Trends in Parasitology

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REVIEWS

Review

Environmental Factors and Host Microbiomes Shape Host–Pathogen Dynamics

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Microorganisms are increasingly recognized as ecosystem-relevant components because they affect the population dynamics of hosts. Functioning at the interface of the host and pathogen, skin and gut microbiomes are vital components of immunity. Recent work reveals a strong influence of biotic and abiotic environmental factors (including the environmental microbiome) on disease dynamics, yet the importance of the host–host microbiome–pathogen–environment interaction has been poorly reflected in theory. We use amphibians and the disease chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* to show how interactions between host, host microbiome, pathogen, and the environment all affect disease outcome. Our review provides new perspectives that improve our understanding of disease dynamics and ecology by incorporating environmental factors and microbiomes into disease theory.

Highlights

In many cases, interactions among host, pathogen, and environment are insufficient to describe disease dynamics in the wild.

The host microbiome plays an important role in host immunity, at the interface between the host and the pathogen, and affects disease outcome.

The environment, as well as environmental microbiomes, influence the host microbiome, and also influence simultaneously the host and the pathogen.

The three-edged disease triangle needs to become a four-edged disease pyramid to study interactions among host, host microbiome, pathogen, and environment.

From Disease Triangle to Disease Pyramid

Directly transmissible and vector-borne pathogens, which are affected by global change, are increasingly seen as risks to humans and wildlife [1,2]. Despite considerable research effort, human malaria continues to be a major health issue for millions of people, and predicting future range expansion with expected climate changes has proved problematic; yet, for other diseases, predictions of how climate changes may alter dynamics is improving [1,2]. For example, the tiger mosquito *Aedes albopictus*, transmitting the Chikungunya viral disease, is currently expanding its geographic range across Europe and the Americas, putting millions of humans at risk. In livestock, the vector-borne bluetongue disease has emerged in northern Europe in response to climate change and is causing the death of millions of animals at massive financial costs [3].

A clear indication that host–pathogen interactions are responding to global change is supported by the global increase of **emerging infectious diseases** (see Glossary) and **re-emerging infectious diseases** of human, wildlife, and plant hosts posing threats to biodiversity and public health [4]. Impacts of the environment on host–pathogen interactions can be subtle and can vary across years. In 2010, a thermal anomaly occurred in Curaçao, causing a dramatic increase in white plague disease and ciliate infection in Caribbean coral *Diploria labyrinthiformis* [1]. In a vastly different system, Clare and colleagues demonstrated that seasonality, in particular the timing of spring ice-thaw of a Pyrenean montane lake, affects the susceptibility of host amphibians to infection by the emerging pathogenic fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) [5]. These examples illustrate how disease risk and dynamics may result from (at least) three factors: the host, the pathogen, and the environment.

In disease ecological theory, a ‘disease triangle’ is a concept that illustrates the outcome of the dynamic interactions consisting of hosts, pathogens, and the environment [6] (see also [7]) (Box 1). It was formalized in plant pathology because plants cannot move to escape unfavorable environmental conditions. We believe that the concept is useful to better understand disease

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dynamics in human and wildlife diseases in a rapidly changing world. It considers the environment in its large sense, including abiotic conditions (e.g., temperature, moisture, pH, soil composition, solar radiation, seasonality, water chemistry), as well as biotic conditions (e.g., social environment, community composition, sex ratio, vegetation cover, abundance and distribution of vectors and intermediate hosts).

In spite of its explanatory power, the disease triangle represents an oversimplified view of the dynamics of infectious diseases; and thus, we must broaden the original concept to include an additional and often neglected aspect of the complex system: the diverse **microbiomes** of the environment and the host. The advent of next-generation sequencing has provided cost-effective ways to describe the highly diverse world of **microorganisms**. We are only beginning to appreciate the distribution, abundance, and diversity of microbial communities, the full extent of which, however, remains highly controversial [8]. The global number of bacteria and archaea are estimated as ~10^{30}, the atmosphere contains ~10^{22} microbial cells, and terrestrial and marine environments each contain ~10^{29} microorganisms [2]. A human body harbors at least 10^{14} microbial cells and 10^{15} viruses [9], with the human gut microbiome alone containing ~1000 bacterial species, but only 150 to 170 predominating in any one person [10]. New data are helping to describe their functional and ecological capacities, as well as the nature of their physiological interactions with hosts [2,9] (Figure 1).

Microbiota and microbiome research has identified microbial communities as important contributors to ecosystem, wildlife, and human health [2,9,11]. We now understand that microbial communities maintain ecosystem health by influencing the global food web, including agriculture, nutrient cycling, nitrogen retention, and CO_2 sequestration [2] (Figure 1). Further, by maintaining ecosystem health, they also contribute to plant, wildlife, and human health. Host microbial communities (i.e., **host microbiome**) can be considered part of the extended phenotype of host defenses. The gut microbiome is involved in energy harvest and storage, brain functioning, development of the intestine and immune system, and susceptibility to disease [9,11]. Not only

Box 1. The Disease Triangle and Former Disease Concepts

The disease triangle concept was developed by Stevens [6] in the context of plant pathology. Disease is the outcome of a dynamic interaction between the host, the pathogen, and the environment, and is illustrated using an equilateral triangle (Figure I) where the gradients of host susceptibility (resistance and tolerance), pathogen pathogenicity (infectivity and virulence), and environmental conditions affect the disease outcome at the center of the triangle. The effect of disease is most intense at the center, where susceptibility, pathogenicity, and environmental conditions favor the pathogen over the host. Disease outcome can vary dramatically if environmental conditions, host resistance or tolerance, or the pathogen's pathogenicity change. Thus, a potentially infectious microorganism does not always invade a host, and a 'pathogen' can be neutral or even a beneficial mutualist under other conditions.

The disease triangle is quite similar to the concept developed by Snieszko for fish diseases, in which infectious disease occurs when a susceptible host is exposed to a virulent pathogen under proper environmental conditions [7] (Figure I). While the environmental microbiome is embedded in the 'environment' component of these models, they lack an important component of disease dynamics: the host microbiome.

Recently, the importance of microorganisms in shaping disease dynamic gained more attention and has been reflected in new theoretical models. Brucker and colleagues proposed to illustrate the interactions between the host, the host microbiome, and the pathogen with a triangle of interactions (response or causation) [17] (Figure I). In this model, the environment experienced by the host, the pathogen, and the host microbiome was not considered. Finally, the concept developed by Snieszko [7] between the host, the pathogen, and the environment was extended to include the microbial community of the pathogen, as the pathobiome concept arose [13] (Figure I). However, in this model, a clear distinction between the host microbiome, the pathogen microbiome, and the environmental microbiome was not made. Interestingly, in this model, the theories applying to hosts can also be applied to vectors of diseases, for example, mosquitoes. In this case, the disease is the consequence of an interaction between the vector, the pathogen, microbiome, and the environment (Figure I).

Glossary

Acclimation response: a reversible change of a physiological trait in response to an environmental change.
Emerging infectious disease: a disease that either has appeared and affected a population for the first time, or has existed previously but is rapidly spreading, either in terms of the number of individuals getting infected, or to new geographical areas. Emerging infectious diseases are caused by pathogens that are increasing in their incidence, geographic or host range, and virulence.
Holobiont: a symbiotic system, composed of the host and its microbial partners (bacteria, archaea, viruses, and eukaryotes).
Host microbiome: microbial communities living on (e.g., skin microbiome) or in (e.g., gut microbiome) the host. Skin and gut microbiomes are currently better described, but other host microbial communities exist, for example, in the mouth, nose, pharynx, and respiratory and urogenital tracts.
Microbiome: the communities of microorganisms including bacteria, yeast, fungi, protists, and archaea in combination with their genomes.
Microbiota: the collection of microorganisms that exists in a given environment, habitat, or host (inside or on the host).
Microorganisms: microscopic organisms that exist as single cells or cell clusters. They include bacteria, protozoa, archaea, microscopic fungi, and microscopic algae. We include here also viruses, which are microscopic but not cellular, although there is ongoing scientific discussion regarding their characterization as 'micro-organisms'.
OTU: 'operational taxonomic unit', used to refer to a cluster of DNA sequences of microorganisms, which are grouped by sequence similarity of a defined taxonomic marker gene present in their DNA.
Pathobiome: initially, the pathobiome concept was defined as the pathogenic agent integrated within its microbial community (i.e., the pathogen interacting with the environmental microbiome [13]). This concept has now evolved to host-associated microorganisms (prokaryotes, eukaryotes, and viruses) negatively impacting the health of the host, with the interaction between the host and its pathobiome inevitably moderated by the environment within the host and immediately surrounding it [14].

