## The Spread of Batrachochytrium Dendrobatidis and its Effect on the Global Amphibian Ecosystem

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Amphibian populations around the world have been experiencing more rapid declines in recent years than any other vertebrae on the planet. Over 44% of amphibian species are currently in decline, according to a 2015 study by the US Fish and Wildlife service. For almost 30 years, scientists had no idea why the decline of frogs, toads, newts, and salamanders was so drastic and so severe. In 1993, the field of herpetology would be forever changed, with the discovery of batrachochytrium dendrobatidis, an infectious fungus that was killing anurans (frogs and toads) at a more rapid rate than any other pathogen in history. What was this destructive microorganism that scientists had just discovered? How did the spread of batrachochytrium dendrobatidis, also known as chytrid or BD, affect amphibian populations in the global ecosystem from its discovery in 1993 to modern day, and how could it affect biodiversity in the future?

To understand the impact of chytrid we must firstly understand the history of BD and its discovery. First identified in Queensland, Australia, BD was found to be the cause of mass amphibian die-offs that had been occurring locally in Australia as well as globally ("CHYTRIDIOMYCOSIS"). To get to the bottom of the devastating amphibian die offs, the researchers first began collecting dead anurans that were perishing in the mass amphibian regressions (Berger et al., 9031). They analyzed the specimens in a multitude of different ways,

performing pathology and microbiology tests from skin scrapings, alginate swabs, and blood smears. The Australian researchers used skin samples to perform electron microscopy, attempting to observe any visual hints as to the cause, and even performed DNA analysis using the skin scrapings of two wild frogs. Eventually, they realized that all signs were pointing to one place. A fungal infection, transmitted from frog to frog via contaminated water. To prove their theory, the Queensland scientists performed a transmission experiment. Captive bred, uninfected Great Barred Frogs were individually housed with gravel and tap water at a stable 24C. The scientists took water infected with spores from the chytrid fungus, and passed the water through filters of different sizes, giving different groups of frogs different samples of water that had passed through specific filter sizes, as well as keeping an uncontaminated control group. 18 days later, all the frogs that had been exposed to the unfiltered chytrid water had died, and when their bodies were analyzed, indisputable evidence of the deadly fungus was found (Berger et al., 9031-9034). The scientists began to apply their findings to retrospective data, seeing evidence of the fungus as far back as 1961, over 30 years prior to their study ("CHYTRIDIOMYCOSIS"). In the next few years, large numbers of sick and dead amphibians were found in western Panama, and when investigated, it was again BD that had caused the devastation (Berger et al., 9036). As research progressed, and scientists realized what to look out for, it was realized that the deadly fungi was remarkably widespread, already being endemic on all 6 continents where amphibians naturally resided (Kilpatrick et al., 109).

Chytrid was the invisible culprit behind these mass die offs of amphibians, but how? What exactly was this killer fungus that had been ravaging amphibian populations for over 30 years? It is important to note that BD isn't the only chytrid fungus out there. Chytrid covers a

large family of fungi, some of which are known to be parasites. However, BD, and its cousin BSal, which is a similar disease that targets salamanders specifically, are the only two in the family known to parasitize vertebrates ("CHYTRIDIOMYCOSIS"). BD has been described as the largest infectious disease threat to biodiversity, and one that is an urgent conservation concern, partially due to the nature of the fungus itself (Kilpatrick et al., 109). As is with most fungi, chytrid reproduces asexually, spreading its spores via environmental forces. In chytrid's case, this environmental force is water ("CHYTRIDIOMYCOSIS"). Water, where amphibians reproduce. Water, where tadpoles live the first portion of their lives. Water, where amphibians wet their skin to allow them to breathe. This location, so vital to the survival of every living species, but amphibians specifically, is where chytrid spreads its infectious spores, with deadly consequences. Batrachochytrium dendrobatidis spores are uniquely adapted to this aquatic environment, and the spores of the fungus were found to survive over three months in water in the lab (Kilpatrick et al., 110). BD is also able to be cultured, that is, to be artificially grown in a lab (Kilpatrick et al., 110). These facts alone provide evidence that it's entirely possible that BD could survive indefinitely without a host for it to parasitize. It could wait, in status, for years. In spite of its strengths, even chytrid isn't invincible. Scientists observed that the changing seasons impacted the frequency and severity of these chytrid outbreaks, leading them to the conclusion that high temperatures act as a mitigating factor in the spread of chytrid

("CHYTRIDIOMYCOSIS"). Despite its temperature weakness, and the ability of antifungal medication to cure chytrid infections, "Chytrid fungus is the most destructive pathogen ever described by science—that's a pretty shocking realization," according to Simon Fraser University biologist Wendy Palen (Greshko).

