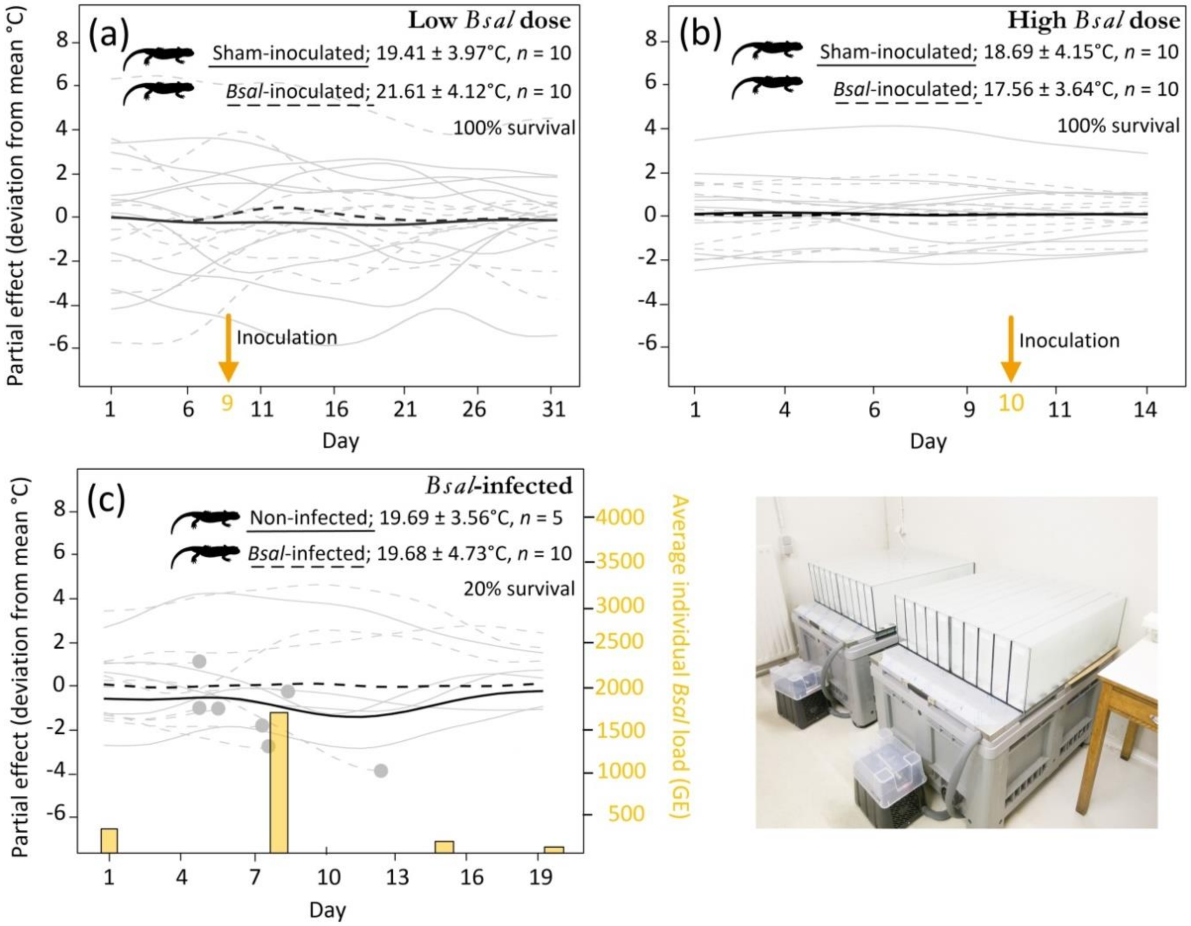

Fig. 1. Temperature selection trends in *Salamandra salamandra* (fire salamander) hosts inoculated (a, b) or infected (c) with *Batrachochytrium salamandrivorans* (*Bsal*). Each panel shows results from a different experiment. Temperature selection is displayed per individual (grey lines) and group (black lines) using GAMM-generated random smoothers that show deviations from the overall mean. Average selected temperature and corresponding SD of each group are indicated in the upper right corner of each panel. In panel (a) and (b), day of inoculation is indicated with an orange arrow. Only panel (c) includes average individual *Bsal* loads, as inoculation in the other two experiments did not result in infection. Grey dots indicate host mortality. The thermal gradient setup used for experimental procedures is displayed on the lower right.

Results

Host thermal behaviour

While *S. salamandra* thermal preference in the different experiments averaged between 18–21°C, individual averages varied up to 12°C (14.7-27°C), resulting in significance of the random effect smooth term for subject in each experiment (Fig. 1, GAMM model summary in Table S1.1). No response to *Bsal* exposure was however apparent, as significant differences in temperature selection trends between fire salamander treatment groups were not detected (Table S1.1, Fig. S1.1). Visual inspection of model estimates consistently revealed considerable overlap of trajectory confidence intervals between treatments (Fig. S1.1). Reorganising the data from the second experiment to compare temperature selection trends between salamanders temporarily exposed to dehydrated gradient enclosures vs. those that were not did reveal a significant difference in the height (intercept) and shape of the predicted trajectories (GAMM, $\beta = -1.48$, SE = 0.51, $t_{280} = -2.91$, $p < 0.001$; Table S1.2, Fig. S1.1c). Visual inspection of model estimates (difference plot, Fig. S1.1c) showed that this difference was caused by an estimated 10°C decrease in temperature preference of salamanders exposed to the dry treatment in contrast to those not exposed to the dry treatment.

Development of chytridiomycosis in control group salamanders and those temporarily housed at 15°C for the third experiment proved that *Bsal* inoculation was successful (Fig. 1c). Yet, none of the salamanders that were inoculated and immediately placed in thermal gradient enclosures (i.e., those in the first two experiments) became infected, regardless of the administered dose.

Five of the infected salamanders that were placed in thermal gradient enclosures as part of the third experiment succumbed to chytridiomycosis during the first seven days (Fig. 1c). *Bsal* loads decreased during the following two weeks, in which two additional hosts died (Table S1.3). One of three persisting hosts maintained infection on the final day of the experiment. Recrudescence of the infection was demonstrated in the two remaining individuals, when housed at 15°C after the experiment.

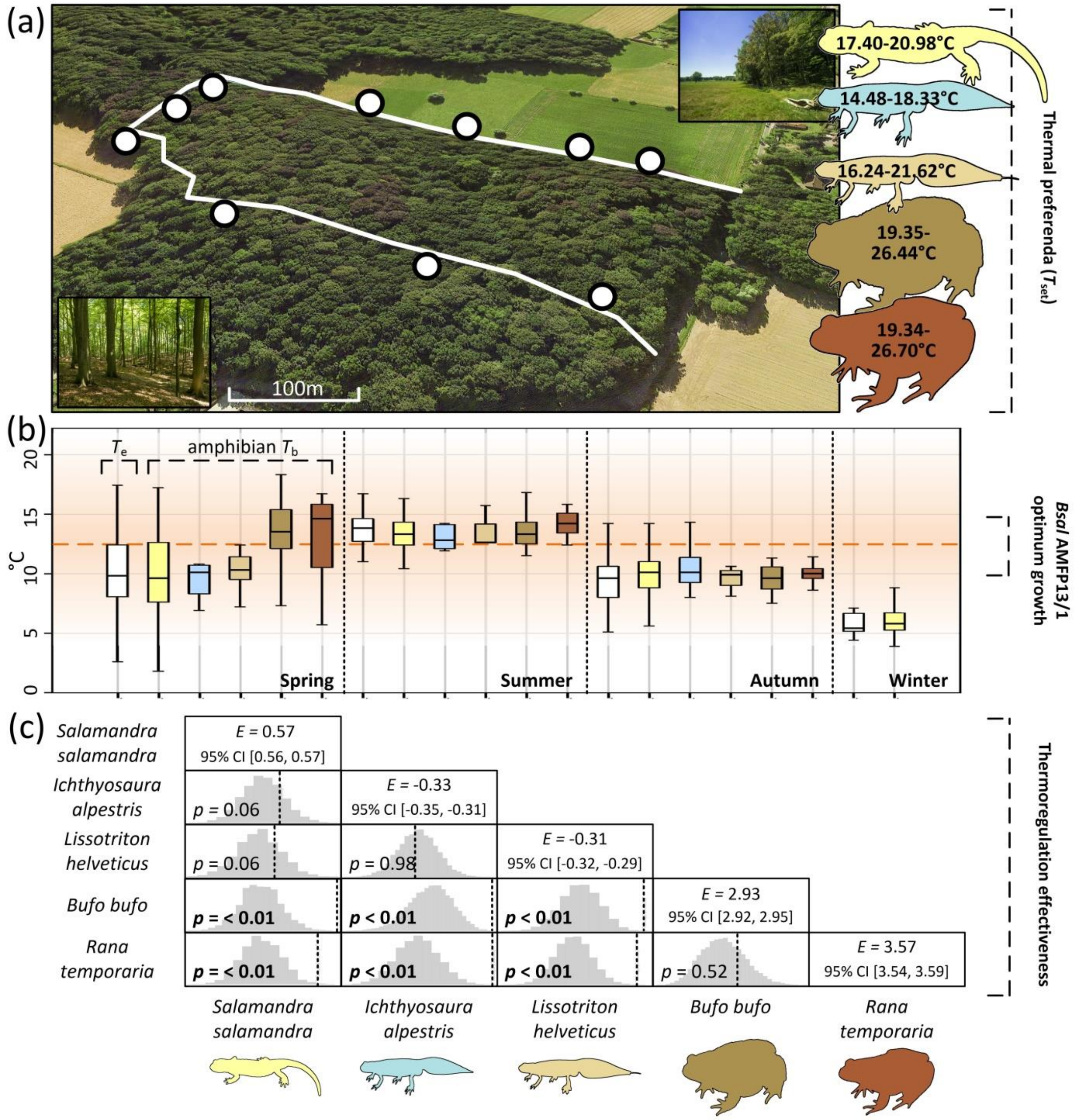

Fig. 2. Active field body and operative temperatures (T_b, T_e), thermal preferenda (T_{set}) and thermoregulation effectiveness (E) of an amphibian assemblage located close to the invasive range of *Batrachochytrium salamandrivorans* (*Bsal*) in Belgium. Nocturnal field surveys were performed along a standardized transect to record T_b and T_e (at white dots, *a*); inset shows T_{set} recorded during earlier thermal preference measurements. The recorded values are arranged per season for overall interpretability in panel (*b*), in relation to *Bsal* thermal growth (orange shading). These data were used in concert to quantify thermoregulation effectiveness (*c*) – *E* values around 0 suggest thermoconformity, while higher numbers represent increasingly effective regulation. Plots below the divide in panel *b* show permutation tests that compare the difference in effectiveness between two species (vertical dotted bar) with a histogram of simulated differences. Aerial imagery © 2018 Google.

Thermal ecology

Thermal preference (T_{set}) of wild *S. salamandra* ranged between 17.40–20.98°C and largely overlapped with that of other salamander species (Fig. 2), from which it did not differ significantly (LMM, β = -2.22, SE = 1.90, $t_{44.50}$ = -1.17, p = 0.24 for *I. alpestris*, β = 0.52, SE = 1.90, $t_{44.98}$ = 0.27, p = 0.79 for *L. helveticus*; Table S2.1). Overall, salamander preferenda were found to be significantly lower than those of anurans (T_{set} 14.48-21.62°C vs. 19.34-26.70°C, respectively; LMM, β = -4.8, SE = 1.23, $t_{49.31}$ = -3.92, p = <0.01; Table S2.2). Indeed, both *B. bufo* and *R. temporaria* showed significantly higher thermal preference than *S. salamandra* (Table S2.1).

When comparing field measurements gathered during the year and season in which thermal preference was determined, interspecific differences were recovered as well (Welch's ANOVA *F*(5,27.94) = 20.47, p = < 0.01). Subsequent post-hoc testing revealed this result to be driven by significantly higher T_b values in anurans, while salamanders generally conform to environmental temperature (full results in Table S2.3, Fig. 2b). Throughout this season, amphibian T_b nevertheless remained consistently below T_{set}. This discrepancy leads to low thermoregulation effectiveness, which ranges between -0.33 to 0.57 *E* in salamanders and 2.93-3.57 *E* in anurans (Fig. 2c).

The lack of overlap between field body temperature and thermal preference held true across the remainder of our field surveys. In fact, of the 2847 active salamander T_b's measured (Fig. 2b), only 11.5% overlaps with the wider T_{set} range as reported above for this group. These however all concern *S. salamandra* T_b that fall below the species-specific lower preference boundary of 17.4°C. LMM analyses performed on the three-year dataset reveal that amphibian T_b shows a borderline significant negative relation with humidity (LMM, β = -0.30, SE = 0.16, $t_{31.34}$ = -1.86, p = 0.07), and is significantly lower where mature forest abruptly turns into meadow (LMM, β = -0.15, SE = 0.06, $t_{3095.21}$ = -2.29, p = 0.02). Body temperature variation among species mirrored that recorded in the season when thermoregulation effectiveness was measured, with salamanders differing little from T_e (full LMM results in Table S2.6) while anuran T_b remained significantly higher (LMM, β = 0.31, SE = 0.10, $t_{3069.63}$ = 3.16, p = 0.002 for *B. bufo*, β = 0.38, SE = 0.13, $t_{3069.31}$ = 2.84, p = 0.004 for *R. temporaria*). These predictors nevertheless only explain about 6% of model variance (R^2m = 0.08), while 92% explained variance is achieved when adding sampling event nested in season as random effect (R^2C = 0.92).

The three *S. salamandra* inhabiting a forest mesocosm rarely attained body temperatures above 17°C during the fourth-month measurement period, which coincided with the 2018 European heat waves (Fig. 3a). Ambient temperature peaked at 34.9°C during this period. While per-individual LM analyses revealed that ambient temperature and humidity significantly and positively predicted salamander T_b, explained variation remained low (23–47%, Table S2.7). A subsequent time-series GAM revealed existence of long periods in which T_b significantly differed between salamanders Table S2.8, Fig. 3b, Fig. S2.3). Nevertheless, average individual T_b measured across the entire period only differed up to 0.47°C (16.07 – 16.54°C, Fig. 3).