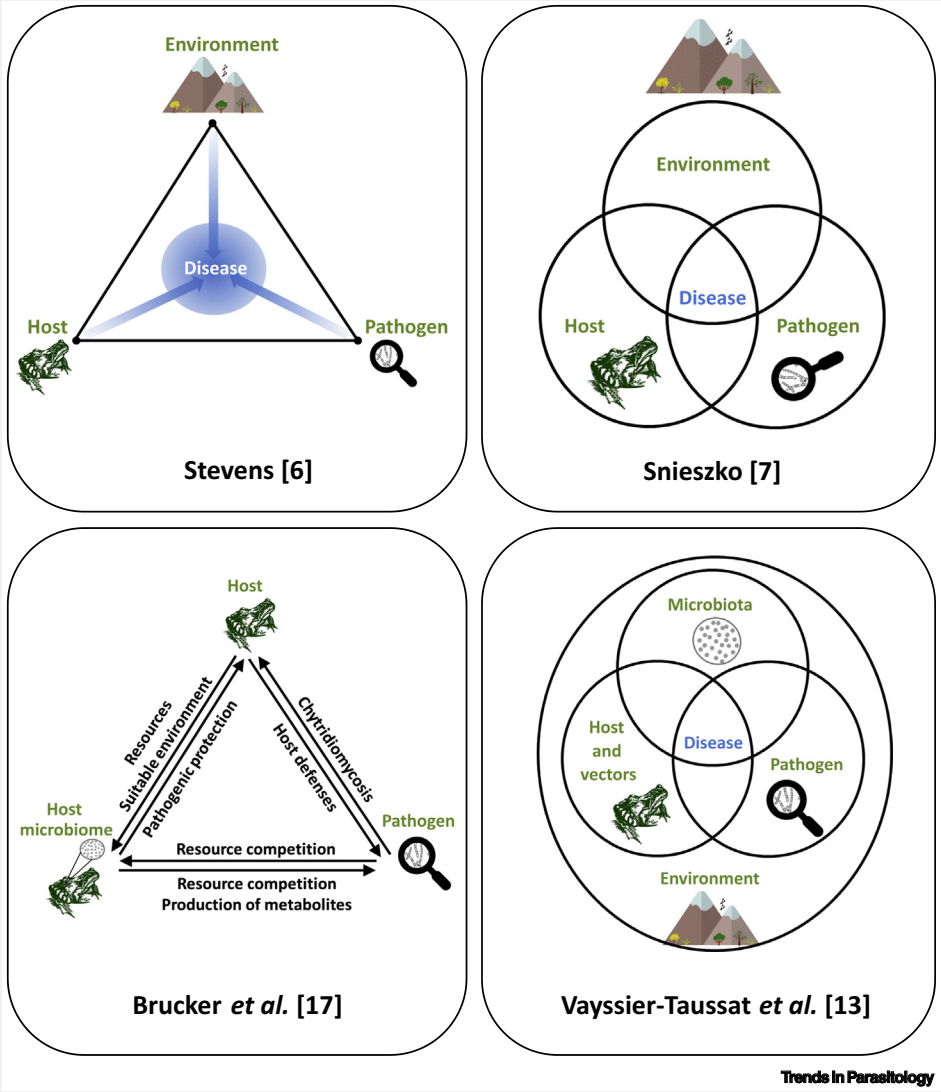

Figure I. Illustration of Former Models [6,7,13,17].

Phenotypic plasticity: the extent to which an organism can change its physiology, behavior, morphology, and/or development in response to environmental cues.

Re-emerging infectious disease: a known infectious disease increasing in incidence after a period of reduced incidence, or even disappearance, of the disease.

does the gut microbiome act as a barrier to infection by defending the host against colonization by pathogens, it also interacts with the host immune system, providing signals to promote maturation of immune cells [9]. The properties of the host microbiome have led to the concept of **holobiont** in which the host and its microbial partners are merged into a symbiotic superorganism [12], and later to the concept of **pathobiome** to further consider microbiome communities in disease dynamics [13,14].

In amphibians, as well as in other vertebrates, the skin microbiome is an integral component of the immune system, acting as a barrier to infection (Box 2). For example, the amphibian chytridiomycosis, a disease caused by the chytrid fungi *Bd* and *Batrachochytrium salamandrivorans*, does not occur simply when the pathogen is present and infects the skin of susceptible species but depends also on the composition of skin microbial communities

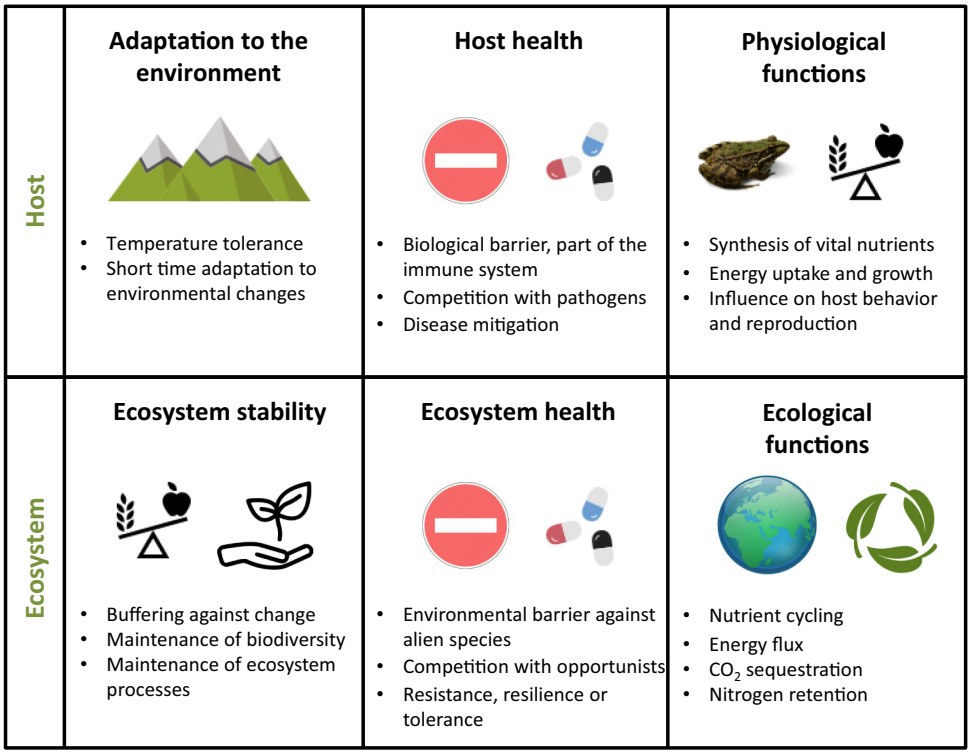

Figure 1. The Importance of the Microbiome to Hosts and Ecosystems. The host microbiome allows the host to adjust to its environment, provides protection against pathogens, and contributes to physiological functions (nutrition, growth, and reproduction). Similarly, the environmental microbiome promotes ecosystem stability and the maintenance of biodiversity by preserving ecosystem health and contributing to important ecological functions (e.g., nutrient cycling, energy uptake, carbon sequestration, and nitrogen retention). Host and environmental microbiomes are interconnected and regularly exchange microorganisms.

[15–17]. Generally, a better understanding of microbiomes in disease dynamics and their appreciation in disease ecology will contribute to disease mitigation.

Here, we review and illustrate the dynamic interaction between the host, the host microbiome, the *Bd* pathogen, and the environment (including the environmental microbiome) (Figure 2, Key Figure) using the well-studied case of the disease chytridiomycosis caused by *Bd*. This four-way interaction highlights the need to adopt a holistic approach to understand disease dynamics and ecology. Only when considering microbiomes in such a holistic approach will we be able to evaluate the impact of a changing environment on ecosystem, wildlife, and human health and overcome limitations of current concepts.

Variety and Roles of Microorganisms

In 1546, Fracastorius suggested that infection is contagious and transmitted by 'particules'. Microscopic organisms were discovered a century later, by Hooke and van Leeuwenhoek who described respectively the microfungus *Mucor*, and 'animalcules' (i.e., protozoa and bacteria). In 1857, Pasteur postulated that infectious diseases are caused by a variety of 'germs' that are everywhere, even in the air. Microorganisms can be found in all habitats (soil, water, air), in the intestines of hosts, as well as on the exterior of organisms [18]. Host-associated microorganisms can be pathogenic, but also can be commensal or beneficial symbionts.

Microorganisms play important ecological roles. Bacteria help to control host populations as they can be pathogenic, benign, or beneficial to hosts (e.g., helping in the digestion of food and the protection of hosts against infections) [19]. Archaea provide major pathways for ammonia oxidation in the environment, in methanogenesis, in sulfur oxidation and in nitrification [20]. Many fungi are also microscopic and are important in nutrient cycling, decomposition, and growth control of other organisms [21,22]. Parasitic and pathogenic fungi cause mycoses, which are increasingly seen as threats to biodiversity [4].

The community of microorganisms is usually described by marker genes and, in rarer cases, their full genome, that is, the microbiome, a term usually used when they are associated with a host individual or species [23]. The composition of the microbiome may vary between species, body areas, and geographic regions. The microbiome performs some main functions, such as disease mitigation [15–17,24], digestion, and has an influence on host behavior, development, and reproduction [25–27] (Figure 1). The microbial taxa shared between the majority of individuals within an animal species is called the core microbiome, and it is probably the main responsible component for performing these essential functions [28]. Perturbations in the composition and function of the microbiome (known as dysbiosis) could interrupt its regular functions and are suggested as a cause for some diseases [14].

The Model System: Amphibian Hosts and *Bd*

In a world where a quarter of all species are threatened with extinction [29], amphibians are the most threatened vertebrates, with rapid population declines detected in 43% of the

Box 2. The Amphibian Immune System

The immune system of amphibians is similar to that of other vertebrates [24] and is complex. It includes three components: barriers (constitutive defenses), innate response, and acquired (adaptive) response (Figure I), whose functions partly overlap.