To discover the impact of batrachochytrium dendrobatidis on amphibians globally, one must understand first how the disease impacts its host on an individual level. For that, we need to understand a little about the anatomy of the amphibians that the fungus is so lethal to. Amphibians have incredibly unique skin. The skin, or epidermis, is the largest organ of these creatures, and is a site of regulated transport for water, ions, electrolytes, and gasses. As these materials pass over and through the amphibian's epidermis, the animal respires. In essence, an amphibian's skin is the vital tool allowing it to breathe and to regulate nutrient levels to remain healthy (Voyles et al, 114). BD attacks the keratin of this delicately balanced organ ("CHYTRIDIOMYCOSIS"). Permeability of the skin varies from species to species, as well as the location on the frog's body, and, unsurprisingly, chytrid is often found in high concentrations on the extremely delicate and porous areas of the skin, specifically the pelvic patch (Voyles et al, 115). When tested, significant amounts of sporangia, the spore producing part of the fungus, were found on the digits, ventral body, and this hypervascularized pelvic "drink patch". Chytrid doesn't burrow deep into the skin, and isn't found to consistently impact internal organs, but it's colonization of the stratum corneum and stratum granulosum, the first layers of the skin, are enough to be lethal (Berger et al, 9034). In essence, B. dendrobatidis kills its victims by disrupting the functions of the skin, leading to a loss of electrolytes due to the creation of an imbalance in osmotic homeostasis (Voyles et al, 116). As chytrid deals death from inside the amphibian, it may begin showing visible symptoms, but often only mere days before the anuran's passing. The frog or toad may exhibit lethargy, including half closed eyes, decreased respiration rate, and low appetite. The skin may also begin to visibly change, becoming dull or greyish on the top of the frog and red underneath, sometimes even showing an accumulation of thickened, cast off skin ("CHYTRIDIOMYCOSIS"). Before the amphibian reaches these clinical

stages, however, frogs can be tested via a polymerase chain reaction (PCR) test. Scientists will also observe irregular skin loss, excess cell growth, known as hyperplasia, in the stratum corneum and stratum intermedium, reduced blood pH, and reduced mineral concentrations (Voyles et al, 115). However, that's only how chytrid impacts fully grown amphibians. Amphibians undergo a metamorphosis early in their life, transforming from a tadpole to a fully developed frog. These tadpoles, who spend their entire time in water, can also be infected by BD, though they experience a relatively low mortality rate that spikes post-metamorphosis. This is due to the fact that tadpoles lack much of the keratinized skin that older frogs have, and since chytrid is only able to affect this skin, the infection isn't nearly as deadly. When the skin of tadpoles was tested for chytrid sporangia, the only place it was present was in the small amount of keratinized skin by the mouth (Voyles et al, 115). While this is somewhat positive that the tadpoles aren't experiencing the mass die offs observed in mature frogs, tadpoles can still spread the fungus, and will often die of it themselves once their skin is keratinized after metamorphosis (Kilpatrick et al, 114).

Batrachochytrium dendrobatidis decimates amphibians on an individual level, preventing their skin from performing the basic biological processes needed for them to survive, leaving frogs with severe electrolyte imbalances, struggling to breathe through their unnaturally thickened skin. BD doesn't attack amphibians one by one, though, and the fungus is just as devastating, if not moreso, on a global scale. On the one hand, while mortality rate of a chytrid infection is dependant on species, individuals from over 700 species of amphibians have found to have been infected, and between BD and it's salamander targeting cousin, BSal, the chytrid fungus has been found to be a significant driving cause in the decline of at least 501 amphibian