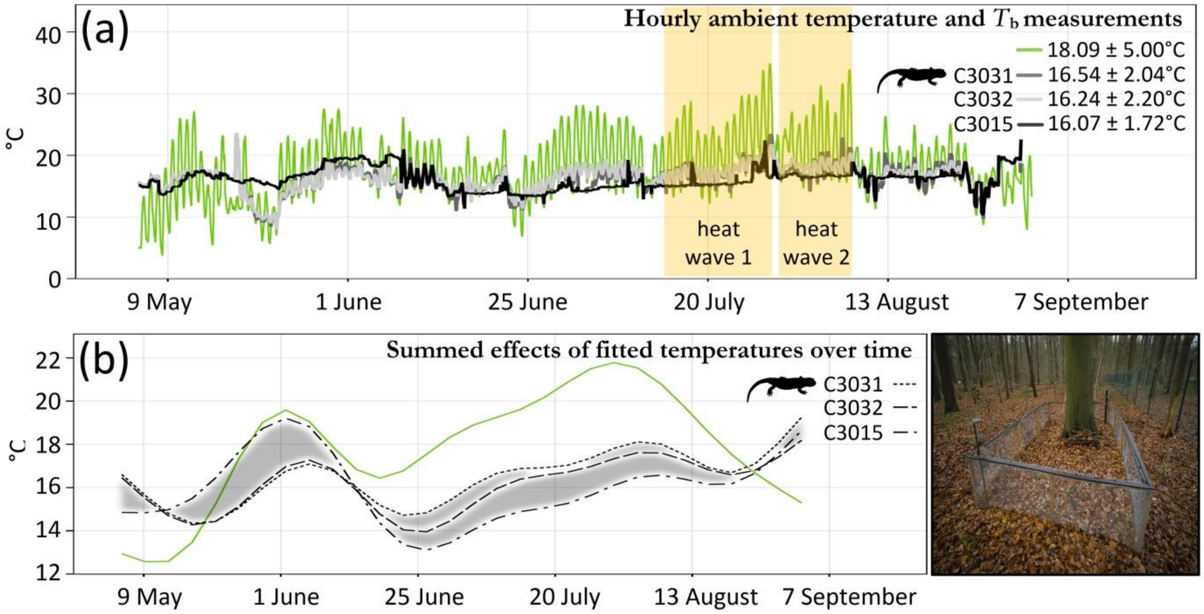

Fig. 3. (*a*) Hourly ambient temperature (green) and body temperature (T_b) of three fire salamanders (*Salamandra salamandra*) fitted with internal data loggers, measured in an outdoor forest mesocosm (lower right) during 2018. Average ambient temperature and individual T_b are ± SD. Panel (*b*) shows time-series General Additive Model (GAM) fitted values for the same three individuals as well as the ambient temperature (green). Dark shading denotes periods during which T_b differed significantly between individuals, determined by iteratively setting each individual as reference to compare with the others in the form of ordered factors. Apart from a short period in early June, ambient temperature consistently differed significantly from salamander T_b (not shown).

Mechanistic predictions

The best parsimonious fit of the *Bsal* thermal growth data was obtained with a fourth order polynomial, from which the coefficients were extracted (Appendix S3.1). The resulting curve was restricted to 0-25°C as both our experiment and Martel *et al.* (2013) found temperatures below 0 or of 25 degrees and above incompatible with *Bsal* growth (Fig. 4a).

Average steady-state *S. salamandra* body temperatures ranged between -1.39–16.89°C across the species' distribution. While higher minimum body temperatures generally approach or overlap with averages (Fig. 4c), higher maximum body temperatures rise well-beyond these, up to 24°C (Fig. 4d).

Areas where *S. salamandra* body temperature may peak above 20°C were predicted to occur in the central Iberian Peninsula and at lower altitudes along the Apennine mountain chain and the southern Balkan Peninsula. Maintaining body temperature above 20°C for at least five consecutive days was however not estimated to be possible in any of these.

Bsal growth was predicted to be possible across the entire *S. salamandra* distribution, but peaks in different seasons in different areas (Fig. 4b). Higher growth is especially estimated to occur in autumn, but this may shift to spring or summer in more continental areas and at higher altitudes, respectively. Across the southern half of the *S. salamandra* distribution, and especially at lower altitudes, growth peaks in winter. Abrupt changes in cell colour in Fig. 4b result from a general high similarity in *Bsal* growth during autumn (brown) and spring (green).

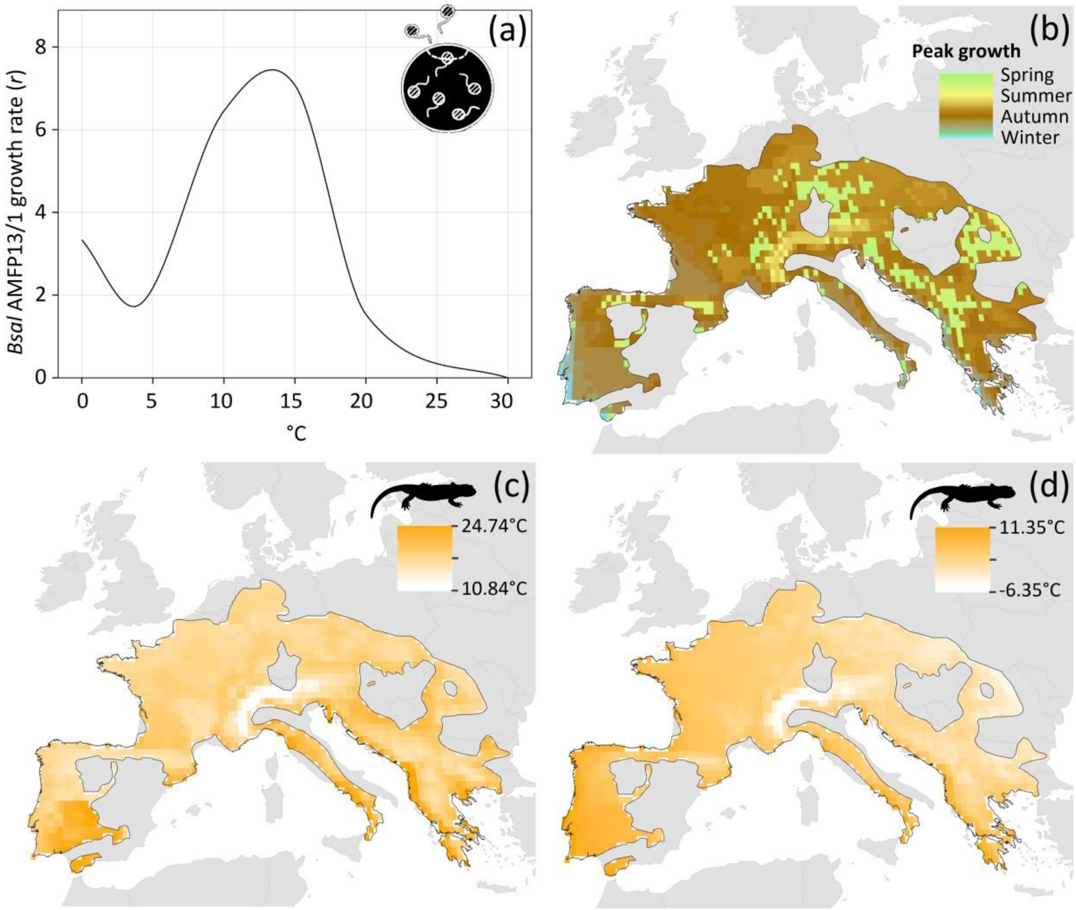

Fig. 4. Thermal growth of *Batrachochytrium salamandrivorans* (*Bsal* AMFP13/1) relative to *Salamandra salamandra* body temperature. *Bsal* thermal growth was determined based on sporangia counts (*a*), and translated across space (*b*) using a mechanistic model of steady-state *S. salamandra* temperature (*c*, *d*). The mechanistic model was built on assumptions of nocturnal/crepuscular activity, and that salamanders retreat underground once relative

humidity falls below 85%. Panel *c* displays maximum daily average body temperatures, panel *d* minima. The investigated area was delineated using the *S. salamandra* distribution. *Bsal* growth in panel *b* was summarized per meteorological season for overall interpretability by determining for each cell at which day of the year growth peaked.

Discussion

We here demonstrate that our focal amphibian host, *S. salamandra*, prefers temperatures that can inhibit *Batrachochytrium salamandrivorans* (*Bsal*) infection and facilitate disease recovery under humidity-saturated conditions. A three-year field study and a mesocosm experiment however reveal that such temperatures are not attained in the wild at our study site, located close to the invasive *Bsal* range. We finally parameterise a mechanistic model that extrapolates our findings, which suggests that possibilities for antifungal thermal behaviour remain similarly limited across the remainder of the wide European *S. salamandra* distribution. Overall, our results confirm that amphibian innate defences against epizootics may remain constrained in the wild, which predisposes to range-wide disease outbreaks and population declines.

The results presented here add to a growing body of research that focuses on local amphibian disease dynamics over time, within environmental context (e.g. Spitzen-van der Sluijs *et al.*, 2017; Stegen *et al.*, 2017; Bosch *et al.*, 2018; Daversa *et al.*, 2018a; Sonn *et al.*, 2019; Stevenson *et al.*, 2020). Notwithstanding the importance of other factors, our focus on thermal biology results in several key findings that merit specific attention, not in the least for mitigation measures (Scheele *et al.*, 2014; Heard *et al.*, 2018; Hettyey *et al.*, 2019).

First, we confirm that innate amphibian thermal behaviour can hold considerable potential to combat fungal disease (Richards-Zawacki, 2010; Rowley & Alford, 2013). Our results show that host routine temperature regulation at temperatures approaching pathogen critical maximum may inhibit infection, and promote disease recovery (see also Blooi *et al.*, 2015; Sonn *et al.*, 2019). While the average of 20°C attained throughout may seem remarkable for a temperate amphibian, similar preferences occur in many European species under humidity-saturated conditions (e.g. Strübing, 1954). The high degree of individual variation is in turn reminiscent of that recently found in several anuran species, which influences susceptibility to chytridiomycosis (Rowley & Alford, 2013; Sauer *et al.*, 2018; Stevenson *et al.*, 2020). What follows is that, in our *Bsal* – *S. salamandra* study system, the temperature limits of the host appear to exceed those of the pathogen, creating a thermal mismatch (Cohen *et al.*, 2017) ready to be exploited by the former. Whereas local adaptation may certainly influence these bounds (Stevenson *et* *al.*, 2013; Cohen *et al.*, 2019b), our captive and wild *S. salamandra* display interestingly similar thermal preferenda to conspecifics tested before (Strübing, 1954). The role of phenotypic plasticity, of both amphibians and hosts, is therefore key to consider in future studies focusing on efficacy of host thermal behaviour.

We however also find that sudden access to local thermal heterogeneity may prolong infection, and thereby promote disease spread. Whereas this outcome is of course study-system dependent, it adds to earlier findings that show how abrupt exposure to increased temperature variation may weaken amphibian hosts, rather than buffer them from disease (e.g. Raffel *et al.*, 2013). This observation is especially relevant from a mitigation perspective. Several studies have recently explored possibilities to combat chytridiomycosis in the wild through habitat manipulation, particularly by creating access to higher operative temperatures than normally available to the host (e.g. Scheele *et al.*, 2014; Heard *et al.*, 2018; Hettyey *et al.*, 2019). While this approach certainly has potential, our results suggest that habitat manipulation in some ecosystems holds most value as an inhibitory strategy taken before disease arrival, rather than as a mitigation measure at existing outbreak sites. The extent to which thermal niches differ between different host and reservoir species in an amphibian assemblage, as we preliminary show here, may furthermore be a critical factor to consider in application of such mitigation approaches.

Knowledge on thermal niche realization in wild amphibians however remains scarce. We here confirm earlier suspicions that thermal behaviour beneficial in mitigating disease may remain constrained, and even fully supressed under natural conditions (Raffel *et al.*, 2010; Becker & Zamudio, 2011; Puschendorf *et al.*, 2011; Sonn *et al.*, 2019). From an ecophysiological viewpoint, these findings are perhaps not unexpected. Specifically, seminal work on amphibian thermal physiology by for instance Brattstrom (1979) already revealed that thermoregulation opportunities of diurnal species by far exceed those of their terrestrial nocturnal or secretive counterparts (see also von May *et al.*, 2018; Cohen *et al.*, 2019b). As the majority of amphibians worldwide are obligate nocturnal (Oliveira *et al.*, 2017), this means that behavioural plasticity may be constrained in many more species than hitherto thought. Beyond disease, such limitations can also affect responses to climate change (Sunday *et al.*, 2014; Gunderson & Stillman, 2015). Nevertheless, the amphibians in our study system appear shielded to at least warming by the microhabitats that limit their thermal behavioural repertoire in the first place. This complexity emphasizes the need to incorporate mechanism not only in models that predict responses to climate change, but also those that determine spatial disease risk.

Our mechanistic model did not identify areas where salamander hosts can maintain body temperature above 20°C for at least five days. Higher *Bsal* growth furthermore appears to shift analogous with salamander phenology through Europe, peaking in spring-summer in mountain ranges and in autumn-winter across the Mediterranean. Notable as well is the relative low degree of predicted body temperature variation throughout the wider *S. salamandra* range, which, at least from a thermal perspective, predisposes to disease. Considering this susceptibility is important in future prioritization efforts, as it means that the south-European genetic refugia of this species, represented by a multitude of different subspecies, are vulnerable to *Bsal* epizootics as well. The same may likely be true for range-limited genetic and ecological relatives such as *Salamandra lanzai*.

Attaining temperatures above fungal pathogen thermal optima for short periods of time may however already slow disease progression, as has been shown for *Bd* (Greenspan *et al.*, 2017c). We therefore expect that a future research focus on relatively warmer, more heterogeneous environments than those studied here could constitute a valuable next step in determining efficacy of amphibian thermal behaviour to combat disease. Such environments, be they at landscape or local level, may also contain valuable information that can help to refine chytridiomycosis mitigation strategies in the wild.

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Author contributions

WB, AM and FP designed the study. WB and JE conducted the thermal inertia pilot. WB collected field data with assistance from JS. WB, AL and KV performed the mesocosm experiment. WB, SVP and AM performed thermal infection experiments. MK collected and processed data on *Bsal in vitro* thermal growth. FFY built mechanistic models with input of WB. WB analyzed all data. WB wrote the first draft of the manuscript with input of AM and FP; all authors contributed to revisions.