The first line of defense consists of (i) a physical barrier comprising an epithelium, such as skin or gut surface, covered by mucus (a matrix of mucopolysaccharides secreted by the mucous glands and complemented by antimicrobial peptides (AMPs), secreted by the granular glands), (ii) a biological barrier (i.e., skin and gut resident microbiomes), and (iii) a chemical barrier (e.g., the acidic pH of the gut, AMPs, and lysozyme in the mucus, and antimicrobial metabolites produced by the resident microbiome). Amphibians use their skin to breathe (many species lack lungs) and to maintain homeostasis. Electrolytes and H_2O, O_2, and CO_2, both actively and passively cross the amphibian epithelium surface and the mucus that act together as a semiporous physical barrier. This increases the importance of its chemical and biological components when acting as a barrier against pathogens. Resident microbiomes, naturally living on the host epithelium, compete for space and resources with pathogens, presumably preventing them from adhering to the skin, proliferating, and entering the host [24]. Host microbiomes actively secrete antimicrobial metabolites, contributing to the chemical barrier that the host creates.

Pathogens that cross those barriers face the innate and adaptive immune response. The innate system includes lysozyme, complement lytic system, and AMPs. It also involves natural killer (NK) cells (which nonspecifically attack and lyse infected cells), and dendritic and macrophage cells (which phagocyte infected cells produce cytokines and activate B and T lymphocytes) [24].

The most advanced response is the adaptive (acquired) response, preventing reinfection. After the initial infection, this system provides a fast secondary response during subsequent exposure. When stimulated by an antigen, B lymphocytes produce antibodies which neutralize pathogens by agglutination, inducing complement activation, and tagging antigen for destruction by phagocytes. T lymphocytes need direct contact with an infected cell to eliminate it. They do not produce antibodies but regulate their production [24]. Several kinds of T lymphocytes exist, including regulatory T cells, T helper cells (which secret cytokines and coordinate antibody production), and cytotoxic T cells (which destroy pathogenic cells by physical and chemical lysis). However, some pathogens, including *Bd*, can suppress the adaptive response [91,92].

In larval amphibians (e.g., the tadpole stage), the immune system is not as well developed as it is in fully metamorphosed individuals, but it is still competent [24].

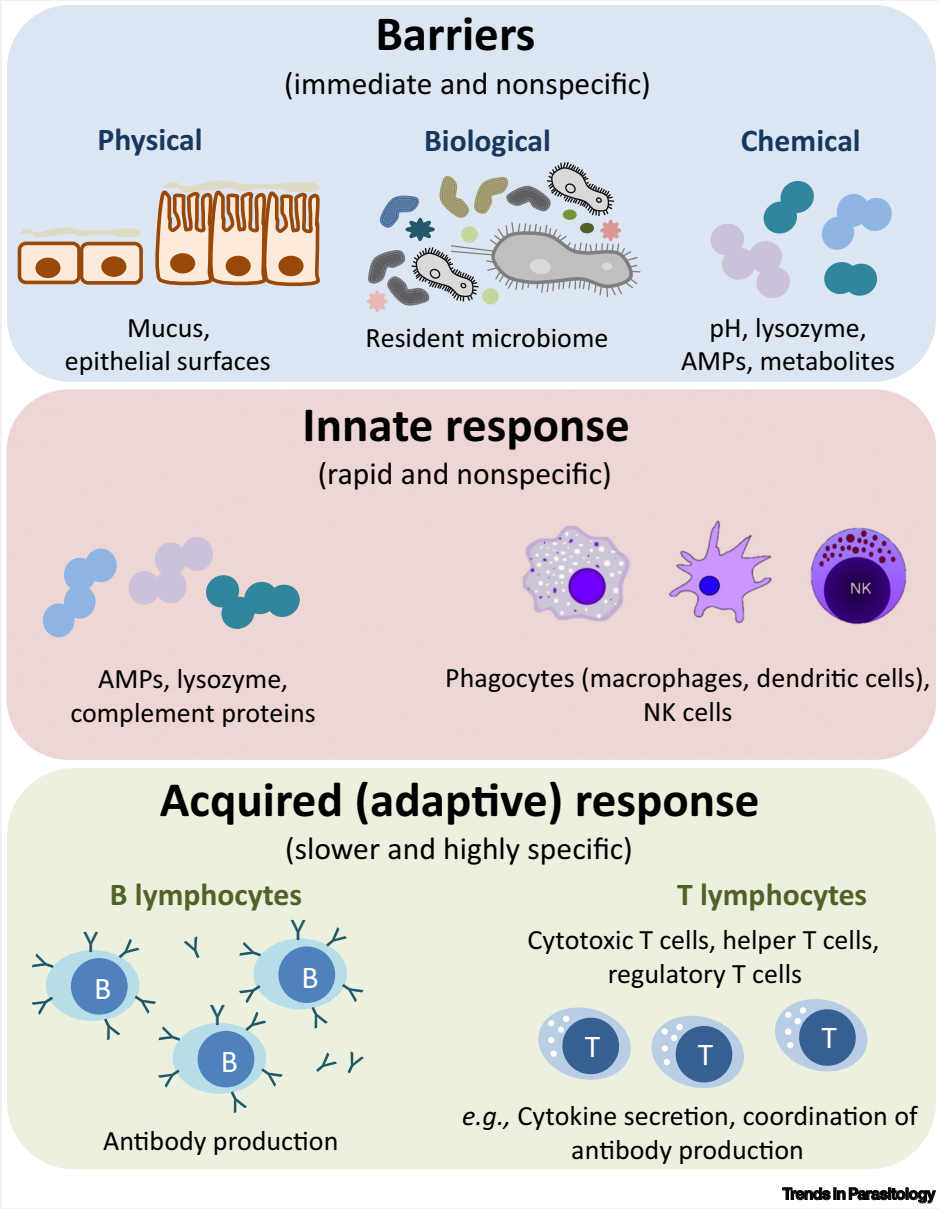

Figure I. The Three Components of the Amphibian and Vertebrate Immune System – Barriers, Innate Response, and Acquired (Adaptive) Response.

>8100 amphibian species described (https://amphibiaweb.org) [4,30]. Loss of amphibians has cascading effects throughout food webs and may alter environmental balance (e.g., water quality and pest control) [31,32]. In some systems (e.g., North American arboreal forests) amphibians are the most abundant terrestrial vertebrates and they facilitate important carbon cycle functions such as leaf litter retention and carbon capture [33], functions that are tied to global cycles.

Together with habitat loss and overexploitation, emerging infectious diseases are the major threats to amphibians. The fungus *Bd* is considered the most destructive pathogen for wildlife,

Key Figure

The Disease Pyramid: A Four-way Interaction between Host, Microbiome, Pathogen, and Environment

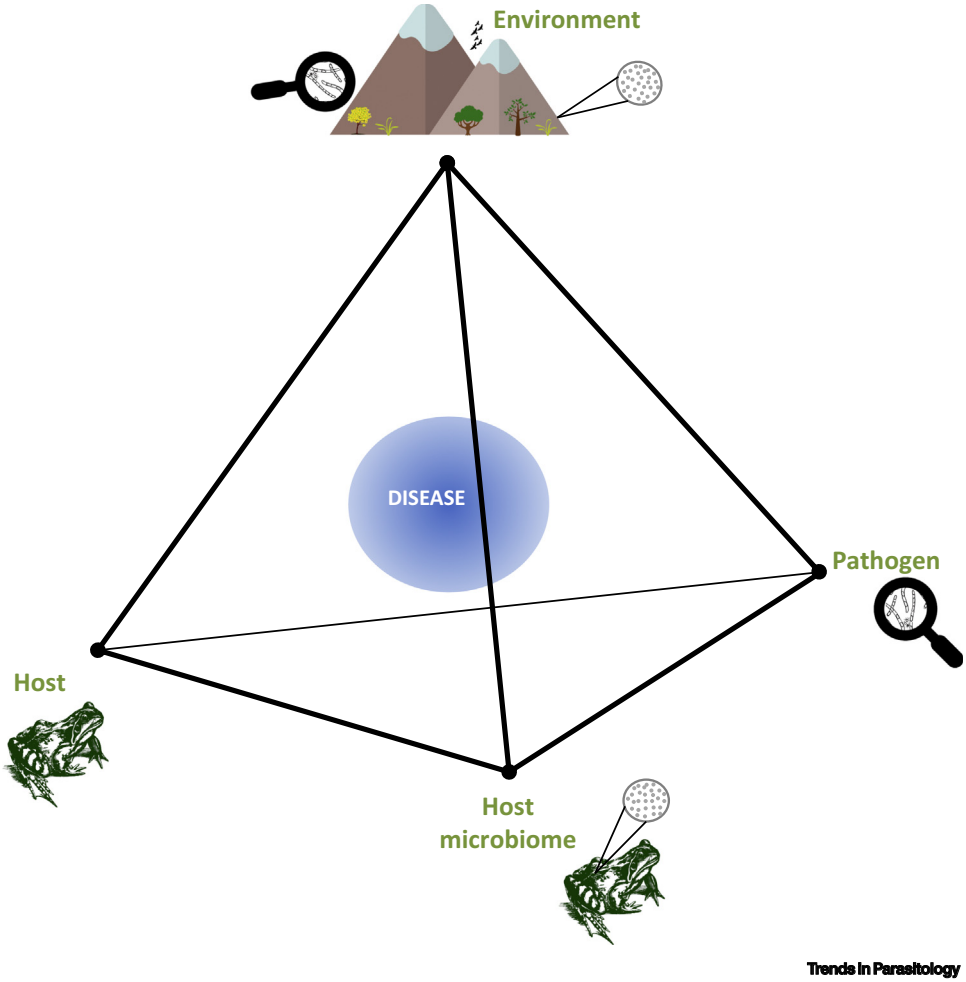

Figure 2.