species (Kilpatrick et al, 116). 124 of those species have experienced a population decline of 90 percent or greater, with another 90 species becoming extinct altogether due to the disease (Greshko). On the other hand, that's just what's known for sure. Some studies have implicated b. dendrobatidis in the extinction of over 200 amphibian species over the 6 continents where amphibians, and BD, reside. In Latin America, batrachochytrium dendrobatidis has been attributed to extinctions of 30 out of the 113 species of harlequin toads (Kilpatrick et al, 116). In Australia, at least 4 species have been completely wiped from the face of the Earth. Chytridiomycosis is the reason you can no longer see either the northern or southern gastric brooding frog, with both species dying out in the 1980s ("CHYTRIDIOMYCOSIS"). "The Global Amphibian Assessment recently argued that the 6000+ species of amphibians are one of the most threatened classes of vertebrates, with 32.5% of species threatened. In addition, 92.5% of the 'critically endangered' group are undergoing 'enigmatic declines' that might be linked to BD" (Kilpatrick et al, 114). As much of a problem as chytrid would pose on its own, it's not the only thing decimating amphibian populations. Furthermore, factors not related to Chytridiomycosis, such as habitat destruction, climate change, overexploitation, and the introduction of harmful predators also put strain on amphibian populations (Voyles et al, 117). According to one hypothesis, known as the chytrid-thermal-optimum hypothesis, or CTOH, mass die offs as a result of chytrid are occurring with much harsher severity and rapidity due to the changing climate. Chytrid isn't even the sole disease that is decimating anuran populations, with other infectious diseases, such as ranavirus, also taking their toll (Kilpatrick et al, 116). The International Union for Conservation of Nature, or ICUN, keeps track of a "red list" a list of species and their population status in the wild. Of the 6,000+ anurans the organization keeps track of, 502 are critically endangered, 851 are endangered, and nearly 1,200 lack significant

data to put them into a category. Only 43% of amphibians listed were categorized as of least concern ("Red List"). Since its discovery in the 1990s, batrachochytrium dendrobatidis has ripped through the already at risk amphibians, but the future holds more questions than it does answers.

There is no changing the past when it comes to Chytrid, but scientists are doing their best to improve the future. Chytrid may already be widespread, but there are certain areas across the world where it has not yet reached. So, herpetologists and environmentalists are attempting to mitigate the spread of disease in the wild. The control of the disease is based more on the protection of unimpacted zones, rather than the decontamination of areas where Chytridiomycosis is already endemic, since it is extremely difficult to cure the disease on a large scale ("CHYTRIDIOMYCOSIS"). While temperature and antifungals can help save frogs in the lab, implementing this on a wide scale could cause disastrous unintended consequences and may not even help, since the disease spreads so rapidly ("CHYTRIDIOMYCOSIS"). However, infection in the wild is not always fatal even if it is in lab populations so there is hope that some resistant frogs may carry on (Kilpatrick et al., 119). While the wild is one place to generate an effect on impacts of Chytrid, labs are another place where scientists are attempting to make a difference.

Laboratories allow researchers to study chytrid in a controlled environment, learning how it works and how the infection impacts its hosts, but it is also where scientists can study amphibians unaffected by chytrid, learning how better to protect them in an uninvasive manner. One of the first steps is figuring out the precise way in which BD kills amphibians (Voyles et al). While we know it is caused by an electrolyte imbalance, it's unclear how exactly this balance

comes about and what can be done to prevent it. Understanding the way in which Chytrid takes its victims may also help scientists understand the variability in mortality rates among different species (Voyles et al). Why are American bullfrogs unaffected, when species like the gastric brooding frog have already become extinct? Another tactic being studied is whether chytridiomycosis is an introduced pathogen or if it is endemic. Depending on the origin of the fungus, different strategies would need to be taken in preventing or curing it so understanding the history of the fungus is vital in determining the next steps that should be taken by humanity (Kilpatrick et al.). As of right now, the leading hypothesis is that the pathogen is novel, meaning it originated in one place and then was introduced via its spores across the world. However, scientists have been unable to locate the initial amphibian populations, where we would expect to see high rates of genetic diversity and resistance to BD. Through this research, however, scientists have discovered that there is low variability in strains worldwide, suggesting that it was the spread of a single strain of chytrid, rather than a multitude of different strains, that has caused this global devastation. If there were many strains, all of which had the same or similar impacts, it would be much more difficult to treat globally, since many strains require many cures. The idea of one or a few strains means that, hypothetically, it would be easier to treat since far less treatments need to be developed, and that only one cure is needed, is needed rather than the many that may be required to treat each different strain (Kilpatrick et al). Scientists also must work on caring for and treating individual amphibians before they cure entire populations. Labs are also used for researching the disease further, as the better we understand it the more ways that we will find to combat it. In vitro, antifungal medication is incredibly helpful for curing Chytrid, but the treatment is unreasonable to do on a wider scale ("CHYTRIDIOMYCOSIS"). Scientists have also discovered that the exposure dosage of Chytrid, temperature, and slight

differences in strains have been found to influence the interactions between pathogen and host, affecting mortality rates (Kilpatrick et al.). Overall, increased doses were found to lead to higher mortality. Temperature wise, however, some species had no change while others experienced higher or lower mortality rates or life span (Kilpatrick et al). Inconclusive study results then require more testing. Being able to understand and further study chytridiomycosis is one of humanities best hopes for protecting the amphibians with whom we share our planet.