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Supporting Information for

Amphibian thermal behaviour beneficial in mitigating disease may remain fully supressed in the wild

S1. Infection experiment details and additional results

Thermal gradients were constructed by placing semi-transparent glass walls at 10cm intervals within four 8mm glass vivaria measuring 100x50x30cm (Fig. 1, inset). Humid paper tissue was used as substrate in the resulting 20 enclosures of 100x10x30cm, on which shelters made from bisected, 50x32mm pvc tubes were placed at 5cm intervals. Each vivarium was covered by an acrylic plate containing small air holes. A one-hand pressure sprayer was used daily to moisten the paper tissue and keep the air humidity inside the enclosures at 100%. Paper tissue was replaced weekly. Cooling on one end was achieved by two 980x600x32mm 500W cold plates (MFC koelplaten, Antwerp, Belgium) placed halfway under the vivaria, connected to a thermostat set to -1°C. Radiant Terramat heat panels of 516x274mm, 23.5W were placed on the other end, resulting in a temperature gradient ranging from 0°C to 35°C. The gradient was placed in a 15°C climate-controlled chamber subject to a natural photoperiod by means of a small window. Temperature selection was recorded twice per day using an infrared thermometer (Omega HHC210). Vitamin-powdered crickets were provided *ad libitum* to avoid that satiation level had an effect on temperature selection.

Bsal zoospore concentrations were adjusted to the desired amount based on hemocytometer counts. Inoculation was performed by placing salamanders for 24h individually in petri dishes containing either zoospores in liquid media or sham inoculum.

We determined if trends in salamander preferred temperature (dependent) differed over time between treatments (independent) using time series Generalized Additive Mixed Models (GAMMs) in R through the *mgcv:: bam()* function (Wood, 2017). Various functions from the *itsadug* package (van Rij *et al.*, 2017) were used for GAMM interpretation. We first averaged preferred temperatures per individual per day and performed data exploration following Zuur *et al.* (2009). Q-Q plots suggested data collected in experiment 1 to be light-tailed, while those collected in experiments 2 and 3 to have an approximately normal distribution. The group of sham-exposed salamanders was used as reference in each experiment, while treatment was converted into an ordered factor to allow testing if the *Bsal* exposed treatment group followed a different trend over time in respect to each sham-exposed group. Subject (individual) was incorporated in the model as random effect through the use of random smooths. Because initial data exploration showed that preferred temperature data in experiment 1 was light-tailed, we initially applied a Scaled t (scat) distribution (Wood *et al.*, 2016), but changed this to a Gaussian distribution given that use of the former inhibited incorporation of an AR1 autocorrelation structure to the GAMM. The number of penalized regression smoothers (*k*) was kept at default in each model.

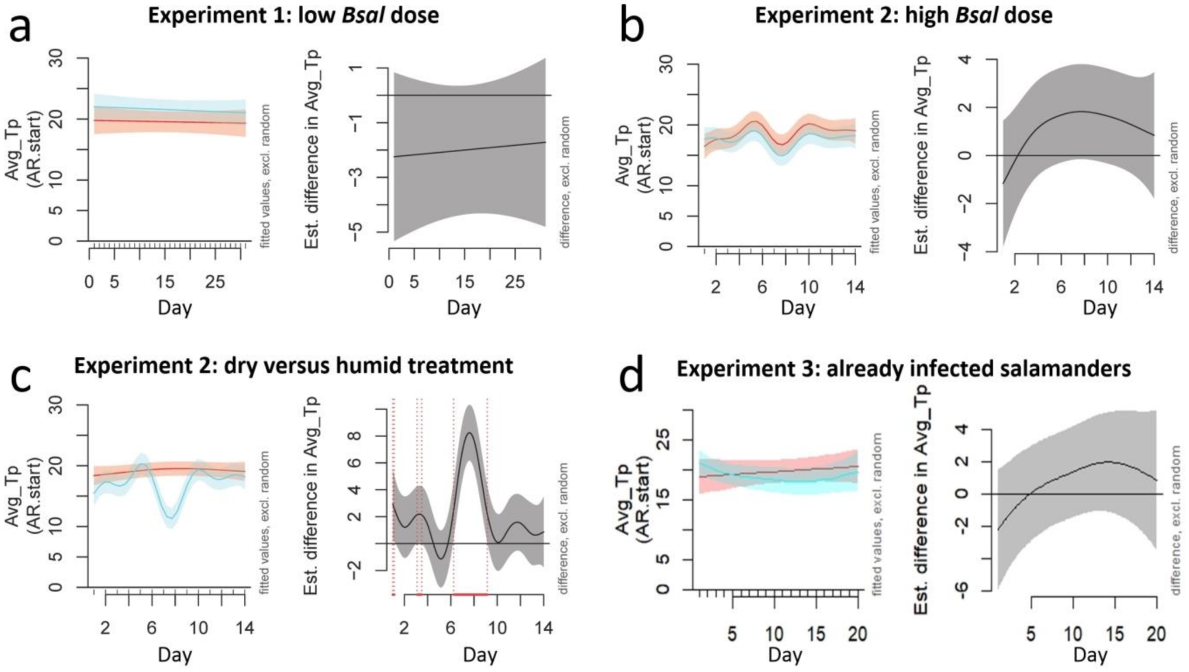

Fig. S1.1 Fitted trajectories of the parametric term treatment produced by time series generalized additive mixed models (GAMMS), comparing temperature selection in groups of non-exposed (red) and *Bsal*-exposed (blue) fire salamanders *Salamandra salamandra* in a series of three experiments (a, b, d). Reorganised data of experiment 2 (b) is shown in panel c, which compares salamanders that were exposed to an initial temporary dry treatment (blue), and those that were not (red). Difference plots show non-(dry)exposed salamanders as a reference (horizontal line at 0), in respect to the exposed treatment (black, wiggly line). Red dotted lines indicate significant differences between the treatments.

Table S1.1 Model summary of time series generalized additive mixed models (GAMMS) performed to infer if trends in fire salamander *Salamandra salamandra* temperature preference over time differed between *Bsal*-exposed vs. sham-exposed treatments. Significance is indicated with * and in **bold**.

Formula	Avg_Tp ~ OFTreatment + s(Day) + s(Day, by = OFTreatment) + s(Day, Indiv, bs = "fs", m = 1)											
	Experiment 1: low *Bsal* dose				**Experiment 3: already infected hosts**				**Experiment 2: high *Bsal* dose**			
Parametric coefficients	*Estimate Std.*	*Error*	*t value*	*Pr(>|t|)*	*Estimate Std.*	*Error*	*t value*	*Pr(>|t|)*	*Estimate Std.*	*Error*	*t value*	*Pr(>|t|)*
(Intercept)	20.54	0.60	34.28	**<0.001***	19.51	0.49	40	**<0.001***	18.12	0.42	43.66	**<0.001***
OFTreatment.L	1.40	0.85	1.70	0.1	-0.20	0.69	-0.28	0.78	-0.77	0.59	-1.31	0.19
Smooth terms	*edf*	*Ref.df*	*F*	*p-value*	*edf*	*Ref.df*	*F*	*p-value*	*edf*	*Ref.df*	*F*	*p-value*
s(Day)	1	1	0.09	0.76	1	1	0.60	0.44	7.36	8.33	4.22	**<0.001***
s(Day):OFTreatment1	1	1	0.07	0.80	1	1	0.63	0.43	2.41	2.97	1.43	0.21
s(Day,Indiv)	63.69	169	2.80	**<0.001***	24.54	163	0.40	**<0.001***	25.41	178	0.42	**<0.001***
R-sq. (adj)	0.45				0.15				0.30			
Deviance explained	50.8%				23.1%				39.3%			
fREML	1570.4				859.42				762.04			
Scale est.	9.71				16.06				10.79			
n	589				300				280			

Table S1.2 Model summary of a time series generalized additive mixed model (GAMM) performed to infer if trends in fire salamander *Salamandra salamandra* temperature preference over time differed between individuals that were temporarily exposed to a dehydrated environment, and individuals that were not. Significance is indicated with * and in **bold**.

	Experiment 2 dry vs. wet			
Parametric coefficients	*Estimate Std.*	*Error*	*t value*	*Pr(>|t|)*
(Intercept)	18.11	0.36	50.34	**<0.00***
OFTreatment.L	-1.48	0.51	-2.91	**<0.00***
Smooth terms	*edf*	*Ref.df*	*F*	*p-value*
s(Day)	1.81	2.23	1	0.37
s(Day):OFTreatment1	8.13	8.79	9.26	**<0.00***
s(Day,Indiv)	23.72	178	0.35	**<0.00***
R-sq. (adj)	0.39			
Deviance explained	46.9%			
fREML	743.36			
Scale est.	9.39			
n	280			

Table S1.3 *Bsal* loads (GE) of infected fire salamander *Salamandra salamandra* individuals (C1-D5). Mortality is displayed in orange. Only individual D3 did not test positive for *Bsal* after the end of the experiment; all individuals apart from D5 were successfully treated.

	Day					
	1	**8**	**15**	**20**	***Bsal* positive post experiment?**	**Treatment**
C1	0	0	544	354	yes	successful
C2	121	4180				
C3	🟧					
C4	🟧					
C5	414	2220	🟧			
D1	🟧					
D2	🟧					
D3	800	840	0	0	no	successful
D4	🟧					
D5	0	0	0	0	yes	🟧

S2. Thermal ecology

S2.1 Thermal preference result details

Table S2.1 Result summary of a Linear Mixed-Effects Model fitted to preferred temperature measurements, with species as fixed effect and individual as random effect; °C ~ species + (1|individual). Significance is indicated with in bold. *p*-values were estimated through *lmerTest* via t-tests using the Satterthwaite approximations to degrees of freedom. Marginal and conditional r^2 were estimated using *MuMIn::r.squaredGLMM*.

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*	*df*
(Intercept)	18.62	1.32	16.02 – 21.21	14.06	**<0.01**	42.48
Ichthyosaura alpestris	-2.22	1.90	-5.93 – 1.50	-1.17	0.24	44.50
Lissotriton helveticus	0.52	1.90	-3.21 – 4.24	0.27	0.79	44.98
Bufo bufo	4.12	1.89	0.41 – 7.83	2.18	**0.03**	44.31
Rana temporaria	4.38	1.90	0.66 – 8.10	2.31	**0.02**	44.73
Random Effects						
σ^2	27.47					
τ_{00} Individual	16.16					
ICC	0.37					
N Individual	50					
Observations	706					
Marginal R^2 / Conditional R^2	0.123 / 0.448					

Table S2.2 Result summary of a Linear Mixed-Effects Model fitted to preferred temperature measurements, with group (anurans and salamanders) as fixed effect and individual as random effect; °C ~ group + (1|individual). Significance is indicated with in bold. *p*-values were estimated through *lmerTest* via t-tests using the Satterthwaite approximations to degrees of freedom. Marginal and conditional r^2 were estimated using *MuMIn::r.squaredGLMM*.

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*	*df*
(Intercept)	22.87	0.95	21.00 – 24.74	24.02	**<0.01**	49.83
Salamanders	-4.81	1.23	-7.22 – -2.41	-3.92	**<0.01**	49.31
Random Effects						
σ^2	27.47					
τ_{00} Individual	15.93					
ICC	0.37					
N Individual	50					
Observations	706					
Marginal R^2 / Conditional R^2	0.109 / 0.436					

Table S2.3 Result summary of pairwise Welch's t-tests comparing field body temperatures and environmental temperatures of our focal amphibian assemblage gathered during spring 2017. Significance is indicated with in bold.

Species pair	*Welch's t-test result*
Salamandra salamandra - operative temperature	*F*(1,150.18) = 71.76, ***p* < 0.01**
Salamandra salamandra - Ichthyosaura alpestris	*F*(1,6.4) = 44.72, ***p* < 0.01**
Salamandra salamandra - Lissotriton helveticus	*F*(1,9.96) = 44.72, ***p* < 0.01**
Salamandra salamandra - Bufo bufo	*F*(1,47.25) = 1.54, *p* = 0.22
Salamandra salamandra - Rana temporaria	*F*(1,8.52) = 0.01, *p* = 0.1
Ichthyosaura alpestris - operative temperature	*F*(1,7.68) = 0.16, *p* = 0.7
Ichthyosaura alpestris - Lissotriton helveticus	*F*(1,9.34) = 0.01, *p* = 0.94
Ichthyosaura alpestris - Bufo bufo	*F*(1,9.92) = 8.54, ***p* = 0.02**
Ichthyosaura alpestris - Rana temporaria	*F*(1,13.75) = 7.37, ***p*= 0.02**
Lissotriton helveticus - operative temperature	*F*(1,16.80) = 0.27, *p* = 0.61
Lissotriton helveticus - Bufo bufo	*F*(1,22.44) = 19.41, ***p* < 0.01**
Lissotriton helveticus - Rana temporaria	*F*(1,12.14) = 10.88, ***p* < 0.01**
Bufo bufo - operative temperature	*F*(1,80.51) = 22.18, ***p* < 0.01**
Bufo bufo - Rana temporaria	*F*(1,11.77) = 0.31, *p* = 0.59
Rana temporaria - operative temperature	*F*(1,10.18) = 10.23, ***p* < 0.01**

S2.2 Thermal inertia pilot study

Thermal inertia of elongate salamanders and compactly-built anurans may differ, which can influence comparative analyses (Olalla-Tárraga & Rodríguez, 2007). A pilot study was therefore performed to determine heat loss rates and thermal time constants between our focal host *S. salamandra* and the phenotypically divergent Common Toad *Bufo bufo*. We temporarily collected 20 individuals of each species of different sizes (Tables S2.3 and S2.4) at our field study site in June 2017. Each amphibian was returned to its place of capture at night during rainy weather conditions after the pilot study. Individuals of both species were randomly divided into two species-specific groups of ten, which were acclimated for seven days at respectively 15°C and 20°C. All individuals were placed into 1 cm deep water 24 hours before the experiment to ensure full hydration. Before the start of the experiment each individual was weighed to the closest 0.01g, after which surface area of salamanders was calculated as $surface\ area\ = 8.42\ x\ weight^{0.694}$, following Whitford and Hutchison (1967). The surface area of toads was calculated as $surface\ area\ = 9.90\ x\ weight^{0.56}$, following McClanahan and Baldwin (1969). The volume (in cm^3) of each individual amphibian was subsequently determined using the displacement method, i.e., by means of quickly submerging the individual in a graduated cylinder partially filled with water. Surface-area-to-volume ratio (in L^{-1}) was calculated by dividing the surface area of each individual between its volume (Tables S2.3 and S2.4).