For a Figure360 author presentation of Figure 2, see the figure legend at https://doi.org/10.1016/j.pt.2020.04.010

The pyramid contains a gradient of host susceptibility (from resistance and tolerance to high susceptibility), host microbiome permeability to the pathogen (from a complete barrier to a fully porous barrier), pathogen pathogenicity (from low to high infectivity and virulence), and environmental conditions (from poorly to highly favorable to the disease) that each feed towards the center of the pyramid to affect disease outcome. A permeable/porous host microbiome does not act as a barrier to pathogens; it allows pathogen establishment, proliferation, and invasion of the host. A given environmental condition interacts simultaneously and independently with the host, the host microbiome, and the pathogen in various ways, modifying each as well as their interactions with each other. The disease is more intense at the center, where the strongest susceptibility, permeability, pathogenicity, as well as the most favorable environmental conditions for the disease are found. Environmental conditions include biotic (e.g., environmental microbiome) and abiotic factors.

implicated in the decline of >500 frog species worldwide [34], and the extirpation of populations and entire species [35]. *Bd* pathogenicity is based on its ability to infect a vital organ, amphibian skin, which is permeable and maintains homeostasis. *Bd* disrupts these fundamental functions,

ultimately resulting in cardiac arrest [36]. It infects hosts as a motile zoospore form in water and colonizes a keratinized or keratinizing part of the host body [37]. In the host skin, *Bd* zoosporangia produce new zoospores that are released into the environment and can either reinfect the same host or infect a new host. Many susceptible amphibians are infected by *Bd* at the host larval stage and die during host metamorphosis, when host immunity is suppressed and keratinized skin cells spread over the body. *Bd* can establish itself in various environments and has been detected on all the continents that amphibians are known to occupy [35]. Moreover, amphibians, being ectotherms, their physiology, including their immunity, is environment-dependent. Finally, the amphibian skin microbiome has attracted considerable attention compared with other wildlife microbiomes, and therefore a large body of literature is available on the interactions between amphibian hosts, the pathogen *Bd*, skin microbiome, and the environment.

Amphibian Microbiome

In amphibians, external (skin) and internal (gut) microbiomes have been described. The amphibian gut microbiome includes protists and bacteria which stimulate growth [38]. The amphibian skin microbiome is composed of different groups, such as bacteria, fungi, and other microeukaryotes [39,40]. The origin of the amphibian gut and skin microbiome is not yet clear. There is some evidence that the amphibian microbiomes may result from both species-specific self-acquisition of microbes in the habitat, as well as social transmission, followed by selection of rare environmental microbiome taxa [15,41–47] (Table 1). Transmission of microorganisms, in this case bacteria, between individual hosts can be vertical or horizontal (for fungi and other microeukaryotes the process is still unknown). Vertical transmission occurs especially in species with parental care, when part of their microbiome is transferred by hosts to eggs and protects them [48]. Horizontal transmission is likely to occur during mating and during congregations of conspecifics or other species [49]. The environmental microbiome also contributes to the host microbiome. Individuals kept in an enclosed habitat are exposed to a poorer environment compared with specimens that live in natural habitats, translating to poorer skin microbiomes [50,51] (Table 1). Salamander larvae experimentally transferred from stream to pond had a modified skin microbial community, likely due to microbes gathering in their habitat [43]. In addition to environmental impacts on the microbiome, species-specificity of microbiomes has also been described [16,52,53].

Gut and skin microbiomes can be influenced by many host factors, including genetics, sex, behavior, and diet [15,53,54] (Table 1). Captive individuals of the Red-eyed tree frog (*Agalychnis callidryas*) that received a diet rich in carotenoid have greater species richness and abundance of skin bacteria compared with those with a carotenoid-free diet [55]. Host health status has been shown to influence the host microbiome, which in turn can influence disease dynamics [54,56,57]. Composition of the skin microbiome also varies with life stage: microeukaryotes are significantly more diverse and abundant in adults and subadults as compared with metamorphs and tadpoles, with a dominance marked by fungal taxa [58,59] (Table 1). During metamorphosis, amphibians undergo many changes: body shape, diet, habitat (from aquatic to more terrestrial), and skin cell composition. During that period, their immune system is suppressed, which apparently creates open niches, inducing a higher bacterial diversity [58] and facilitating the proliferation of, and infection by, opportunistic pathogens.

Environmental Impacts on Amphibian Immunity

In amphibians, as in all vertebrates, the first protection against pathogens is the physical, chemical, and biological barriers of the epithelium (Box 2). Amphibians have a permeable skin that allows exchange of gases, water, and electrolytes. The resident microbiome present on the skin is a dynamic community that competes with exogenous microorganisms for space and resources and can prevent pathogens from adhering to the skin (Box 2). The host

Table 1. Overview of Recent Studies Focusing on the Amphibian Skin Microbiome and Host-, Pathogen (*Batrachochytrium dendrobatidis*, *Bd*)-, Environment-Related Variables

Host species	Sampling site	Molecular marker	Target microbial group	Interaction	Main findings	Refs
Host–microbiome						
Anaxyrus boreas	Colorado, USA	V4 region 16S rRNA gene	Bacteria	Life stages	Skin communities changed across life stages	[59]
Agalychnis callidryas, Dendropsophus ebraccatus	Panama	V4 region 16S rRNA gene	Bacteria	Year, date of sampling	Skin communities differing across years on both species. Differences in relative abundance of key OTUs explained by rainfall	[47]
Host–microbiome–environment						
Acquisition/maintenance of microbiome						
Cymops pyrrhogaster	Breeding facility and pond in Japan	V4 region 16S rRNA gene	Bacteria	Wild/captive conditions	Wild individuals had more diverse communities, but similar richness in OTUs	[50]
Atelopus zeteki	Panama	V4 region 16S rRNA gene	Bacteria	Wild/captive conditions	Wild population had three times more unique OTUs	[51]
Plethodon cirineus	Controlled environment	V4 region 16S rRNA gene	Bacteria	Environmental soil vs. sterile media	Diversity across treatments and decreased in the sterile media	[42]
Plethodon cirineus	Controlled environment	V4 region 16S rRNA gene	Bacteria	Cage with or without bacteria reservoir	Bacteria related to antifungal isolates were more likely to persist on salamanders, regardless the environment	[93]
Plethodon jordani	North Carolina, USA	V4 region 16S rRNA gene	Bacteria	Host vs. environmental microbiomes	OTUs highly associated with salamanders tended to be absent/too rare in the environment	[94]
Rana catesbeiana, Notophthalmus viridescens	Virginia, USA	V2 region 16S rRNA gene	Bacteria	Host vs. environmental microbiomes	Relative abundance of OTUs shared by amphibians and environment was inversely related	[41]
Atelopus certus, Craugastor fitzingeri, Colostethus panamansis, Espadarana prosoblepon, Strabomantis bufoniformis	Serrania del Sapo, Panama	V4 region 16S rRNA gene	Bacteria	Host vs. environmental microbiomes	Microbiome communities were enriched with rare environmental OTUs, and high percentage of OTUs shared between frogs and habitat	[44]
Cryptobranchus alleganiensis alleganiensis	Indiana, West Virgiana, North California, Tennessee and Georgia, USA	V2 region 16S rRNA gene	Bacteria	Host vs. environmental microbiomes	Variation in community diversity among populations and in proportion of shared OTUs between animals and river	[95]
89 species of frog	Madagascar	V4 region 16S rRNA gene	Bacteria	Host vs. environmental microbiomes	Host microbial communities were different and less diverse than environmental ones	[96]
Rana marina	Puerto Rico and Costa Rica	V4 region 16S rRNA gene	Bacteria	Geographic location	Significant environmental influence in composition, richness, and abundance of microbiome taxa	[45]
genera *Ensatina* and *Batrachoseps*	California, USA	V3–V4 region 16S rRNA gene	Bacteria	Geographic location	Strong site differences in bacterial communities	[46]
Rana pipiens, Pseudacris triseriata, Ambystoma tigrinum	Colorado, USA	V2 region 16S rRNA gene	Bacteria	Altitudinal gradient	Host species community similarity	[52]
Eleutherodactylus coqui	Puerto Rico	V4 region 16S rRNA gene	Bacteria	Altitudinal gradient, intact/disturbed forest	Diversity changed with site, elevation, and land use	[97]
Lissotriton boscai,	Galicia, Spain	V4 region 16S	Bacteria	Life stage,	Terrestrial adults had more	[98]

Table 1. (continued)

Host species	Sampling site	Molecular marker	Target microbial group	Interaction	Main findings	Refs
Telmatobius marmoratus		rRNA gene		aquatic/terrestrial environment	diverse and richer bacterial communities	
Anaxyrus boreas, Pseudacris regilla, Taricha torosa, Rana catesbeianus	California, USA	V4 region 16S rRNA gene	Bacteria	Species, geographic location	Amphibian skin identity was the strongest predictor of microbiome composition	[16]
Plethodon glutinosus, P. cinereus, P. cylindraceus	Central Appalachians, US	V3–V5 region 16S rRNA gene	Bacteria	Species, altitudinal gradient	Diversity changed with elevation, and co-occurring salamanders had similar microbiome structure	[99]
Ensatina eschscholtzii xanthoptica	San Francisco and Sierra Nevada, USA	V3–V4 region 16S rRNA gene	Bacteria	Life stage, sex, geographic location	Isolated populations had similar communities, which were significantly different from the environment	[100]
Possible environmental stressors						
Pelophylax perezi	Portugal	Nearly full length of the 16S rRNA gene	Bacteria	Metal contamination, salinity	Low diversity and density on individuals from metal-contaminated population	[101]
Pseudacris crucifer	Controlled environment	V4 region 16S rRNA gene	Bacteria	Coal combustion waste	Little impact from acute exposure to fly ash on the bacterial communities	[102]
205 amphibian species	13 countries	V4 region 16S rRNA gene	Bacteria	Thermal stability, habitat class (aquatic, terrestrial, arboreal), elevation	Bacterial richness decreased in warmer and more stable environments, and in arboreal hosts	[68]
Atelopus zeteki	Controlled environment	V4 region 16S rRNA gene	Bacteria	Microbiome, *Bd*	Survival to *Bd* infection was related to initial composition of the skin bacterial community	[77]
12 amphibian species	Costa Rica	Nearly full length of the 16S rRNA gene	Bacteria	Microbiome, *Bd*	11% of the bacterial isolates collected from the species exhibited *Bd* inhibition and 2.2% enhanced *Bd* growth	[78]
Dendrobates sp.	Aquarium and animal-care facility, USA	V4 region 16S rRNA gene + ITS	Bacteria and fungi	*Bd* inhibition and enhancement	Abundance of cutaneous fungi contributed more to *Bd* defense than bacteria; different	[39]
Bombina orientalis	Controlled environment	V4 region 16S rRNA gene + V9 region 18S rRNA gene	Bacteria and micro-eukaryotes	Life stage, microbiome, *Bd*	Major change in community until 15 days after metamorphosis; richness diverged between aquatic and terrestrial stages	[103]
Anaxyrus boreas	Colorado, USA	V4 region 16S rRNA gene + V9 region 18S rRNA gene	Bacteria	Life stage, microbiome, *Bd*	Life stage had the largest effect on microbiome; diversity of micro eukaryotes was lowest in tadpoles	[58]
Rana sierra	California, USA	V3–V4 region 16S rRNA gene	Bacteria	Life stage, microbiome, *Bd*	Skin microbiome of highly infected juveniles had reduced richness and lower variation between individuals	[79]
Rana cascadae	Northern California, USA	V4 region 16S rRNA gene + V9 region 18S rRNA gene	Bacteria and micro-eukaryotes	Life stage, microbiome, *Bd*	*Bd* was significantly lower on tadpoles and highest on subadults	[40]
Lithobates yavapaiensis, Eleutherodactylus coqui	Arizona, USA and Puerto Rico	V4 region 16S rRNA	Bacteria	Life stage, season, *Bd*	Winter-sampled individuals exhibited higher diversity; hosts with higher bacterial diversity carried lower *Bd* loads	[104]

(continued on next page)

Table 1. (continued)

Host species	Sampling site	Molecular marker	Target microbial group	Interaction	Main findings	Refs
Craugastor fitzingeri, Agalychnis callidryas, Dendropsophus ebraccatus	Panama	V4 region 16S rRNA gene	Bacteria	Different species, *Bd*	Tree frogs had a significantly higher number of culturable *Bd*-inhibitory OTUs than terrestrial species	[105]
Rana catesbeiana, Notophthalmus viridescens, Pseudacris crucifer, Anaxyrus americanus	Virginia, USA	V4 region 16S rRNA gene	Bacteria	Different species, *Bd*	Dominant bacteria had higher *Bd* inhibition in bullfrog and newt. Dominant and rare bacteria did not differ in inhibition in spring peeper and toad, in which *Bd* was lower	[106]
Rana sierra	California, USA	V1–V2 region 16S rRNA gene	Bacteria	Resistant/non-resistant population to *Bd*, *Bd*	Different bacteria richness between resistant/non-resistant populations	[107]
Rana sierra	Controlled environment	V1–V2 region 16S rRNA gene	Bacteria	Resistant/non-resistant population to *Bd*, *Bd*	Frogs housed in water from resistant populations had greater bacterial richness than those housed in non-resistant population water	[56]
Rana sierra	California, USA	V1–V2 region 16S rRNA gene	Bacteria	*Bd* (epizootic/enzootic)	100% mortality of postmetamorphic frogs during *Bd* epizootic; several bacterial taxa showed the same response to *Bd* across multiple field populations	[108]
Rana catesbeiana	Controlled environment	V4 region 16S rRNA gene	Bacteria	Before/after exposure to *Bd*	Microbial community of frogs prior to *Bd* exposure influenced infection intensity	[109]
Anaxyrus boreas	controlled environment	V4 region 16S rRNA gene + V9 region 18S rRNA gene	Bacteria and micro eukaryotes	Four probiotic treatments, *Bd*	Amphibians in captivity lost the *Bd*-inhibitory bacteria; inoculations of the *Bd*-inhibitory probiotic increased survival	[110]
Host-microbiome-pathogen-environment						
Bombina orientalis	South Korea	V3 region 16S rRNA gene	Bacteria	Wild/captive conditions, *Bd*	*Bd* infection intensity was correlated neither with richness nor diversity indices; diversity was greater, and microbiome structure more complex in wild toads	[111]
Agalychnis callidryas, Dendropsophus ebraccatus, Craugastor fitzingeri	Panama and USA	V4 region 16S rRNA gene	Bacteria	*Bd*, geographic location	No clustering of OTUs based on *Bd* infection status	[53]
Agalychnis callidryas, Dendropsophus ebraccatus, Silverstoneia flotator, Craugastor fitzingeri, Rana catesbeiana, Pseudacris crucifer, Notophthalmus viridescens, Anaxyrus americanus	Panama and USA	16S rRNA gene database and ITS1	Bacteria and fungi	Species, *Bd*, geographic location	The host species was more important in determining microbiome composition than was geographic location or *Bd* load	[112]
Silverstoneia flotator	Panama	V4 region 16S rRNA gene	Bacteria	*Bd*, elevational gradient	Similar skin communities across elevations; richness varied with *Bd* presence; severe outbreaks occurred at high elevation	[85]
Rana sphenocephala	Controlled	V4 region 16S	Bacteria	*Bd*, controlled	Efforts to maintain a normal skin	[88]

Table 1. (continued)

Host species	Sampling site	Molecular marker	Target microbial group	Interaction	Main findings	Refs
	environment	rRNA gene		mesocosm	community using seminatural mesocosms failed to provide long-term protection	
Bolitoglossa (3 spp.), *Pseudoeurycea* (3 spp.), *Plectrohyla* (4 spp.)	Mexico and Guatemala	V3-V4 region 16S rRNA	Bacteria	*Bd*, forest type	Phylogeny influence of diversity and structure of microbiome at higher taxonomic levels; the habitat predominated on lower scales	[89]
Dendropsophus. minutus	São Paulo and Rio Grande do Sul, Brazil	V4 region 16S rRNA gene	Bacteria	*Bd*, land cover, forest connectivity	Bacterial diversity and *Bd* loads increased towards natural vegetation	[90]
Ichthyosaura alpestris, Lissotriton vulgaris, Triturus cristatus	Kleiwiesen and Elm, Germany	V4 region 16S rRNA gene	Bacteria	*Bd*, water temperature	Skin microbe fluctuations not correlated with fluctuations of pond microbiota; significant correlation between water temperature and newt bacterial community structure	[86]
Acris blanchardi	Ohio and Michigan, USA	V3 region 16S rRNA gene	Bacteria	*Bd*, pH, $CaCO^3$, conductivity, natural/managed habitat	Microbiome composition associated with water conductivity, ratio of natural to managed land, and latitude	[87]
Craugastor fitzingeri	Panama	Metagenome	Bacteria	*Bd* positive and negative sites	Bacterial communities in positive sites were less diverse than in negative ones	[113]

microbiome can also actively defend the host by producing antimicrobial peptides or metabolites. For example, *Janthinobacterium* spp., a symbiotic skin bacterium, produces the antifungal metabolite violacein [17]. Violacein was isolated from amphibian skin and transferred to the *Bd*-susceptible Mountain yellow-legged frog (*Rana muscosa*), resulting in a successful bioaugmentation that increased host survival [60].

Many factors are known to affect amphibian immunity, and thus host–pathogen interactions (e.g., Table 1). Temperature is likely the most important environmental factor that affects microbiomes. Since amphibians are ectotherms, all of their physiological responses are temperature-dependent, including immunity. Several studies have found that *Bd* infection probability and impacts of chytridiomycosis are higher at lower temperatures (e.g., [37,61,62]). For that reason it could be suggested that climate change may be beneficial for hosts that suffer from *Bd* infections. However, the interaction between the host, the pathogen, and the environment is complex. Climate change predictions suggest large increases in the variability of climatic conditions that may favor chytridiomycosis [63,64]. Controlled-temperature experiments on the Cuban tree frog (*Osteopilus septentrionalis*) revealed that the temperature-dependent growth of *Bd* on hosts was greater at warmer temperatures than the pattern of *Bd* growth in culture, emphasizing the importance of accounting for the host–pathogen interaction when predicting climate-dependent disease dynamics [64]. Another striking result was the demonstration that global warming is predicted to cause an increase in susceptibility to *Bd* in some amphibian species due to earlier ice-thaw of montane lakes in spring [5].

Besides temperature, additional environmental parameters that may alter amphibian immune responses include pH and chemicals in the environment. Studies have also shown that the presence of heterospecifics, intra- and inter-specific competition, predation, food availability,

and pond drying, can all cause stress in hosts that may suppress the immune response [24]. In the Pyrenees, the presence of zooplankton in lake water also protects amphibians against *Bd*, since zooplankton predation diminishes the population of the infectious stage (zoospores) of the pathogenic fungus [65]. The list of factors is not exhaustive, and we believe additional impacts of environmental conditions will be discovered as the impacts of human activities on the environment continue to increase.