Given that environmental heat sources are absent during our nocturnal amphibian surveys, and because interspecific comparisons of amphibian heating rates may be inappropriate due to differences in evaporative cooling (Wygoda, 1989), we restricted ourselves to calculating cooling rates only. Cooling was achieved by using two 980x600x32mm stainless steel 500W cold plates containing 0.3kg Tetrafluoroethane (www.koelplaten.be), which were connected to a thermostat set to 0°C. Condensation was removed from the cold plate before measurements took place, after which a toad or salamander was placed on the plate under a low mesh cover for ten minutes. Mid-dorsal body temperature (T_b, in °C) was measured every minute using a type K thermocouple connected to an Omega HHC201 Dual Thermocouple Thermometer. Thermal time constants (τ_{cool}) were subsequently obtained per individual by first determining the slope of a linear regression of $\ln(T_b - T_{cold\ plate})$ as a function of time, and subsequently calculating the inverse of the slope (Dzialowski & O'Connor, 2001). Agar models, used to obtain operative temperatures during subsequent field surveys (Navas & Araujo, 2000), were subjected to the same procedure to confirm that these displayed similar thermal properties as the amphibians. Heat loss rates were not found to differ significantly after controlling for surface-to-volume ratio (ANCOVA $F(2, 35) = 0.07$, $p = 0.94$; Fig. S2.1a). Similarly, thermal time constants did not differ significantly between species (τ_{cool} 15.63 ± 4.19 for *S. salamandra*, 17.02 ± 5.14 for *B. bufo*, values are mean ± sd) or acclimation treatments (τ_{cool} 16.90 ± 4.71 at 15°C, 15 ± 5.73 at 20°C) according to a one-way ANOVA ($F(3,36) = 0.82$, $p = 0.49$; Fig. S2.1b).

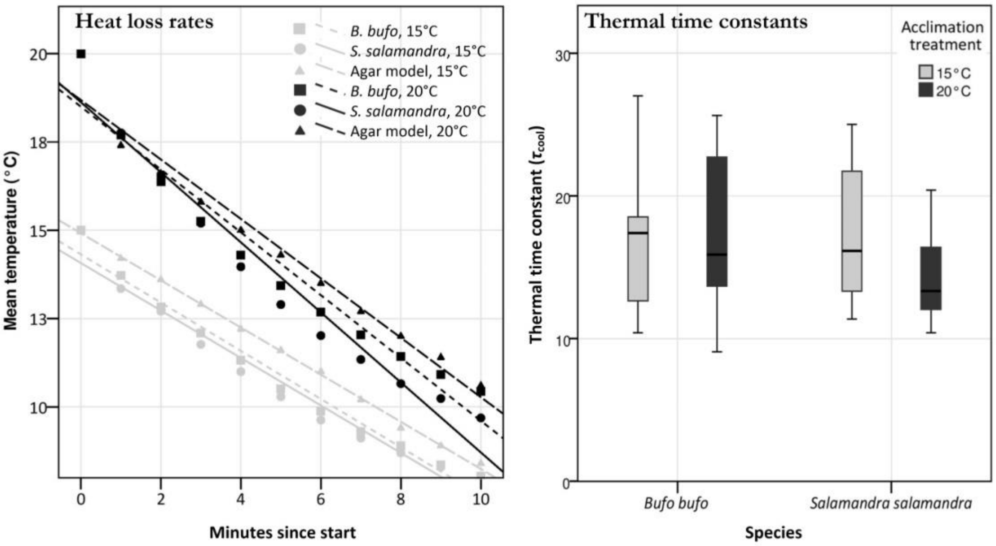

Fig. S2.1. Per-acclimation treatment heat loss and thermal time constants (τ_{cool}) for fire salamanders *Salamandra salamandra*, common toads *Bufo bufo,* and agar models used to obtain operative temperatures during field surveys.

Table S2.4 Weight (g), surface, volume (cm^3), surface-area-to-volume ratio (L^{-1}), acclimation temperature (T_i, in °C) and body temperatures ($T_1 - T_{10}$) measured while cooling each amphibian from 15°C during a period of 10 minutes. M is the slope of a linear regression of ln($T_\mathrm{b} - T_{\mathrm{cold\ plate}}$) as a function of time, whereas τ is the cooling constant.

Species	**Sex**	**Weight**	**Surface**	**Volume**	**L^{-1}**	T_i	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	M	τ
Bufo bufo	NA	13.21	42.01	17	2.47	15	14.5	13.8	13.2	12.6	11.8	11.1	10.3	9.8	8.8	8.6	-0.058	17.241
Bufo bufo	NA	15.18	45.41	19	2.39	15	13.5	12.8	12.2	11.8	11	10.3	9.6	9.4	8.8	8.5	-0.055	18.182
Bufo bufo	NA	30.04	66.55	40	1.66	15	13.3	12.8	12.6	11.9	11.4	10.6	10.1	9.2	8.9	8.6	-0.054	18.519
Bufo bufo	J	6.72	28.77	10	2.88	15	13	11.8	10.6	9.6	8.7	8.1	7.4	7.1	6.4	5.9	-0.090	11.111
Bufo bufo	NA	18.5	50.73	19	2.67	15	13.8	12.9	12.2	11.6	10.8	10	9.4	9	8.3	8	-0.063	15.873
Bufo bufo	NA	35.75	73.36	45	1.63	15	14	13.4	12.9	12.4	11.7	11	10.4	10	9.5	9	-0.050	20.000
Bufo bufo	J	3.47	19.87	5	3.97	15	12.9	11.1	9.7	8.5	7.2	6.8	6.4	6.2	5.8	6	-0.096	10.417
Bufo bufo	NA	16.32	47.29	19	2.49	15	14	13.6	12.8	11.9	11	10.3	9.8	9.4	9.1	8.6	-0.057	17.544
Bufo bufo	NA	22.93	57.21	20	2.86	15	14.2	13.7	13.4	12.9	12.4	12.2	11.6	11.2	10.6	10.2	-0.037	27.027
Bufo bufo	J	2.97	18.21	2	9.11	15	14.1	12.4	11.3	10	9.1	8.4	8.1	7.7	7.4	7	-0.079	12.658
Salamandra salamandra	M	40.31	109.51	30	3.65	15	13.9	13.7	13.1	12.6	12.1	11.4	10.9	10.3	9.8	9.4	-0.046	21.739
Salamandra salamandra	J	13.09	50.17	14	3.58	15	13.2	12.7	12.1	10.8	9.7	9.2	8.6	7.9	7.6	7	-0.075	13.333
Salamandra salamandra	J	7.45	33.93	8	4.24	15	13.4	12.5	11	9.9	9.3	8.3	7.9	7.3	6.9	6.8	-0.082	12.195
Salamandra salamandra	J	14.69	54.35	16	3.40	15	13.3	12.1	11.1	10.3	9.4	8.6	8	7.7	7.3	7.2	-0.075	13.333
Salamandra salamandra	F	40.78	110.40	35	3.15	15	13	13.1	12.6	12.1	11.5	11	10.5	10.3	9.9	9.8	-0.040	25.000
Salamandra salamandra	J	13.45	51.13	13	3.93	15	13.2	11.5	10.3	9.1	8.3	7.6	7.3	6.8	6.4	6.3	-0.088	11.364
Salamandra salamandra	M	25.93	80.63	27	2.99	15	13.4	12.9	11.6	11.3	10.3	9.8	9	8.6	8.4	7.8	-0.064	15.625
Salamandra salamandra	J	6.42	30.60	9	3.40	15	13.3	12.4	11.4	10.7	10.2	9.6	9.2	8.9	8.3	7.9	-0.060	16.667
Salamandra salamandra	F	24.63	77.80	27	2.88	15	13.5	13.3	12.5	11.9	11.5	10.9	10.5	10.3	9.9	9.5	-0.043	23.256
Salamandra salamandra	J	10.51	43.08	11	3.92	15	13.3	12.9	12	11.3	10.6	9.9	9.3	8.9	8.5	8.3	-0.059	16.949
Agar model	NA	43.82	63.62	35	1.81	15	14.2	13.6	12.9	12.2	11.6	11	10.2	9.4	8.9	8.4	-0.059	16.950

Table S2.5 Weight (g), surface, volume (cm^3), surface-area-to-volume ratio (L^{-1}), acclimation temperature (T_i, in °C) and body temperatures ($T_1 - T_{10}$) measured while cooling each amphibian from 20°C during a period of 10 minutes. M is the slope of a linear regression of ln($T_b - T_{\text{cold plate}}$) as a function of time, whereas τ is the cooling constant.

Species	**Sex**	**Weight**	**Surface**	**Volume**	**L^{-1}**	**T_i**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**	**T_{10}**	**M**	**τ**
Bufo bufo	NA	20.06	53.08	20	2.65	20	18	16.9	15.9	15.4	14.7	14.2	13.8	13.2	12.7	12.5	-0.044	22.727
Bufo bufo	NA	13.42	42.38	19	2.23	20	17.6	16.5	15.8	15	14.2	13.4	12.7	12.4	11.7	11.5	-0.053	18.868
Bufo bufo	J	9.3	34.51	12	2.88	20	18	16.6	15.5	14.5	13.5	12.8	12	11.4	10.8	10.5	-0.064	15.625
Bufo bufo	J	3.34	19.45	4	4.86	20	16.3	14.3	12.4	10.6	9.9	9.2	8.1	7.5	6.9	6.3	-0.110	9.091
Bufo bufo	J	3.25	19.16	5	3.83	20	17.1	14.9	12.9	11.3	10.9	9.2	9	8.3	8.1	7.2	-0.098	10.204
Bufo bufo	NA	38.14	76.07	40	1.90	20	18.5	17.7	16.6	16.2	15.6	15	14.4	13.9	13.5	13	-0.041	24.390
Bufo bufo	NA	17.6	49.33	20	2.47	20	17.7	16.5	15.5	14.3	13.3	12.9	12.1	11.6	11	10.5	-0.062	16.129
Bufo bufo	NA	17.28	48.83	21	2.33	20	17.5	16.4	15.4	14.5	13	12.4	11.6	10.4	10.2	9.4	-0.073	13.699
Bufo bufo	NA	28.65	64.81	29	2.23	20	18.3	17.5	16.9	16.3	15.8	15.2	14.7	14.2	13.5	13.1	-0.039	25.641
Bufo bufo	NA	15.93	46.65	16	2.92	20	18	16.5	15.6	14.9	13.5	12.6	12	11.4	10.8	10.4	-0.065	15.385
Salamandra salamandra	M	25.2	79.05	27	2.93	20	17.8	17.2	16.2	14.9	14	13.3	12.4	11.7	11.2	10.6	-0.062	16.129
Salamandra salamandra	F	29.58	88.35	29	3.05	20	18.1	17.3	16.2	15.5	14.5	14	13.5	13.1	12.6	11.9	-0.049	20.408
Salamandra salamandra	J	5.45	27.31	5	5.46	20	18.7	17.2	15.3	13.4	11.3	10.9	10	9.3	8.5	8.1	-0.096	10.417
Salamandra salamandra	F	27.92	84.87	29	2.93	20	18.4	17.5	16.5	15.9	14.9	14	12.6	12	11.7	10.8	-0.061	16.393
Salamandra salamandra	J	9.28	39.52	11	3.59	20	17.2	15.5	13.8	13.5	12.3	11.7	10.7	10.2	9.5	9.1	-0.075	13.333
Salamandra salamandra	J	5.33	26.89	5	5.38	20	17	15	13.1	11.7	10.6	10	9.2	8.6	8.6	7.8	-0.091	10.989
Salamandra salamandra	F	16.91	59.93	15	4.00	20	17.5	15.7	14.4	13.3	12	11.3	10.5	10	9.7	9.5	-0.075	13.333
Salamandra salamandra	M	18.03	62.66	14	4.48	20	17.9	16.8	15.5	13.5	12.3	10.7	10.3	9.8	9.5	8.9	-0.083	12.048
Salamandra salamandra	M	15.45	56.29	15	3.75	20	17.5	16.3	14.9	13.1	12.4	11	10.6	10	9.6	9.2	-0.078	12.821
Salamandra salamandra	M	26.5	81.85	26	3.15	20	17.6	17.2	16.1	14.9	14.7	13.3	12.6	11.9	11.5	11	-0.058	17.241
Agar model	NA	43.57	63.62	50	1.27	20	17.4	16.6	15.8	15	14.3	13.5	12.7	12	11.4	10.6	-0.058	17.240

S2.3 Field survey results details

Table S2.6 Result summary of a Linear Mixed-Effects Model fitted to temperature measurements, with fixed effects of species, humidity, time since sundown, and location along the transect (sector, Fig. 2.2). Sampling event was nested in season as random effects; °C ~ species + humidity + time since sundown + sector + (1|SamplEvent/Season). Significance is indicated with in bold. *p*-values were estimated through *lmerTest* via t-tests using the Satterthwaite approximations to degrees of freedom. Marginal and conditional r^2 were estimated using *MuMIn::r.squaredGLMM*.