Environmental Impacts on Host–Host Microbiome Interactions

The skin and gut microbiome can be seen as a miniature ecosystem at equilibrium. Just like any ecosystem, a stressor may alter the balance of the associated microbiome. As a response, the richness and composition of the microbiome may either resist or shift to a new stage. It may stay permanently at this new equilibrium as an **acclimation response**, or it may exhibit resilience and return to the former equilibrium. From the perspective of the host, when environmental modifications occur, the microbiome may exhibit flexibility. This flexibility might increase the host adaptive capacity by providing the host with higher **phenotypic plasticity**, compared with the one acquired only through the host genome or through a static gut/skin microbiome [66] (Figure 1). The bacterium *Serratia symbiotica*, for example, provides a reproductive advantage (higher fecundity) to the insect pea aphid after an event of heat stress, compared with control individuals [67]. Hence, the environment has the potential to influence the outcome of host–microbiome interactions because it can impact both the host and the host microbiome.

The composition, richness, and diversity of the host microbiome can be modified directly by biotic and abiotic environmental factors (Table 1). It can also be modified indirectly, through the host physiology, as the host–microbiome interactions create the environment in which the microbiome lives. A recent study analyzed samples from over 200 amphibian species (>2300 individuals) across a broad biogeographic range to investigate how climatic variables (temperature, precipitations, and seasonality), elevation, latitude, and microhabitat class relate to skin microbial communities [68]. Cold winter temperatures and seasonality were the best predictors of richness and composition of skin bacterial communities at the global scale. Latitude (with a decreased richness at lower latitudes) was also important, likely due to an intercorrelation between latitude and low temperatures [68]. Finally, skin bacterial richness was also influenced by microhabitat. Bacterial richness on amphibian hosts appeared higher in more seasonal environments, especially those with colder winters during which the amphibian immunity is suppressed for longer periods of time. Thus, there might be selection for low-temperature resistance in the amphibian skin microbiome, counteracting the low internal immunity of amphibians [68].

Additional environmental stressors of importance for the host–microbiome interaction include habitat degradation, ultraviolet radiation, environmental pollution, invasive species, and climate change [69–71]. In particular, climate change is predicted to impact many terrestrial and aquatic host species, and this may allow pathogens to spread into new geographic areas under optimal temperatures [72,73]. Habitat degradation, such as deforestation and fragmentation, decreases biodiversity in macro-organisms, and this pattern extends to their associated microbiomes [74]. Habitat degradation thus impairs vertical and horizontal transmission of microbiomes and induces lower microbiome skin diversity. Human activities can also modify the communities of environmental microorganisms [74]. For example, livestock grazing provides copious amounts of livestock feces, which can enrich the environment for microorganisms, or, on the contrary, can deposit antibiotics and antifungal compounds that degrade the habitat for natural microorganisms. Finally, other pathogens in the environment may also change the outcome of host–microbiome–pathogen interactions though alteration of the host immunity or condition, or through pathogen–pathogen competition for hosts [24].

Host–Host Microbiome–Pathogen Interactions

Microorganisms in the environment, including potentially pathogenic microorganisms, represent an important source of disturbance to the natural skin and gut microbiomes of hosts. These potential pathogenic microorganisms may also be favored or inhibited by the microbiome composition of the host and/or vector [75]. For example, the susceptibility of the mosquito *Anopheles gambiae* to *Plasmodium falciparum* infection is inversely correlated to the abundance of bacteria of the family Enterobacteriaceae in their gut [76]. In the *Bd*–amphibian system, there is a significant relationship between *Bd* infection and the microbiome composition of amphibian skin (e.g., [77–79]) (Table 1). Hosts with greater microbiome diversity are more resistant to this invasive pathogen, while populations that coexist with *Bd* for a long time have a higher proportion of anti-*Bd* skin bacteria than populations which are declining due to the disease and never had contact with *Bd* before [79,80]. This evidence confirms that pathogens can influence, and be influenced by, the amphibian skin microbial community.

Most research conducted so far on the amphibian skin microbiome focused on bacteria (Table 1), even though skin microbial communities also comprise eukaryotic microorganisms such as protists and fungi [39,40]. Two recent studies suggest that fungi might be more efficient or have a higher competitive capacity against other invasive fungi [39,58], opening new avenues on the role played by fungal communities in protecting amphibians against *Bd*. Bacterial viruses (phages), as key drivers for mortality and diversity of bacterial communities, represent another so far unexplored frontier in disease mitigation.

To our knowledge, research conducted on amphibian–host microbiome–pathogen interactions has been almost exclusively conducted on *Bd*. So far, only a few studies explored this interaction on different pathogens. A study on FV3-like ranavirus unravels the importance of habitat–microbiome interactions on disease outcome in the amphibian host *Rana temporaria* and supports the idea that the amphibian skin microbiome likely also protects amphibians against pathogens other than *Bd* [81]. In contrast, research on the newly discovered fungus *B. salamandrivorans* suggests that the skin microbiome of salamanders contains inhibitory bacteria at numbers too low to confer sufficient protection against the pathogen and even constitutes a source of opportunistic pathogens contributing to pathogenesis [82–84].

Environmental Impacts on Host–Host Microbiome–Pathogen Interaction

Due to recent technological breakthroughs, research on wildlife microbiomes is relatively new, and thus it is not surprising that the number of studies available to address the four-way interaction (the host–microbiome–pathogen–environment interaction) in disease ecology is very limited. Our review reveals that most studies have only considered the geographic location as a potential source of variation in the outcome of the host–microbiome–pathogen interaction (Table 1). Regarding abiotic factors, studies have investigated the effects of elevational gradient [85], water temperature [86], and pH, $CaCO_3$ and conductivity [87] on the skin microbiome of amphibians. Few studies take biotic factors, such as land cover, forest type, connectivity, and habitat management into account when comparing skin microbiomes of amphibians [87–90]. We expect future studies will include climatic variables, elevation, latitude, and microhabitat characteristics at a large geographic scale as is seen in Kueneman *et al*. [68]. Interestingly, to our knowledge, no study has considered the potential role that stochasticity may play in shaping the relationship between the host, the host microbiome, the pathogen, and the environment.

There is now substantial evidence that the host microbiome plays an important role in modulating pathogen invasion and disease outcome. This is likely because host microbiomes are located precisely at the interplay between host, pathogen, and environment. In some cases, the

microbiome is considered as an extension of the innate immune system of amphibian hosts [24], and the host and its microbiome are fused into a holobiont [12]. As such, the host microbiome is considered to be a part of the host/holobiont when implementing the disease triangle concept. Such a representation is restrictive since it undermines the fundamental interaction between two different entities (the host and its microbiome) and the fact that the environment can affect both separately and independently, with various outcomes on disease dynamics. Growing evidence from multiple studies demonstrates the importance of environmental conditions in modulating the host–microbiome–pathogen interaction. Any environmental condition is imposed simultaneously on the host, the microbiome, and the pathogen; however, all three factors may respond in different ways, independently of each other. We propose that a holistic approach, in which the disease triangle is extended to a disease pyramid, can best capture the complex nature of these interactions (Figure 2).

A disease triangle may allow the eventual linking of both single and multiple pathogen systems as coinfections become more widespread. *Bd* and ranaviruses can affect the same host population, requiring the immune system to act against two pathogenic targets simultaneously. The pathobiome concept, representing a consortium of microbes acting altogether as a pathogenic entity, is meant to capture the idea of coinfection [13,14]. In this concept, the pathogen and either environmental or host microbe are assembled, leading to similar issues raised for the holobiont. Because the environmental microbiome and host microbiome have a different community composition, and different physiological and ecological functions (Figure 1), we recommend considering them in a disease pyramid as two separated factors, yet connected and interdependent, with microbes potentially moving from one community to another. In our model, additional microbes acting with the pathogen can either come from the environment (environmental microbiome), or already live on the host (host microbiome). Generally speaking, the pathobiome concept makes it more difficult to apprehend the pathogen at all of its life stages. When the pathogen is colonizing the host, the pathobiome is what we call the host microbiome (as, at this stage, the pathogen is living on the host). However, many pathogens have free life stages, outside the host, and it is unclear what the pathobiome is at that moment (environmental microbiome or host microbiome?). Our pyramid does consider the life of pathogens outside of the host and the various interactions it might have.

We need to move from the reductive vision of a three-edged disease triangle to the broader vision of a four-edged disease pyramid that considers the host, the microbiome, the pathogen, and the environment as interdependent components that affect disease dynamics. Any modification in any one of the vertices means a change in all other relationships; thus, the disease pyramid considers the role of the environment without isolating the other entities (host, microbiome, pathogen) from the whole system of which all are essential parts. In addition, the theory developed for and applied to hosts in the disease pyramid is also entirely valid for disease vectors such as, for example, mosquitoes (see also [13] and Box 1).

Concluding Remarks

Human activities are profoundly changing the environment in numerous ways. Therefore, if we want to maintain host health and control wildlife diseases, it is important that we develop a research approach that includes both biotic and abiotic factors in studies of host–microbiome–pathogen interactions (see Outstanding Questions). Research on the amphibian–*Bd* system provides an opportunity to better understand the complex relationships between the host, the microbiome, the pathogen, and the environment, depicted by the suggested disease pyramid. Given the current threats pathogens pose to biodiversity, it is necessary to extend this research to other wildlife pathogens. For amphibians, it means, for example, *B. salamandrivorans*,

Outstanding Questions

Among the potential biotic and abiotic environmental parameters, which ones impact the host–host microbiome–pathogen interaction, how do they impact it, and which ones have the strongest influence?

Do evolutionary trajectories of the host, host microbiome, pathogen, and environment in the pyramid scale up to changes in infection and mortality risk?

Which role does stochasticity play in the four-way interactions of host–host microbiome–pathogen–environment?

How to translate knowledge acquired from studying all interactions of the disease pyramid into disease prediction risks and in disease mitigation?

ranaviruses, and herpesviruses. Harrison and colleagues studied the impact of different habitat characteristics of garden ponds on the *Rana temporaria* microbiome–ranavirus interaction [81]. They found that more diverse microbiomes were more resistant to the virus and that individuals living in more complex habitats had lower rates of mortality.

Future research on wildlife pathogens should consider the disease pyramid as a foundation by which to yield the necessary insights to understand and mitigate disease impacts and their different components. The disease pyramid provides an overarching comprehensive framework that can be applied to many hosts (animals, plants, humans), their microbiomes, their pathogens, and can theoretically incorporate environmental conditions as well as varied responses to environmental changes. Such a comprehensive framework will be increasingly necessary in medical sciences and disease ecology in a rapidly changing world.

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References

1. Altizer, S. *et al.* (2013) Climate change and infectious diseases: from evidence to a predictive framework. *Science* 341, 514–519
2. Cavicchioli, R. *et al.* (2019) Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* 17, 569–586
3. Jones, A.E. *et al.* (2019) Bluetongue risk under future climates. *Nat. Clim. Chang.* 9, 153
4. Fisher, M.C. *et al.* (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484, 186–194
5. Clare, F.C. *et al.* (2016) Climate forcing of an emerging pathogenic fungus across a montane multi-host community. *Phil. Trans. R. Soc. Lond. B* 371, 20150454
6. Stevens, R.B. (1960) In *Plant Pathology, an Advanced Treatise* (Horsfall, J.G. and Dimond, A.E., eds), pp. 357–429, Academic Press
7. Snieszko, S.F. (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. *J. Fish Biol.* 6, 197–208
8. Louca, S. *et al.* (2019) A census-based estimate of Earth's bacterial and archaeal diversity. *PLoS Biol.* 17, e3000106
9. Clemente, J.C. *et al.* (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* 148, 1258–1270
10. Qin, J.J. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–70
11. Nicholson, J.K. *et al.* (2012) Host-gut microbiota metabolic interactions. *Science* 336, 1262–1267
12. Margulis, L. (1993) *Symbiosis in Cell Evolution*, W.H. Freeman
13. Vayssier-Taussat, M. *et al.* (2014) Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Front. Cell. Infect. Microbiol.* 4, 29
14. Bass, D. *et al.* (2019) The pathobiome in animal and plant diseases. *Trends Ecol. Evol.* 34, 996–1008
15. Jiménez, R.R. and Sommer, S. (2017) The amphibian microbiome: natural range of variation, pathogenic dysbiosis, and role in conservation. *Biodiv. Cons.* 26, 763–786
16. Kueneman, J.G. *et al.* (2014) The amphibian skin-associated microbiome across species, space and life history stages. *Mol. Ecol.* 23, 1238–1250
17. Brucker, R.M. *et al.* (2008) Amphibian chemical defense: Antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*. *J. Chem. Ecol.* 34, 1422–1429
18. Finlay, B.J. and Esteban, G.F. (2001) Ubiquitous microbes and ecosystem function. *Limnetica* 20, 31–43
19. Grice, E.A. and Segre, J.A. (2011) The skin microbiome. *Nat. Rev. Microbiol.* 9, 244–253
20. Jarrell, K.F. *et al.* (2011) Major players on the microbial stage: why archaea are important. *Microbiology* 157, 919–936
21. Frenken, T. *et al.* (2017) Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. *Environ. Microbiol.* 19, 3802–3822
22. van der Linde, S. *et al.* (2018) Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature* 558, 243–248
23. Marchesi, J.R. and Ravel, J. (2015) The vocabulary of microbiome research: a proposal. *Microbiome* 3, 31
24. Rollins-Smith, L.A. and Woodhams, D.C. (2012) Amphibian immunity: Stay in tune with the environment. In *Ecoimmunology* (Demas, G.E. and Nelson, R.J., eds), pp. 92–143, Oxford University Press
25. Heijtz, R.D. *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3047–3052
26. Waite, D.W. and Taylor, M. (2015) Exploring the avian gut microbiota: current trends and future directions. *Front. Microbiol.* 6, 673
27. Colombo, B.M. *et al.* (2015) Microbiota and mucosal immunity in amphibians. *Front. Immunol.* 6, 1–15
28. Turnbaugh, P.J. and Gordon, J.I. (2009) The core gut microbiome, energy balance and obesity. *J. Physiol.* 587, 4153–4158
29. IPBES (2019) In *Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services* (Díaz, S. *et al.*, eds), IPBES secretariat, Bonn, Germany
30. Stuart, S.N. *et al.* (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786
31. DuRant, S.E. and Hopkins, W.A. (2008) Amphibian predation on larval mosquitoes. *Can. J. Zool.* 86, 1159–1164
32. Whiles, M.R. *et al.* (2013) Disease driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16, 146–157
33. Best, M.L. and Welsh, H.H., Jr (2014) The trophic role of a forest salamander: impacts on invertebrates, leaf litter retention, and the humification process. *Ecosphere* 5, 16
34. Scheele, B.C. *et al.* (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363, 1459–1463
35. Fisher, M.C. *et al.* (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annu. Rev. Microbiol.* 63, 291–310

36. Voyles, J. *et al.* (2011) Interactions between *Batrachochytrium dendrobatidis* and its amphibian hosts: a review of pathogenesis and immunity. *Microbes Infect.* 13, 25–32
37. Berger, L. *et al.* (2005) Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.* 68, 51–63
38. Pryor, G.S. and Bjorndal, K.A. (2005) Effects of the nematode *Gyrinicola batrachiensis* on development, gut morphology, and fermentation in bullfrog tadpoles (*Rana catesbeiana*): a novel mutualism. *J. Exp. Zool. A Comp. Exp. Biol.* 303, 704–712
39. Kearns, P.J. *et al.* (2017) Fight fungi with fungi: antifungal properties of the amphibian mycobiome. *Front. Microbiol.* 8, 2494
40. Kueneman, J.G. *et al.* (2017) Composition of micro-eukaryotes on the skin of the Cascades frog (*Rana cascadae*) and patterns of correlation between skin microbes and *Batrachochytrium dendrobatidis*. *Front. Microbiol.* 8, 2350
41. Walke, J.B. *et al.* (2014) Amphibian skin may select for rare environmental microbes. *ISME J.* 8, 2207–2217
42. Loudon, A.H. *et al.* (2014) Microbial community dynamics and effect of environmental microbial reservoirs on red-backed salamanders. *ISME J.* 8, 830–840
43. Bletz, M.C. *et al.* (2016) Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. *Nat. Commun.* 7, 13699
44. Rebollar, E.A. *et al.* (2016) Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *ISME J.* 10, 1682–1695
45. Abarca, J.G. *et al.* (2018) Characterization of the skin microbiota of the Cane toad *Rhinella cf. marina* in Puerto Rico and Costa Rica. *Front. Microbiol.* 8, 2624
46. Bird, A.K. *et al.* (2018) Skin microbiomes of California terrestrial salamanders are influenced by habitat more than host phylogeny. *Front. Microbiol.* 9, 14
47. Estrada, A. *et al.* (2019) Skin bacterial communities of neotropical treefrogs vary with local environmental conditions at the time of sampling. *PeerJ* 7, e7044
48. Walke, J.B. *et al.* (2011) Social immunity in amphibians: evidence for vertical transmission of innate defenses. *Biotropica* 43, 396–400
49. Bletz, M.C. *et al.* (2013) Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. *Ecol. Lett.* 16, 807–820
50. Sabino-Pinto, J. *et al.* (2016) Composition of the cutaneous bacterial community in Japanese amphibians: effects of captivity, host species and body region. *Microb. Ecol.* 72, 460–469
51. Becker, M.H. *et al.* (2014) The effect of captivity on the cutaneous bacterial community of the critically endangered Panamanian Golden frog (*Atelopus zeteki*). *Biol. Conserv.* 176, 199–206
52. McKenzie, V.J. *et al.* (2012) Cohabiting amphibian species harbor unique skin bacterial communities in wild populations. *ISME J.* 6, 588–596
53. Belden, L.K. *et al.* (2015) Panamanian frog species host unique skin bacterial communities. *Front. Microbiol.* 6, 1171
54. Rebollar, E.A. *et al.* (2016) Using 'Omics' and integrated multi-omics approaches to guide probiotic selection to mitigate chytridiomycosis and other emerging infectious diseases. *Front. Microbiol.* 7, 68
55. Antwis, R.E. *et al.* (2014) *Ex situ* diet influences the bacterial community associated with the skin of red-eyed tree frogs (*Agalychnis callidryas*). *PLoS One* 9, 1–8
56. Jani, A.J. and Briggs, C.J. (2018) Host and aquatic environment shape the amphibian skin microbiome but effects on downstream resistance to the pathogen *Batrachochytrium dendrobatidis* are variable. *Front. Microbiol.* 9, 487
57. Song, S.J. *et al.* (2019) Engineering the microbiome for animal health and conservation. *Exp. Biol. Med.* 244, 494–504
58. Kueneman, J.G. *et al.* (2016) Inhibitory bacteria reduce fungi on early life stages of endangered Colorado boreal toads (*Anaxyrus boreas*). *ISME J.* 10, 934
59. Prest, T.L. *et al.* (2018) Host-associated bacterial community succession during amphibian development. *Mol. Ecol.* 27, 1992–2006
60. Harris, R.N. *et al.* (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME J.* 3, 818–824
61. Walker, S.F. *et al.* (2010) Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in Iberia. *Ecol. Lett.* 13, 372–382
62. Forrest, M.J. and Schlaepfer, M.A. (2011) Nothing a hot bath won't cure: Infection rates of amphibian chytrid fungus correlate negatively with water temperature under natural field settings. *PLoS One* 6, e28444
63. Rohr, J.R. and Raffel, T.R. (2010) Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proc. Natl. Acad. Sci. U. S. A.* 107, 8269–8274
64. Raffel, T.R. *et al.* (2013) Disease and thermal acclimation in a more variable and unpredictable climate. *Nat. Climate Change* 3, 146–151
65. Schmeller, D.S. *et al.* (2014) Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Curr. Biol.* 24, 176–180
66. Feldhaar, H. (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecol. Entomol.* 36, 533–543
67. Russell, J.A. and Moran, N.A. (2005) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. Lond. B* 273, 603–610
68. Kueneman, J.G. *et al.* (2019) Community richness of amphibian skin bacteria correlates with bioclimate at the global scale. *Nat. Ecol. Evol.* 3, 381–389
69. Blaustein, A.R. and Kiesecker, J.M. (2002) Complexity in conservation: lessons from the global decline of amphibian populations. *Ecol. Lett.* 5, 597–608
70. Blaustein, A.R. *et al.* (2003) Ultraviolet radiation, toxic chemicals and amphibian population declines. *Divers. Distribut.* 9, 123–140
71. Beebee, T.J.C. and Griffiths, R.A. (2005) The amphibian decline crisis: a watershed for conservation biology? *Biol. Conserv.* 125, 271–285
72. Epstein, P.R. (2001) Climate change and emerging infectious diseases. *Microbes Infect.* 3, 747–754
73. Walther, G.R. *et al.* (2002) Ecological responses to recent climate change. *Nature* 416, 389–395
74. Trevelline, B.K. *et al.* (2019) Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proc. R. Soc. B* 286, 20182448
75. Bahrndorff, S. *et al.* (2016) The microbiome of animals: implications for conservation biology. *Int. J. Genomics* 2016, 5304028
76. Boissière, A. *et al.* (2012) Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS Pathog.* 8, e1002742
77. Becker, M.H. *et al.* (2015) Composition of symbiotic bacteria predicts survival in Panamanian Golden frogs infected with a lethal fungus. *Proc. R. Soc. Lond. B* 282, 20142881
78. Madison, J.D. *et al.* (2017) Characterization of *Batrachochytrium dendrobatidis* inhibiting bacteria from amphibian populations in Costa Rica. *Front. Microbiol.* 8, 290
79. Ellison, S. *et al.* (2019) Reduced skin bacterial diversity correlates with increased pathogen infection intensity in an endangered amphibian host. *Mol. Ecol.* 28, 127–140
80. Lam, B.A. *et al.* (2010) Proportion of individuals with anti-*Batrachochytrium dendrobatidis* skin bacteria is associated with population persistence in the frog *Rana muscosa*. *Biol. Conserv.* 143, 529–531
81. Harrison, X.A. *et al.* (2019) Stability dynamics of the amphibian skin microbiome and susceptibility to a lethal viral pathogen. *Front. Microbiol.* 10, 2883
82. Bletz, M.C. *et al.* (2018) Disruption of skin microbiota contributes to salamander disease. *Proc. R. Soc. B* 285, 20180758
83. Smith, H.K. *et al.* (2018) Skin mucosome activity as an indicator of *Batrachochytrium salamandrivorans* susceptibility in salamanders. *PLoS One* 13, e0199295