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*	*df*
(Intercept)	38.15	14.9	8.96 – 67.35	2.56	**< 0.01**	31.23
Salamandra salamandra	0.02	0.06	-0.10 – 0.13	0.26	0.79	3070.88
Ichthyosaura alpestris	-0.13	0.18	-0.48 – 0.22	-0.71	0.47	3069.25
Lissotriton helveticus	0.14	0.25	-0.36 – 0.63	0.55	0.58	3069.17
Bufo bufo	0.31	0.1	0.12 – 0.50	3.16	**< 0.01**	3069.63
Rana temporaria	0.38	0.13	0.12 – 0.64	2.84	**< 0.01**	3069.31
Humidity (%)	-0.3	0.16	-0.61 – 0.02	-1.86	0.06	31.34
Time since sundown (min)	0	0	-0.00 – 0.00	0.85	0.40	2958.33
Sector 2	-0.15	0.06	-0.27 – -0.02	-2.29	**0.02**	3095.21
Sector 3	0.01	0.09	-0.16 – 0.18	0.12	0.90	3079.91
Sector 4	-0.11	0.12	-0.34 – 0.12	-0.96	0.34	3074.3
Sector 5	0.19	0.13	-0.06 – 0.44	1.52	0.13	3032.88
Random Effects						
σ^2	0.89					
τ_{00} Season:Sample event	0.43					
τ_{00} Sample event	9.58					
ICC	0.92					
N Season	4					
N Sample event	33					
Observations	3112					
Marginal R^2 / Conditional R^2	0.08 / 0.93					

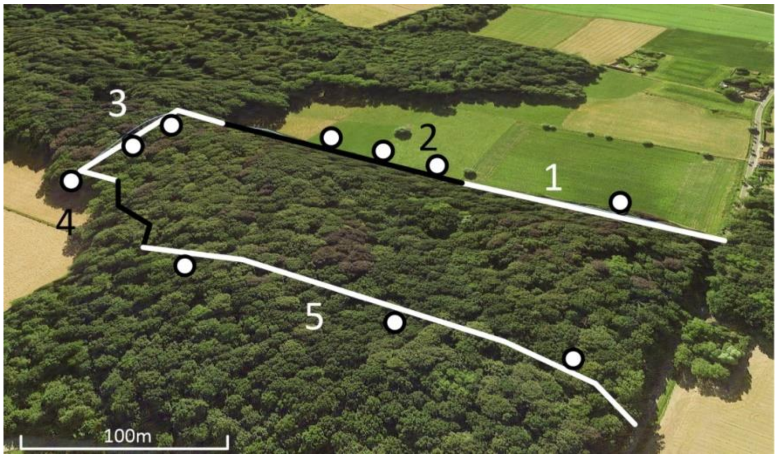

Figure S2.2 Location of sectors along the transect. White dots denote locations of agar models used to measure operative temperature. Sectors were delineated to reflect differences between gradual forest edges (1, 4), an abrupt change from mature forest into meadow (2) and mature forest (3, 5).

Active T_b, T_e and T_{set} measurements recorded in spring 2017 were combined to calculate mean thermal habitat quality ($\bar{d}_e$), the mean accuracy of temperature regulation ($\bar{d}_b$), and the effectiveness of temperature regulation (E) per species (see Hertz *et al.*, 1993 for a full methodological description), for which we used the R package *ectotemp*. Following Blouin-Demers and Nadeau (2005) we used the protocol of Hertz *et al.* (1993) to calculate $\bar{d}_e$ and $\bar{d}_b$, while estimating E using a variant on the original formula provided by Blouin-Demers and Weatherhead (2001). The rationale for this mix of approaches is that the latter authors demonstrated that interpretation of the original formula to estimate E (i.e., from Hertz *et al.*, 1993) is confounded by the fact that different combinations of $\bar{d}_e$ and $\bar{d}_b$ might lead to similar E values. Specifically, two organisms characterized by different thermal environments, and distinct strategies to regulate their body temperature, may show the same E value if the ratios between $\bar{d}_e$ and $\bar{d}_b$ are the same. The variant proposed by Blouin-Demers and Weatherhead (2001) simply quantifies the extent of departure from perfect thermoconformity. Positive E values indicate active temperature regulation, negative values represent active avoidance of suitable thermal habitat, and values around 0 suggest thermoconformity.

Differences in seasonal acclimation and reproductive state may influence preferred temperature of the amphibian species studied here (e.g. Degani, 1982, 1984; Gvoždík, 2005, 2012; Toufarová & Gvoždík, 2016). We therefore calculated $\bar{d}_e$, $\bar{d}_b$ and E only for the meteorological season during which T_{set} was measured, comprising the months of March, April and May. First, the thermal quality of the habitat (d_e) from the perspective of each amphibian species was determined as follows: if a T_e value fell within the concerning species' T_{set}, d_e was considered to be zero, while if T_e fell below the lower bound or above the upper bound of T_{set}, d_e was determined as the difference between T_e and the thermal bound in question. Environments in which the mean d_e ($\bar{d}_e$) is zero fall within the set point range, and are therefore regarded as having high thermal quality. A higher $\bar{d}_e$ signifies lower quality of the thermal environment. The accuracy of temperature regulation (d_b) was subsequently determined as d_e, but by using T_b instead of T_e. The mean d_b ($\bar{d}_b$) is a measure of the average degree to which a species experiences T_b outside T_{set}, with a low $\bar{d}_b$ indicating much time spent within T_{set}, while a high $\bar{d}_b$ suggests the opposite.

Finally, as mentioned above, E was calculated following Blouin-Demers and Weatherhead (2001) as $E = \bar{d}_e - \bar{d}_b$. The function *ectotemp::bootstrap_E()* was used to obtain 95% confidence interval for E for each species. In order to test for differences in E between different species, Hertz *et al.* (1993) suggested comparing paired estimates of E obtained through bootstrapping. However, because sample sizes of T_b or T_e may be small and/or could differ in size and variance, possibly leading to non-normality, we used two-sided permutation testing (*ectotemp::compare_E()*) instead of bootstrapping to build and compare *E* value distributions.

S2.4 Mesocosm results details

Table S2.7 Result summary of three Linear Models fitted to hourly body temperature measurements of three *S. salamandra* (C3031, C3032, C3015) inhabiting a forest mesocosm, with fixed effects of ambient temperature (°C), humidity (%), and rainfall (mm). Significance is indicated with in bold.

C3031

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*
(Intercept)	2.49	0.05	2.40 – 2.58	55.09	**<0.01**
Temperature (°C)	0.05	0.00	0.05 – 0.05	46.88	**<0.01**
Humidity (%)	0.01	0.00	0.01 – 0.01	20.95	**<0.01**
Rainfall (mm)	0.09	0.05	-0.01 – 0.19	1.74	0.08
Observations	2924				
R^2 / R^2 adjusted	0.47 / 0.46				

C3032

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*
(Intercept)	7.25	0.33	6.61 – 7.90	22.10	**<0.01**
Temperature (°C)	0.35	0.01	0.33 – 0.36	43.84	**<0.01**
Humidity (%)	0.04	0.00	0.04 – 0.05	14.38	**<0.01**
Rainfall (mm)	0.21	0.36	-0.50 – 0.92	0.58	0.56
Observations	2925				
R^2 / R^2 adjusted	0.46 / 0.46				

C3015

Predictors	*Estim. (β)*	*std. Error*	*CI*	*T statistic*	*p*
(Intercept)	6.80	0.33	6.16 – 7.45	20.69	**<0.01**
Temperature (°C)	0.22	0.01	0.21 – 0.24	28.01	**<0.01**
Humidity (%)	0.07	0.00	0.07 – 0.08	24.94	**<0.01**
Rainfall (mm)	0.31	0.36	-0.40 – 1.03	0.86	0.39
Observations	2924				
R^2 / R^2 adjusted	0.23 / 0.22				

Table 2.8 Time-series Generalized Additive Model results showing the extent to which variance in *Salamandra salamandra* body temperature measured with internal data loggers is explained by smooth terms for outside environmental temperature, humidity, and rainfall (mm).

Formula	C ~ OFType + s(Datetime) + s(Datetime, by = OFType) + s(Humid) + s(Rain)			
Parametric coefficients	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*
(Intercept)	18.11	0.04	439.40	**<0.01**
OFTypeC3015	-2.05	0.06	-35.10	**<0.01**
OFTypeC3031	-1.88	0.06	-32.23	**<0.01**
OFTypeC3032	-1.58	0.06	-26.98	**<0.01**
Smooth terms	*edf*	*Ref.df*	*F*	*p-value*
s(Datetime)	9.000	9.00	425.76	**<0.01**
s(Datetime):OFTypeC3015	8.91	8.99	247.49	**<0.01**
s(Datetime):OFTypeC3031	8.96	8.99	143.91	**<0.01**
s(Datetime):OFTypeC3032	8.96	8.99	132.09	**<0.01**
s(Humid)	8.65	8.97	347.26	**<0.01**
s(Rain)	2.58	3.23	5.15	**<0.01**
R-sq. (adj)	0.49			
Deviance explained	49.7%			
fREML	5.03			
Scale est.	5.01			
n	11732			

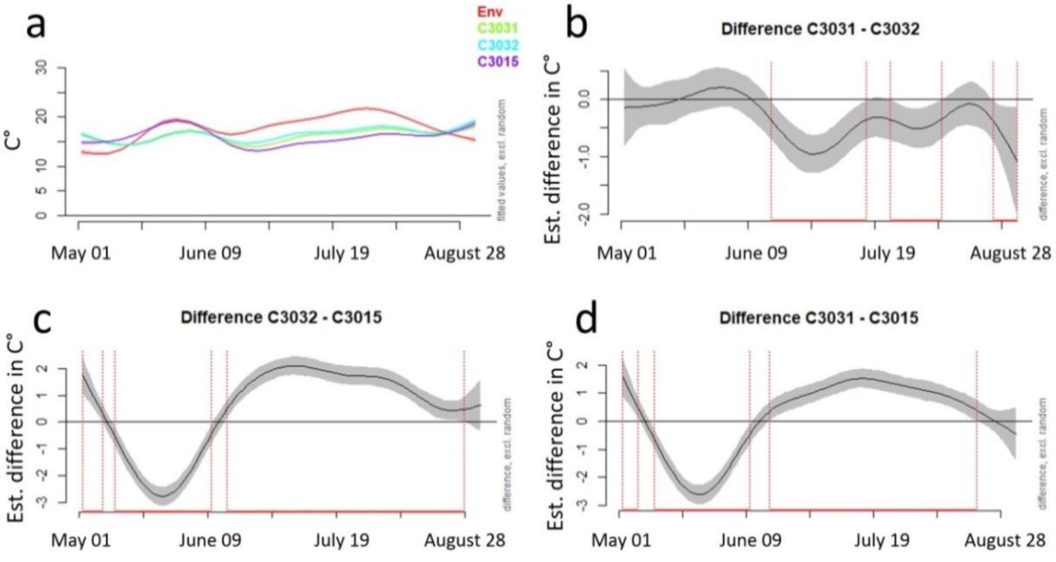

Fig. S2.3 Fitted trajectories of the parametric term 'OFType' produced by a time series Generalized Additive Models (GAM), comparing body temperature between ambient temperature ('Env') and three *Salamandra salamandra* individuals (C3032, C3031, C3015, a). Difference plots (b, c, d) show comparisons by setting one individual as a reference (horizontal line at 0) in respect to another individual (black line). Red dotted lines indicate significant differences in body temperature between individuals.

S3. Mechanistic model

S3.1 Bsal thermal growth and fitting thermal growth curves

Pathogen growth is ideally measured *in vivo*, which may differ from *in vitro* growth owing to interactions with the host immune system (Sonn *et al.*, 2017). Obtaining *Bsal* growth data *in vivo* across a broad spectrum of *S. salamandra* body temperatures would however require large numbers of experimental animals, which was logistically infeasible and ethically questionable given the availability of *in vitro* measurements as an alternative. To obtain a detailed overview of *Bsal* thermal growth we therefore conducted a 10-day *in vitro* experiment by exposing *Bsal* AMFP13/1 to 6 different temperatures ranging between 0 and 30°C. *Bsal* was cultured on TGhL medium as described by Martel *et al.* (2013) and Martel *et al.* (2014). Sixteen hours prior to setting up the experiment the medium was replaced by 0.8% Tryptone TGhL to facilitate spore attachment. Fifty milliliter from the original TGhL medium was placed at each test temperature (0, 5, 10, 15, 20 and 30°C) to equilibrate. Collected spores were filtered using a 10µm cell strainer (Pluristrainer, https://www.pluriselect.com) and diluted to the desired concentration of 5x10^5 spores per ml. One milliliter of this spore solution was then added to 2 wells of three separate 24 well plates per temperature, totaling six wells for each temperature. The plates were subsequently wrapped in aluminum foil to standardize daylight exposure, and placed at the designated test temperatures for three hours. After three hours the medium was removed from the wells, and replaced with 1ml 1.6% tryptone TGhL acclimatized to the concerning test temperature. We considered this as time zero, as all non-attached spores had been removed. One plate was removed at this point and processed for qPCR analysis, while the two remaining plates were photographed using a Leica DMi1 microscope and 20x magnification with which photographs of three random positions of the base of the well were taken. These plates were subsequently wrapped in aluminum foil and returned to their experimental temperature. Photographing was repeated on day 3, 5, 7 and 10. At each time point, the number of motile spores was categorized as 0 (zero motile spores), A (fewer than 7 spores), B (more than 7 spores), or B+ (individual spores cannot be distinguished due to the large total amount of spores). Photographing each plate was performed as quickly as possible to minimize acclimatization to ambient temperature. On day 5, 1 ml of 16% tryptone TGhL media acclimatized to the experimental temperature was added to each well. The experiment finished on day 10 with all wells being processed for qPCR analysis.

A 1000x1000 pixel square centered at pixel 500x500 was analyzed from each photo using the software ImageJ (Schneider *et al.*, 2012). The number of spores, the number of sporangia and the area of each sporangia was measured; then the minimum, maximum, mean and standard deviation of sporangia size was calculated for each photograph. All analyses were performed by the same person, with photographs labeled only with an uninformative number.

Growth curves were fitted to sporangia count per well and the intrinsic growth rate, r, extracted for each well using *growthcurver* in R (Sprouffske, 2018). Whereas optical density has been repeatedly used in the past to fit growth curves for e.g. *B. dendrobatidis* (e.g. Voyles *et al.*, 2017; Stevenson *et al.*, 2020) we found that preliminary results based on this technique did not correlate with culture growth rates. Our choice therefore fell on sporangia counts instead, which we preferred over spore counts, sporangia coverage or qPCR results because of the following reasons. First, sporangia count represents the number of reproductive bodies. Second, spore count can be falsely inflated by spores slow or failing to mature (i.e., a poorly growing culture) and reduced by spores that have matured into sporangia (illustrating a successfully growing culture). Third, we refrained from selecting sporangia coverage because in *Bsal* there is no known correlation between sporangia size and infectivity. Also, we observed during the experiment that at temperatures beyond its thermal optimum *Bsal* sporangia were enlarged, perhaps due to a stress response. These bodies did however not appear to be as productive as normal full, mature sporangia. Fourth, we did not use qPCR results as these were only available for day 0 and day 10, while growth curves illustrated that the cultures at 10°C and 15°C had reached carrying capacity much earlier in the experiment whereas lower temperatures were still in exponential growth phase, see Fig. S1.3.

We fitted a range of polynomial curves (1 to 4 degrees), to the extracted r values using *stats::lm*() and *stats::poly*() in R. The best-fitting curve was identified using *stats::anova* (fourth order polynomialism, R^2 of different degrees of polynomialism: 1^{st}=0.02692, 2^{nd}=0.2851, 3^{rd}=0.2964, 4^{th}=0.6022), from which the coefficients were extracted. The curve was restricted to 0-25°C as both our experiment and Martel *et al.* (2013) found temperatures below 0 or of 25 degrees and above incompatible with growth.

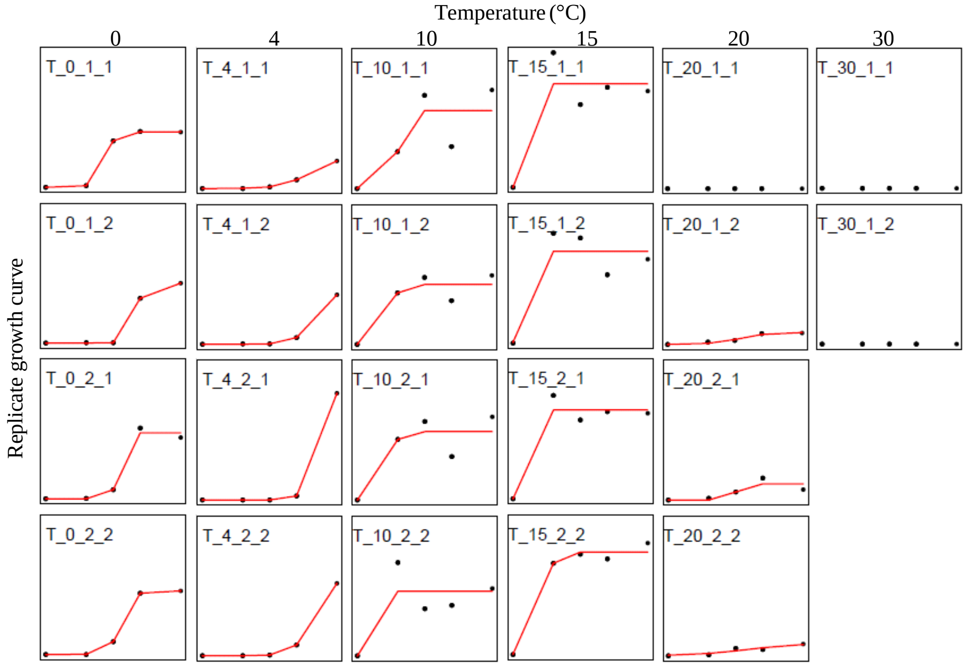

Figure S3.1 *Bsal* growth rates based on sporangia counts, plotted by the R package *growthcurver* per well. Each graph is labelled such that the first number represents the temperature, the second represents the plate number and the third number represents the replicate number per plate.

S3.2 NicheMapR environmental input data collection

Topographic parameters were assembled by first obtaining an altitude layer in the form of the SRTM v4.1 digital elevation model (http://srtm.csi.cgiar.org/srtmdata/), from which slope and aspect parameters were derived through the *terrain()* function in the *raster* R package. Climate data were obtained from the ERA5 dataset, which gives access to hourly estimates of a large number of atmospheric, land and oceanic climate parameters covering the Earth from surface up to a height of 80km (Copernicus Climate Change Service, 2017). We queried the ERA5 dataset describing hourly data on single levels from 1979 to present to obtain 2m temperature, 2m dew point temperature, 10m u- and v-components of wind, total cloud cover, total precipitation and volumetric soil water in soil layer 1 (0 - 7cm depth) for the period ranging from 01-01-2010 to 31-12-2018. All data were downloaded in .netcdf format and processed using the *ncdf4* and *raster* packages in R. We derived wind speed and relative humidity parameters through use of *rWind::uv2ds()* and *humidity::RH()* in R. Please refer to table S1.3 for a detailed overview.

We used a point-based grid (fishnet) of 0.25 degree resolution ranging between 59.88-34.26 and -10.97-29.08 degrees to extract data from all parameters using *raster::extract()* in R, which was used to inform NicheMapper. This extent covers most of Europe and parts of the Mediterranean Basin, including the distribution of *S. salamandra*.

S3.3 NicheMapR parameterization

Salamander steady-state temperature was calculated using the *NicheMapR* R package (Kearney, 2019). We used the microclimate module (Kearney & Porter, 2017) to estimate the micro-environmental conditions based on the parameters indicated above, which was used as input for the ectotherm module (Kearney & Porter, 2020) that provided steady-state body temperature of *S. salamandra* throughout Europe. The model was run hourly for the middle day of each month for an organism of 40g and the shape of a lizard. Evaporative water loss was taken into account by considering the surface of the organism as a free-water exchanger. The salamander was assumed to have nocturnal and crepuscular activity, and the percent of shaded conditions was set between 90 and 100%. Further details on the parameterization of the model can be found in Table S1.4.

Table S3.1 Topographic and climate parameters used to inform a NicheMapper mechanistic model of fire salamander *S. salamandra* body temperature. Parameters indicated in *italics* were used in the final model.

Parameter	**Unit**	**Dataset**	**Details**	**Link**
Daily mean minimum & maximum air temperature	Kelvin (K), converted to Celsius (°C)	ERA5 hourly data on single levels	Derived from daily 2m temperature	http://thredds.northwestknowledge.net:8080/thredds/terraclimate_aggregated.html
Daily mean air temperature	Kelvin (K), converted to Celsius (°C)	ERA5 hourly data on single levels	Derived from daily 2m temperature	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily mean minimum & maximum dewpoint temperature	Kelvin (K), converted to Celsius (°C)	ERA5 hourly data on single levels	Derived from 2m dew point temperature	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily total precipitation	mm	ERA5 hourly data on single levels	-	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily average minimum & maximum cloud cover	Dimensionless	ERA5 hourly data on single levels	Derived from daily total cloud cover	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily volume of water in soil layer 1 (0 - 7cm, the surface is at 0cm)	$m^3 m^{-3}$	ERA5 hourly data on single levels	-	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily average minimum & maximum relative humidity	%	ERA5 hourly data on single levels	Calculated from ERA5 min and max (dew point) temperature	-
Daily average minimum & maximum u- and v-components of wind	$m s^{-1}$	ERA5 hourly data on single levels	Derived from daily 10m u- and v-components	https://cds.climate.copernicus.eu/cdsapp#!/dataset/reanalysis-era5-single-levels?tab=form
Daily average minimum & maximum wind speed	$m s^{-1}$	ERA5 hourly data on single levels	Calculated from min 10m u and v wind components	-
Altitude	m	SRTM v4.1	-	http://srtm.csi.cgiar.org/srtmdata/
Slope	Degrees	-	Derived from altitude layer	-
Aspect	Degrees	-	Derived from altitude layer	-

Table S3.2. Details on *NicheMapR* parameterization. Names in the left column follow those of the examples provided in Kearney (2019), the center column shows the used values and the right column provides a short explanation and units when applicable. When there was no information available on certain parameter, those provided in examples of Kearney (2019) were used.

Options	**Value**	**Details and Units**
General options		
microdaily	0	Run as normal, 3 iterations since initial conditions.
runshade	1	Run once for each shade level.
runmoist	1	Run the soil moisture model.
snowmodel	0	Snow model was not run.
hourly	0	Run from non-hourly climate data.
rainhourly	0	Run from non-hourly climate data.
IR	0	Clear sky longwave radiation computed using Campbell and Norman (1998).
message	0	Warning messages from Fortran integrator not allowed.
fail	24	Number of restarts of the integrator before Fortran program quits.
Temporal and geographical options		
doynum	12	Number of time intervals (days) to make predictions.
doy	15, 46, 74, 105, 135, 166, 196, 227, 258, 288, 319, 349	Julian days to make the prediction (middle day of each month).
idayst	1	First month.
ida	12	End month.
HEMIS	1	Northern hemisphere.
EC	0.0167238	Eccentricity of earths orbit.
Wind scaling parameters		
RUF	0.004	Roughness height (m).
Refhyt	2	Reference height of wind speed (m).
Usrhyt	0.01	Height above ground to which wind speed is scaled (m).
ZH	0	Heat transfer roughness height (m).
D0	0	zero plane displacement correction factor (m).
Emissivity, reflectivity, atmospheric water and extinction		
SLE	0.96	Substrate emissivity (decimal).

REFL	0.1	Substrate solar reflectivity (decimal).
CMH2O	1	Precipitable H2O in air column, moist (cm).
TAI	**	Aerosol extinction coefficient profile.
hori	0	Horizon angles (24 angles, all set to zero).
minshade	90	Minimum shade available (%).
maxshade	100	Maximum shade available (%).
PCTWET	0	Percentage of surface area acting as a free water surface (%).
DEP	0, 2.5, 5, 10, 15, 20, 30, 50, 100, 200	Depth of soil nodes (cm).
ERR	1.5	Integrator error for soil calculations.
TIMINS	0,0,1,1	Vector of times of minimum values for Air temperature and wind speed relative to sunrise, and for humidity and cloud cover relative to solar noon.
TIMAXS	1,1,0,0	Vector of times of maximum values for Air temperature and wind speed relative to solar noon, and for humidity and cloud cover relative to sunrise.
Soil thermal parameters		
Thcond	1.25	Thermal conductivity of soil minerals (W/mC).
Density	2.56	Density of soil minerals (Mg/m3).
SpecHeat	870	Specific heat of soil minerals (J/kg-K).
BulkDensity	2.56	Soil density (Mg/m3).
SatWater	0.26	Saturation volumetric water content (m3/m3).
PE	0.7	Air entry potential (J/kg) from Campbell and Norman (1998), parameters for Sand in Table 9.1.
KS	0.0058	Saturated conductivity (kg*s/m3) from Campbell and Norman (1998), parameters for Sand in Table 9.1.
BB	1.7	Soil B parameter from Campbell and Norman (1998), parameters for Sand in Table 9.1.
BD	1.3	Bulk density of soil (Mg/m3)
L	0, 0, 8.2, 8.0, 7.8, 7.4, 7.1, 6.4, 5.8, 4.8, 4.0, 1.8, 0.9, 0.6, 0.8, 0.4 ,0.4, 0, 0	Root density at each soil node times 10000 (mm/m3).
R1	0.001	Root radius (m).
RW	2.50E+10	Resistance of root (m3/kg*s).
RL	2.00E+06	Resistance of leaf (m3/kg*s).

PC	1500	Critical leaf water potential for stomatal closure (J/kg).
SP	10	Stability parameter for equation for stomatal closure.
IM	1.00E-06	Maximum allowed error for mass balance (kg).
MAXCOUNT	500	Maximum number of iterations for mass balance.
LAI	0.1	Leaf area index.
rainmult	1	Rain multiplier (to impose catchment).
maxpool	10	Maximum depth of water (mm).
evenrain	1	Rain is spaced evenly throughout the day.
SoilMoist_Init	0.2	Initial soil water content (in this case it is the same for each node)(m3/m3).

** Values extracted from the Global Aerosol Data Set (GADS) (described in d'Almedia *et al.* (1991) and Koepke *et al.* (1997) for the location of Madison.

The vector of values of the profile is: 0.269904738,0.266147825, 0.262442906, 0.258789404, 0.255186744, 0.251634356, 0.248131676, 0.2412732, 0.234606887, 0.228128378, 0.221833385, 0.215717692, 0.20977715, 0.204007681, 0.198405272, 0.187685927, 0.177588357, 0.168082846, 0.159140695, 0.150734206, 0.142836655, 0.135422274, 0.128466227, 0.12194459, 0.115834329, 0.110113284, 0.104760141, 0.099754417, 0.09507644, 0.090707328, 0.086628967, 0.082823998, 0.07927579, 0.075968428, 0.072886691, 0.070016034, 0.067342571, 0.064853053, 0.062534858, 0.060375964, 0.058364941, 0.056490925, 0.054743609, 0.053113222, 0.051590514, 0.050166738, 0.046408775, 0.045302803, 0.044259051, 0.043271471, 0.042334415, 0.041442618, 0.040591184, 0.039775572, 0.038991583, 0.038235345, 0.037503301, 0.036792197, 0.036099067, 0.034101935, 0.033456388, 0.032817888, 0.032184949, 0.031556287, 0.030930816, 0.030307633, 0.029065372, 0.027825562, 0.027205981, 0.026586556, 0.025967391, 0.025348692, 0.024114005, 0.023498886, 0.021669152, 0.021066668, 0.019292088, 0.018144698, 0.016762709, 0.015451481, 0.014949794, 0.014224263, 0.013093462, 0.012670686, 0.012070223, 0.011164062, 0.010241734, 0.009731103, 0.009507687, 0.009212683, 0.008965785, 0.008827751, 0.008710756, 0.008574128, 0.008462605, 0.008446967, 0.008539475, 0.009015237, 0.009748444, 0.010586023, 0.011359647, 0.011901268, 0.012062153, 0.011735443, 0.010882215, 0.009561062, 0.007961182, 0.006438984, 0.005558204, 0.006133532, 0.009277754

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General discussion

General discussion

The general goal of this PhD was to assess the role of the environment, and particularly temperature, in shaping *Batrachochytrium salamandrivorans* (*Bsal*) disease dynamics in north-western Europe. Emphasis was furthermore given to improve spatial predictions of *Bsal* spread. In the discussion below, I focus on two broadly defined issues which reflect findings in the preceding chapters that merit specific attention, especially in the light of future research efforts.

I. Narrow salamander niche breadth increases vulnerability

In Chapter 4, we showed that the bioclimatic niche of invasive *Bsal* populations is a small subset of its much wider native East-Asian niche. About a third of the salamander species inhabiting the Western Palearctic were nonetheless found to show significant bioclimatic niche overlap with invasive *Bsal*. This result suggests that most salamanders occupy similar climatic conditions at macroscale, despite their occurrence in different regions of this large study area (Vieites *et al.*, 2009). It may be argued that such similarity increases vulnerability to *Bsal*, which would find little resistance in terms of climate when novel hosts occur in conditions comparable to areas where the pathogen has already established. However, does bioclimatic niche similarity hold at local levels?