84. Bates, K.A. *et al.* (2019) Captivity and infection by the fungal pathogen *Batrachochytrium salamandrivorans* perturb the amphibian skin microbiome. *Front. Microbiol.* 10, 1834
85. Medina, D. *et al.* (2017) Variation in metabolite profiles of amphibian skin bacterial communities across elevations in the Neotropics. *Microb. Ecol.* 74, 227–238
86. Bletz, M.C. *et al.* (2017) Amphibian skin microbiota exhibits temporal variation in community structure but stability of predicted *Bd*-inhibitory function. *ISME J.* 11, 1521
87. Krynak, K.L. *et al.* (2016) Landscape and water characteristics correlate with immune defense traits across Blanchard's cricket frog (*Acris blanchardi*) populations. *Biol. Conserv.* 193, 153–167
88. Holden, W.M. *et al.* (2015) Skin bacteria provide early protection for newly metamorphosed southern leopard frogs (*Rana sphenocephala*) against the frog-killing fungus, *Batrachochytrium dendrobatidis*. *Biol. Conserv.* 187, 91–102
89. Ellison, S. *et al.* (2019) The influence of habitat and phylogeny on the skin microbiome of amphibians in Guatemala and Mexico. *Microb. Ecol.* 78, 257–267
90. Becker, C.G. *et al.* (2017) Land cover and forest connectivity alter the interactions among host, pathogen and skin microbiome. *Proc. R. Soc. Lond. B* 284, 20170582
91. Fites, J.S. *et al.* (2013) The invasive chytrid fungus of amphibians paralyzes lymphocyte responses. *Science* 342, 366–369
92. McMahon, T.A. *et al.* (2014) Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature* 551, 224–227
93. Loudon, A.H. *et al.* (2016) Vertebrate hosts as islands: dynamics of selection, immigration, loss, persistence, and potential function of bacteria on salamander skin. *Front. Microbiol.* 7, 333
94. Fitzpatrick, B.M. and Allison, A.L. (2014) Similarity and differentiation between bacteria associated with skin of salamanders (*Plethodon jordani*) and free-living assemblages. *FEMS Microbiol. Ecol.* 88, 482–494
95. Hernández-Gómez, O. *et al.* (2017) Cutaneous microbial community variation across populations of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). *Front. Microbiol.* 8, 1379
96. Bletz, M.C. *et al.* (2017) Host ecology rather than host phylogeny drives amphibian skin microbial community structure in the biodiversity hotspot of Madagascar. *Front. Microbiol.* 8, 1521–1534
97. Hughey, M.C. *et al.* (2017) Skin bacterial microbiome of a generalist Puerto Rican frog varies along elevation and land use gradients. *PeerJ* 5, e3688
98. Sabino-Pinto, J. *et al.* (2017) Temporal changes in cutaneous bacterial communities of terrestrial-and aquatic-phase newts (Amphibia). *Environ. Microbiol.* 19, 3025–3038
99. Muletz Wolz, C.R. *et al.* (2018) Effects of host species and environment on the skin microbiome of Plethodontid salamanders. *J. Anim. Ecol.* 87, 341–353
100. Prado-Irwin, S.R. *et al.* (2017) Intraspecific variation in the skin-associated microbiome of a terrestrial salamander. *Microb. Ecol.* 74, 745–756
101. Costa, S. *et al.* (2016) Diversity of cutaneous microbiome of *Pelophylax perezi* populations inhabiting different environments. *Sci. Total Environ.* 572, 995–1004
102. Hughey, M.C. *et al.* (2016) Short-term exposure to coal combustion waste has little impact on the skin microbiome of adult Spring Peepers (*Pseudacris crucifer*). *Appl. Environ. Microbiol.* 82, 3493–3502
103. Bataille, A. *et al.* (2018) Skin bacterial community reorganization following metamorphosis of the fire-bellied toad (*Bombina orientalis*). *Microb. Ecol.* 75, 505–514
104. Longo, A.V. *et al.* (2015) Seasonal and ontogenetic variation of skin microbial communities and relationships to natural disease dynamics in declining amphibians. *R. Soc. Open Sci.* 2, 140377
105. Rebollar, E.A. *et al.* (2019) Integrating the role of antifungal bacteria into skin symbiotic communities of three Neotropical frog species. *ISME J.* 13, 1763–1775
106. Walke, J.B. *et al.* (2017) Dominance-function relationships in the amphibian skin microbiome. *Environ. Microbiol.* 19, 3387–3397
107. Jani, A.J. *et al.* (2017) Epidemic and endemic pathogen dynamics correspond to distinct host population microbiomes at a landscape scale. *Proc. R. Soc. Lond. B* 284, 20170944
108. Jani, A.J. and Briggs, C.J. (2014) The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. *Proc. Natl. Acad. Sci. U. S. A.* 111, e5049–e5058
109. Walke, J.B. *et al.* (2015) Community structure and function of amphibian skin microbes: An experiment with bullfrogs exposed to a chytrid fungus. *PLoS One* 10, e0139848
110. Kueneman, J.G. *et al.* (2016) Probiotic treatment restores protection against lethal fungal infection lost during amphibian captivity. *Proc. R. Soc. Lond. B* 283, 20161553
111. Bataille, A. *et al.* (2016) Microbiome variation across amphibian skin regions: implications for chytridiomycosis mitigation efforts. *Microb. Ecol.* 71, 221–232
112. Medina, D. *et al.* (2019) Amphibian skin fungal communities vary across host species and do not correlate with infection by a pathogenic fungus. *Environ. Microbiol.* 21, 2905–2920
113. Rebollar, E.A. *et al.* (2018) The skin microbiome of the neotropical frog *Craugastor fitzingeri*: inferring potential bacterial-host-pathogen interactions from metagenomic data. *Front. Microbiol.* 9, 466