Intuitively, one would expect that for instance salamanders in Mediterranean Europe are exposed to higher local temperatures than those living in the north of the continent. The ensuing variation in body temperature may provide an extra buffer against *Bsal*, of which fitness rapidly declines above 20°C. Is this however truly the case? Research into thermal ecology of European and Mediterranean salamanders is surprisingly rare, which makes it challenging to answer this question. Nevertheless, a few studies exist that shed some light on the matter. Lunghi *et al.* (2015) presented data on operative (environmental) temperatures from caves inhabited by *Speleomantes italicus* in central Italy. The body temperature of these salamanders differs little from their surroundings (Lunghi *et al.*, 2016), similar to what we found for *S. salamandra* in Chapter 5. As shown in Figure 1, operative temperatures in this habitat differ little from those experienced by terrestrial salamanders close to the invasive *Bsal* range (Chapter 5, Fig. 2, 3). While caves are admittedly a highly buffered environment, Busack (1978) again recorded similar body temperatures in active *S. salamandra* measured during August in northern-central Spain. Together, these studies support our mechanistic model presented in Chapter 5 by showing that at least terrestrial salamanders throughout Europe may be characterized by similar, narrow niche breadths that keep them from overheating or dehydration. From an ecophysiological viewpoint this is not unexpected (see also Chapter 5, discussion). What follows is that the bioclimatic niche similarity recovered in Chapter 4 may indeed translate across spatial scales – in fact, at local scales salamander thermal niches may even be more similar than identifiable by macroecological analyses as a result of microhabitat buffering.

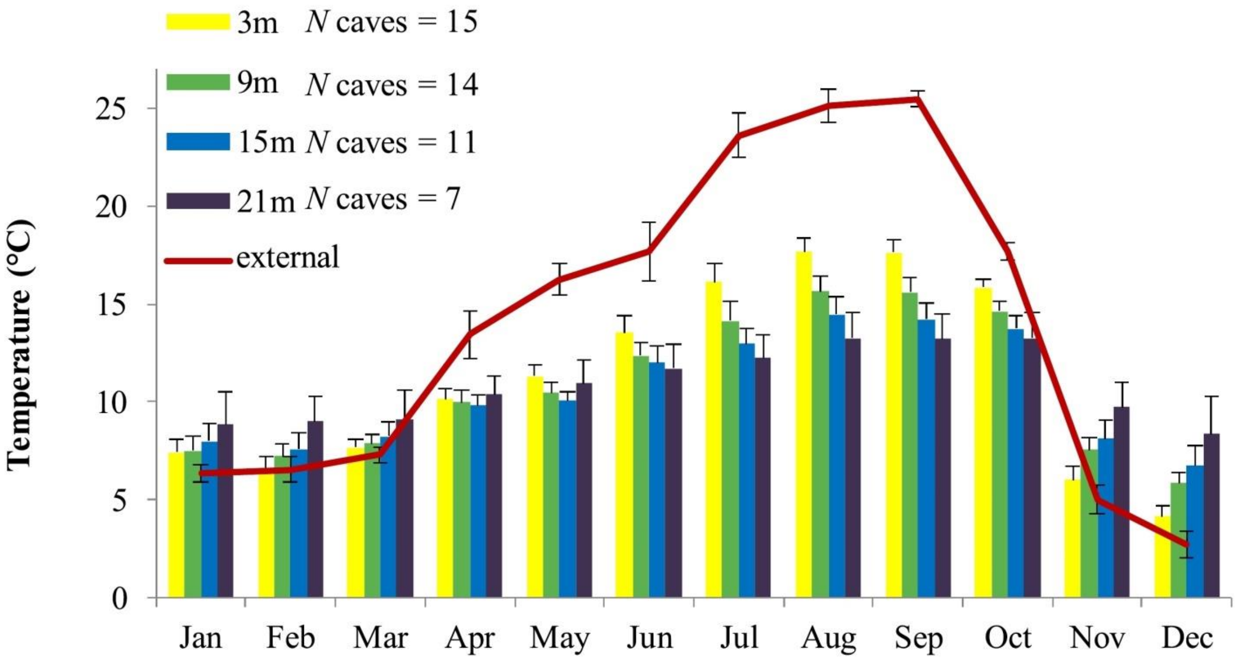

Fig. 1. Annual operative temperatures of cave habitats inhabited by *Speleomantes italicus*, modified from Lunghi *et al.* (2015).

An additional realization that stems from the existence of high similarity and narrow thermal niche breadths among salamanders is that especially under temperate conditions *Bsal* outbreaks may occur throughout the year, rather than in specific seasons (Bozzuto & Canessa, 2019). Salamander reproductive activity promotes *Bsal* spread and mainly takes place during the shoulder seasons, which increases risk for disease spread (Stegen *et al.*, 2017). Nevertheless, climatic conditions remain suitable for *Bsal* growth throughout summer and winter in our study area presented in Chapter 5.

Finally, to gain better insight into thermal disease dynamics between *Bsal* and salamander hosts across Europe it is also critical to obtain better understanding of *Bsal* phenotypic plasticity, and the true width of its thermal niche. Specifically, within its native range, *Bsal* has been found on the skin of Vietnamese *Tylototriton* and *Paramesotriton* newts that inhabited ponds and streams in subtropical forest ranging between 20- 26.5°C. Such temperatures would be lethal to the *Bsal* strain that emerged in the Netherlands (Martel *et al.*, 2013), but, perhaps not to other strains that originate from other introductions, such as that in Catalonia, Spain.

II. Risk assessments – where to go from here

Early responses to *Bsal* emergence in Europe included creation of ecological niche models and epidemiological models, which were used to predict pathogen spread and estimate its impact on host populations, respectively (Feldmeier *et al.*, 2016; Schmidt *et al.*, 2017; EFSA Panel on Animal Health and Welfare *et al.*, 2018; Katz & Zellmer, 2018; Bozzuto & Canessa, 2019; Chapter 4). Application of such models is challenging, as usually much remains unknown about the ecology of a pathogen once it emerges (Peterson, 2006; Desprez-Loustau *et al.*, 2007; Hay *et al.*, 2013; Tompkins *et al.*, 2015). This lack of information often necessitates model building based on various assumptions, which in case of *Bsal* results in ongoing uncertainty about pathogen invasiveness and environmental invasibility (Feldmeier *et al.*, 2019). To improve future risk assessments it is hence valuable to summarize what has been learned up to now, and what knowledge is still missing. I do so below with a particular focus on ecological niche models, as these have been frequently used to model habitat suitability for *Bsal*.

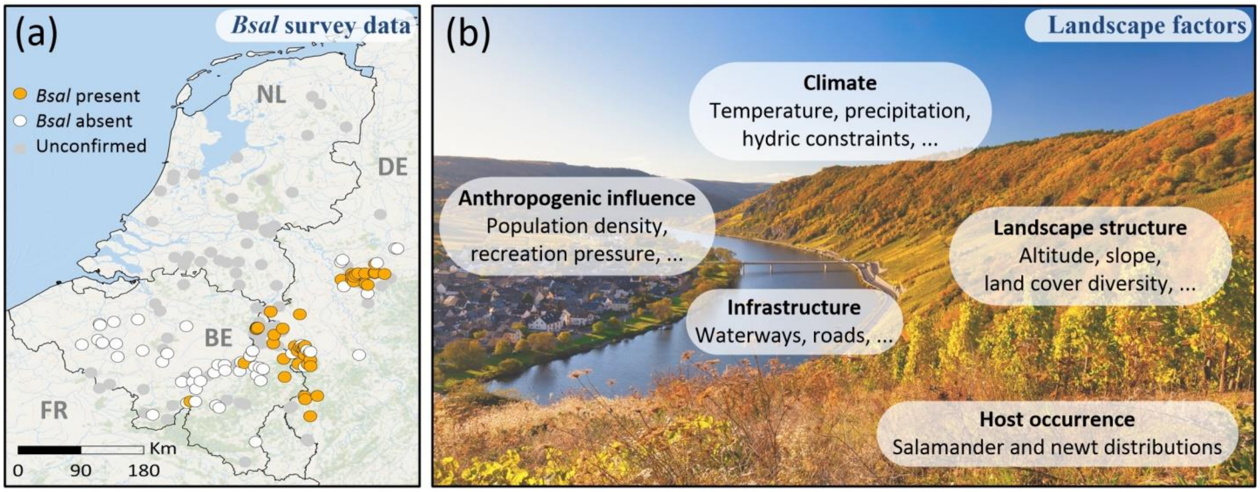

Fig. 2. An overview of *Bsal* presence-absence data created in early 2020 (a), and a summary of factors that could drive or constrain the presence of this pathogen at a landscape scale (b).

Ecological niche models have been fitted to *Bsal* distribution data to i) identify climatic drivers and estimate regional invasibility (Feldmeier *et al.*, 2016), ii) in the context of a criticised exercise to produce models with restricted input data (Katz & Zellmer, 2018; Feldmeier *et al.*, 2019), iii) to estimate continent-wide invasibility (EFSA Panel on Animal Health and Welfare *et al.*, 2018), and iv) in our Chapter 4 to assess if and how differences in native and invasive bioclimatic niches may influence risk assessments. Most of these approaches were parameterized only with climate data, and across study areas of varying extent, which may influence model outcome (Soberón & Nakamura, 2009; Elith *et al.*, 2010; Lobo *et al.*, 2010). These models provide valuable preliminary information on where climate may allow for further *Bsal* spread and colonisation, relative to already occupied conditions. Nevertheless, it is important to realise that an empirical assessment of landscape invasibility to *Bsal* throughout the wider northwest European infection front remains outstanding, as exploratory predictions have restricted to the immediate, original outbreak area (Feldmeier *et al.*, 2016). Furthermore, little attention has been given to the relative contribution of environmental factors other than climate in driving or inhibiting the *Bsal* advance.

Two approaches may be beneficial in improving invasibility estimates to *Bsal* in the Western Palearctic. First, given that the results in Chapter 4 revealed that considerable differences exist between the native and invasive *Bsal* niches, it would be possible to accommodate uncertainty on further niche filling by informing local predictions with a model of the global *Bsal* niche. This approach is increasingly applied in invasive species distribution modelling, where it has been proven to benefit model predictive accuracy (Dullinger *et al.*, 2009; Jiménez-Valverde *et al.*, 2011; Gallien *et al.*, 2012; Václavík & Meentemeyer, 2012). It is however possible that a global niche may not account for local environmental conditions that shape the invasive range (e.g. Dullinger *et al.*, 2009). Also, it remains unknown if the *Bsal* strain(s) that emerged in Europe are able to attain the full range of conditions occupied across the wide native distribution in East-Asia (Martel *et al.*, 2013; Laking *et al.*, 2017). Fitting a realized regional niche model using increased *Bsal* distribution data and a wide range of environmental parameters may therefore be valuable to better understand which factors drive or constrain this pathogen at landscape scale (Lötters *et al.*, 2018; Schulz *et al.*, 2018; Wagner *et al.*, 2019; Fig. 2)

Future epidemiological models may particularly benefit from a focus on host assemblages rather than single species, as already put forward by Canessa *et al.* (2019). Recent research on differences in host susceptibility, disease-related switching between terrestrial and aquatic habitats in newts, and potential of the midwife toad *Alytes obstetricans* to act as disease reservoir has clearly demonstrated that chytridiomycosis outbreaks at local level are influenced by many more factors than just climate (Stegen *et al.*, 2017; Daversa *et al.*, 2018b). Finding out how these factors work in synergy will be critical to development of strategies that mitigate the impact of *Bsal* on salamander diversity.

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Summary

Summary

Amphibian biodiversity worldwide is under threat by environmental change. The role of emerging infectious disease in this crisis is significant, and grows as globalisation increasingly facilitates pathogens to emerge beyond their native range. A recent example is formed by *Batrachochytrium salamandrivorans* (*Bsal*), an East-Asian chytrid fungus that has become established in Europe. While *Bsal* appears to occur in stable co-existence with salamander hosts in its native range, infection leads to death in most non-Asian salamanders. *Bsal* outbreaks in the invasive range may cause population decline, and can lead to local extinction of salamander populations. Currently, *Bsal* threatens an increasing number of salamander populations in Belgium, the Netherlands, and Germany. A considerable risk for further spread exists as this pathogen is able to persist in the environment for extended periods without hosts.

In order to mitigate the impact of *Bsal* it is crucial to gain practical understanding of its disease ecology, which may be used to direct further research and prioritize conservation efforts. The aim of this thesis was therefore to assess the role of the environment, and particularly temperature, in shaping *Bsal* disease dynamics in north-western Europe. Emphasis was furthermore given to improve spatial predictions of *Bsal* spread. After introducing the focal pathogen and host, the fire salamander *Salamandra salamandra* (Chapter 1 and 2), I first provide an overview of the invasive *Bsal* distribution (Chapter 3). These data are used together with climate parameters to generate the invasive and native bioclimatic niches of *Bsal*. By calculating overlap between these, and niches of all Western Palearctic salamanders, I explored how differences in climate and niche structure may affect predictions of pathogen impact (Chapter 4).

Finally, I zoom in to local disease dynamics to assess if fire salamanders can use temperature variation in their habitat to fight *Bsal* infection. To this end, temperature selection of these salamanders before and after contact with *Bsal* was measured under controlled conditions. Temperature selection of wild fire salamanders, under natural conditions, was then mapped during three-year period through fieldwork and a mesocosm experiment. As a final step, the findings were extrapolated across Europe using a mechanistic model of fire salamander body temperature. By adding information about the thermal growth rate of *Bsal* at different temperatures to this model, a refined spatial prediction of *Bsal* invasion risk was generated (Chapter 5).

Despite several decades of research on chytridiomycosis it remained ambiguous how many amphibian species have been affected by this disease. In **Chapter 1** we therefore combined evidence in the form of

published data, IUCN assessments and expert opinion to classify declined species into severity categories based on reductions in abundance. We found that at least 501 amphibian species have been affected by chytridiomycosis, of which 90 (18%) are confirmed or presumed extinct in the wild, with a further 124 (25%) experiencing a >90% reduction in abundance. These results showed that decline severity was greatest for larger-bodied species, those occurring in consistently wet regions, and those strongly associated with perennial aquatic habitats. Declines were less severe for species with large geographic and elevational ranges. We conclude that even though some species show signs of recovery, considerable risk for further outbreaks remains.

The invasion of *Bsal* goes hand in hand with precipitous declines among populations of fire salamanders *Salamandra salamandra*, which are therefore increasingly used as models to assess or predict the impact of this pathogen. Fire salamanders are however not just interesting in the context of disease research. In fact, members of the genus *Salamandra* display extensive phenotypic polymorphism in body sizes, color patterns and reproductive modes, and therefore constitute fascinating subjects for a plethora of biological studies. In **Chapter 2** we take a popular-scientific approach to introduce these animals in the form of a Current Biology quick guide.

Even though the discovery of *Bsal* in the Netherlands was quickly followed by its detection in adjacent Belgium, it remained unknown if this pathogen had spread to other areas as well. In **Chapter 3** we therefore present results of a series of field surveys performed over a 6-year period, which reveal *Bsal* to be present at various sites in the southern Netherlands, the German Eifel, and eastern Belgium. In addition to fire salamanders, we found that alpine newts *Ichthyosaura alpestris* may fall victim to *Bsal* as well.

We used the previously gathered distribution data to map the invasive and native bioclimatic *Bsal* niches in **Chapter 4,** through ordination in PCA-bound environmental space. We then used overlap tests to compare these niches with those of 56 putative Western Palearctic host species, and built ecological niche models for each *Bsal* niche to assess if, and how differences between niches influenced predictions of *Bsal* spread. Our results show that the invasive *Bsal* niche is a conservative, partially filled subset of its native niche. Pathogen-host overlap measured using the narrow invasive niche was nevertheless found to be significantly higher than that of the native niche, which is partly situated in subtropical conditions absent from the Western Palearctic. Ecological niche models created using the native niche predict high suitability for *Bsal* throughout Europe. Conversely, a more restricted range was predicted using the invasive niche, which coincides with presence of oceanic climates in north-western Europe and several

Mediterranean mountain ranges. We conclude that unequal relative availability of environments in native and invasive ranges along with partial niche filling may lead to uncertainty when creating predictions of potential pathogen spread. *Bsal* is nevertheless already present in conditions shared by numerous European host species, and thereby poses considerable risk to salamanders beyond its invasive range.

In the final **Chapter 5** we zoom in to local level to study how local microclimates steer the ability of fire salamanders (*Salamandra salamandra)* to fight the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) using thermal behaviour. Laboratory trials reveal that not behavioural fever, but salamander thermal preference can inhibit *Bsal* infection and facilitate disease recovery, but only under humidity saturated conditions. Yet, a three-year field study and a mesocosm experiment demonstrate absence of such antifungal thermal behaviour under natural conditions. A mechanistic model estimating fire salamander body temperatures year-round predicts equally low environmental conduciveness to antifungal behaviour throughout the species' wide European range. Our results show that amphibian innate defences against epizootics may remain constrained in the wild, which predisposes to range-wide disease outbreaks and population declines.

Samenvatting

Samenvatting

Amfibieën worden wereldwijd bedreigd door veranderingen in hun leefomgeving. Infectieziekten spelen een aanzienlijke rol in deze crisis, niet in de laatste plaats omdat globalisering steeds vaker leidt tot introductie van ziekteverwekkers in nieuwe gebieden. Een recent voorbeeld wordt gevormd door *Batrachochytrium salamandrivorans* (*Bsal*), een van oorsprong Oost-Aziatische schimmel die zich heeft gevestigd in Europa. Terwijl *Bsal* in het natuurlijke verspreidingsgebied vreedzaam lijkt samen te leven met salamandergastheren, leidt infectie met dit pathogeen tot sterfte bij de meeste niet-Aziatische salamanders. Een *Bsal* -uitbraak kan zodoende populatieafname veroorzaken, en tot lokaal uitsterven van salamander-populaties leiden. Op dit moment bedreigt *Bsal* een toenemend aantal salamander-populaties in België, Nederland en Duitsland. Het risico van verdere verspreiding is reëel omdat *Bsal* ook zonder gastheer lange tijd in de omgeving kan overleven.

Om de opmars van *Bsal* te stoppen is het cruciaal praktisch inzicht te krijgen in de ecologie van deze schimmel. Zulke kennis kan gebruikt worden om verder onderzoek aan te sturen, en om prioriteiten voor beschermingsmaatregelen te stellen. Het doel van dit doctoraat was zodoende na te gaan hoe de natuurlijke omgeving, en met name temperatuur, de ecologie van *Bsal* in Noordwest-Europa beïnvloed. Nadruk werd daarbij gelegd op het verbeteren van ruimtelijke predicties van *Bsal* -invasierisico. Na de introductie van de focale pathogeen en gastheer, de vuursalamander *Salamandra salamandra* (Hoofdstuk 1 en 2), werd eerst de *Bsal*-verspreiding in kaart gebracht (Hoofdstuk 3). Met behulp van deze distributiedata en klimaatparameters werden de invasieve en inheemse bioklimatische niches van *Bsal* gegenereerd. Door overlap te berekenen tussen deze niches en die van alle West-Palearctische salamanders werd nagegaan hoe verschillen in klimaat en niche-structuur invloed hebben op het bepalen van *Bsal*-invasierisico op regionale schaal (Hoofdstuk 4).

Ten slotte werd ingezoomd naar lokaal niveau om na te gaan in hoeverre vuursalamanders temperatuurverschillen in hun leefgebied kunnen gebruiken om *Bsal*-infectie te bestrijden. Hiertoe werd eerst temperatuurkeuze van deze salamanders voor en na contact met *Bsal* gemeten onder gecontroleerde omstandigheden. Vervolgens werd temperatuurkeuze van wilde vuursalamanders, onder natuurlijke omstandigheden, gedurende een periode van drie jaar in kaart gebracht middels veldwerk en een mesocosm-experiment. Als laatste stap werden de bevindingen geëxtrapoleerd naar geheel Europa op basis van een mechanistisch model van vuursalamander-lichaamstemperatuur. Door informatie over groeisnelheid van *Bsal* op verschillende temperaturen toe te voegen aan dit model werd een verfijnde ruimtelijke predictie van *Bsal*-invasierisico gegenereerd (Hoofdstuk 5).

Ondanks tientallen jaren onderzoek naar chytridiomycose bleef het onduidelijk hoeveel amfibieën door deze ziekte zijn aangetast. In **Hoofdstuk 1** combineerden we daarom bewijs uit publicaties, IUCN-beoordelingen en expert opinion om de achteruitgaande soorten in ernstcategorieën op te delen, op basis van reducties in abundantie. We ontdekten dat minstens 501 amfibiesoorten zijn aangetast door chytridiomycose, waarvan 90 (18%) uitgestorven zijn het wild, terwijl een verdere 124 (25%) een vermindering van ten minste 90% in abundatie hebben doorgemaakt. De resultaten toonden aan dat achteruitgang het grootst was voor relatief grotere soorten, soorten die in nattere gebieden voorkomen, en soorten die geassocieerd zijn met permanente aanwezigheid van water. Soorten die relatief grotere verspreiding bezetten en in zowel lage als hoge gebieden voorkomen worden minder sterk getroffen. We concludeerden dat, hoewel sommige soorten tekenen van herstel vertonen, er nog een aanzienlijk risico bestaat voor toekomstige ziekte-uitbraken.

De invasie van *Bsal* gaat hand in hand met een terugname van populaties vuursalamanders *Salamandra salamandra*, die daarom in toenemende mate worden gebruikt als model om de impact van deze ziekteverwekker te beoordelen of te voorspellen. Vuursalamanders zijn echter niet alleen interessant in het kader van ziektenonderzoek. Omdat leden van het geslacht *Salamandra* gekarakteriseerd worden door verregaand fenotypisch polymorfisme, bijvoorbeeld in lichaamsgrootte, kleurpatroon en voortplantingsgedrag, vormen deze dieren fascinerende subjecten voor een overvloed aan biologische studies. In **Hoofdstuk 2** pasten we een populair-wetenschappelijke benadering toe om deze dieren te introduceren in de vorm van een Current Biology Quick Guide.

Hoewel na ontdekking in Nederland *Bsal* ook snel in aangrenzend België gevonden werd, bleef het onbekend of deze ziekteverwekker zich ook naar andere gebieden had uitgebreid. In **Hoofdstuk 3** presenteerden we de resultaten van een reeks veldonderzoeken uitgevoerd gedurende een periode van 6 jaar, waaruit bleek dat *Bsal* aanwezig is op verschillende locaties in Zuid-Nederland, de Duitse Eifel en Oost-België. Naast vuursalamanders beschreven we dat alpenwatersalamanders *Ichthyosaura alpestris* ook slachtoffer kunnen worden van *Bsal*.

We gebruikten deze verzamelde distributiegegevens vervolgens in **Hoofdstuk 4** om de invasieve en inheemse bioklimatische *Bsal*-niches in kaart te brengen op basis van ordinatie in topologische ruimte. De mate van overlap tussen deze niches werd daarna vergeleken met die van 56 vermeende gastheersoorten in het West-Palearctisch gebied, waarna niche-modellen voor beide *Bsal* niches opgesteld werden om na te gaan in hoeverre verschillen in opbouw voorspellingen van de potentiële *Bsal*-verspreiding beïnvloedden. Onze resultaten lieten zien dat de invasieve *Bsal*-niche een conservatieve, gedeeltelijk gevulde subset van de oorspronkelijke niche is. De overlap tussen gastheren en de kleinere, invasieve niche is echter groter dan die van de wijdere inheemse niche, aangezien laatstgenoemde zich gedeeltelijk bevindt in subtropische omstandigheden die afwezig zijn in het West-Palearctisch gebied. Niche-modellen op basis van de inheemse niche voorspellen hoge habitatgeschiktheid voor *Bsal* in geheel Europa. Een kleiner potentieel leefgebied werd voorspeld op basis van de invasieve niche, dat zich beperkt tot oceanische klimaten in Noordwest-Europa en verschillende mediterrane bergketens. We concludeerden dat zowel ongelijke relatieve beschikbaarheid van omgevingswaarden binnen de inheemse en invasieve *Bsal*-verspreidingen als gedeeltelijke nichevulling kunnen leiden tot onzekerheid tijdens creatie van voorspellingen met betrekking tot potentiële uitbreiding van pathogenen. *Bsal* is niettemin al aanwezig in klimatologische omstandigheden die door vele Europese gastheersoorten worden gedeeld, en vormt daardoor een aanzienlijk risico voor salamanders in de rest van Europa.

In **Hoofdstuk 5** zoomen we in tot lokaal niveau. We kwantificeren temperatuurafhankelijk gedrag van vuursalamanders als reactie op contact met *Bsal*, en gaan na in hoeverre dit gedrag infectie kan tegengaan onder natuurlijke omstandigheden. Eerst brengen we gedurende drie jaar de thermische ecologie van een *Bsal*-gastheergemeenschap in kaart op basis van veldwerk en een mesocosm-experiment. De resulterende informatie wordt gecombineerd met experimentele gegevens over temperatuurafhankelijk gedrag van vuursalamanders tijdens *Bsal*-contact. Onze resultaten laten zien dat vuursalamanders brede, aangeboren variatie in temperatuurvoorkeur bezitten dat *Bsal*-infectie kan voorkomen, maar de benodigde temperaturen blijven in het wild op onze onderzoekslocatie gedurende het gehele jaar onbereikbaar. We extrapoleren vervolgens onze bevinden met behulp van een mechanistich model naar geheel Europa om na te gaan waar, en wanneer lokale klimaatomstandigheden het toelaten *Bsal* invasie tegen te gaan. De mogelijkheden hiervoor zijn beperkt, gezien habitatkeuze van salamanders over algemeen sterk overeenkomt. Dit kenmerk compromiteert het tegengaan van ziekte door middel van temperatuurafhankelijk gedrag, ondanks de potentie van dit soort reacties om infectie te verlichten.

Addendum

Addendum

I. Curriculum Vitae

Wouter Beukema was born on July 5, 1987 in Eindhoven, the Netherlands. He received his BSc degree in Wildlife Management from the Van Hall Larenstein University of Applied Sciences in Leeuwarden in 2010, largely based on research into the systematics, biogeography and conservation of Moroccan amphibians in collaboration with Cadi Ayyad University, Marrakesh. The basis for his current research was formed during an MSc in Geo-Informatics and Earth Observation (2010-2012) at the University of Twente, where he specialized in Natural Resource Management.

After working for a short time as an ecological advisor in the Netherlands, he took up a position as research assistant at the Cátedra Rui Nabeiro-Biodiversidade, University of Évora, Portugal. Fascinated by research into how wild animals react to changes in their environment, and how the environment can enhance or limit these responses, he started a PhD in 2015 at Wildlife Health Ghent, part of the Department of Pathology, Bacteriology and Poultry Diseases at Ghent University. This study was financed through a predoctoral fellowship from the Research Foundation – Flanders (FWO).

Wouter Beukema is author or co-author of 41 scientific publications and reports. He was a speaker at various symposia and actively participated in several national and international conferences.

II. Selected publications

Please refer to Google Scholar for a full publication overview; https://scholar.google.com/citations?user=vewDCdQAAAAJ

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These four years have flown by. It feels like yesterday that I arrived in Ghent, ready to set out on a new adventure. It has been fascinating (and still is!) to work on such a timely topic as *Bsal*, not in the least because this took place at the forefront of wildlife disease research. In addition to my main topic I was also able to contribute to Flemish and global chytridiomycosis assessments, and continued collaborating on several other research projects, most notably Iberian lizard resilience to climate change. Finally, together with a passionate team of authors and a talented artist we also published a field guide on European reptiles and amphibians during this period, which is now available in four languages. Finishing this PhD therefore feels like the end of a chapter in more than one way. Indeed, the roots that formed the basis of some work included here stretch back a decade – and many people are to be thanked for their involvement, help, and support.

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During my PhD, I experienced how our research group at the Faculty of Veterinary Medicine grew from just a few people into the much larger Wildlife Health Ghent that we are now. This was quite a rollercoaster, during which I particularly enjoyed spending time in an international environment with people from so many different backgrounds. Our trip to Han-sur-Lesse was probably the best example of how fun and stimulating this can be. A massive thank you goes to past and present members including Mark, Elin, Lieze, Gwij, Tijn, Keely, Alex, Queenie, Zhimin, Moira, Jesse, Robby and of course Stefano and Annemarieke, for making this a memorable journey. In de afgelopen jaren heb ik amper tijd doorgebracht op de bacteriologie – ik stond vanaf het begin van mijn doctoraat vaker ’s nachts in het veld dan overdag in het lab. Niettemin, dikke merci aan Marleen, Gunter, Jo en Koen voor alle logistieke hulp en positiviteit. Sarah, misschien was het voor jou all in a day’s work, maar je hulp en inzet hebben me in de afgelopen jaren enorm geholpen!

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