Herpetologists are not the only ones who can create an impact though. Monitoring and surveillance of frog populations helps us provide further information on Chytrid, and many states and countries have ways for citizens to report data on amphibian populations, general health, and breeding habits ("CHYTRIDIOMYCOSIS"). This citizen science can help the professionals collect more knowledge on the impact of the disease on certain frog populations, as well as allowing for detection and early action in response to new outbreaks, establishing disease-free quarantine areas, and monitoring the success of control strategies, without the need for them to hire more manpower or receive further funding ("CHYTRIDIOMYCOSIS"). It just requires a little bit of help from the community. Testing and observing populations before any chytridinduced decline can also help researchers study how chytrid affects amphibians in the wild, and how in turn, the amphibian decline affects the surrounding ecosystem (Kilpatrick et al.). Another vital part of handling the Chytridiomycosis epidemic is the safe and ethical handling of amphibians. While pet frogs might not be the most common, regulations around the pet trade can help Chytrid from spreading. Making sure to purchase pet frogs from certified Chytrid-free breeders, never releasing pets into the wild, and not purchase or keeping wild-caught frogs will help limit the spread. Scientists encourage citizens to disinfect their hands and use single-use

gloves or plastic bags when handling wild frogs, and remind those who have come into contact with frogs to always wash their hands afterwards ("CHYTRIDIOMYCOSIS"). This can not only help with Chytrid, but the fact that frogs have incredibly delicate skin and the oils from human hands can impact its effectiveness, so by handling frogs safely and carefully, frogs are prevented from dying in other ways. Another way citizens can help the cause, without having to dedicate time is by donating. Despite the fact that amphibians are undergoing the most rapid extinction rate out of any animal species out there, they tend to receive little funding from governments or other conservation organizations ("About"). This means that much of the funding has to be raised on its own, meaning that further discovery into the disease is slow, and can even be stalled due to a lack of resources. Organizations like Save the Frogs aim to combat this, and spread information about Chytrid and the other ways that amphibians are being harmed ("About"). And finally, there's the completely free option of spreading awareness. Many people are unaware of Chytrid, its dangers, and the massive effect it's already had, simply because amphibians fail to receive as much attention as other species. Chytrid isn't as deadly as it is because nothing can be done, but the fact that so little is being done.

Chyridiomycosis also doesn't just impact amphibians. On December 2nd, 2020 the American Geophysical Union published a paper showing that prevalence of Chytrid in the 90's and 2000's in Central America drastically decreased frog populations, something scientists already knew. However, the paper showed how these decreased frog populations allowed for increased mosquito populations (American Geophysical Union). This in turn created a rise in malaria cases. Preventing Chytrid isn't just about saving the frogs, it's also about saving human lives.

But the study of Chytrid is not only advancing herpetology but science in general. The two species of gastric brooding frog, a small family of frogs that lay and fertilize their eggs, then swallow them and gave birth out of their mouth, both went extinct due to Chytrid in the 1980s. In 2013, scientists began efforts to bring the frog back from extinction (Pilcher). They went as far as to get an embryo, but it failed to develop it fully into a tadpole and was therefore unable to grow into an adult frog. However, it was the first time in 30 years that a gastric brooding frog had been seen anywhere on the face of the Earth. It was the second time that an animal had gone from extinct, to un-extinct and then back to extinct again, when the embryo eventually failed (Pilcher). We as a people cannot rely on our ability to clone to reverse our actions, however. While cloning and genetic modification does provide hope for some, the ethical concerns of cloning, as well as its high cost and the low genetic diversity that it results in provide massive barriers for using this method in practice. Along with that, the cloned animals would not be exactly the same species as the extinct animal (Pilcher). To clone a creature, there must be an unfertilized egg and a sperm. While these can be genetically modified to carry the traits of an extinct creature, They will not be exactly the same as those that would have come from the creature. so, while some see de-extinction as the best option, others understand that we must first focus on saving the anurans that are still with us. Certain groups are helping to breed endangered frogs and reintroduce them into the wild, saving their species from extinction. Many groups aren't just breeding random frogs though, but selectively breeding animals that show a resistance to chytrid in an attempt to create innate immunity in a species ("CHYTRIDIOMYCOSIS"). While methods like these, involving raising and breeding frogs, are helpful, they aren't addressing the root of the problem. Who is to say that once the frogs are released they won't just

contract chytrid again? While it may be effective later down the line, the approach of breeding frogs to save them is not as cost effective or addressing the root of the problem.

Batrachochytrium dendrobatidis has ravaged amphibian populations in the global ecosystem from its discovery in 1993 to modern day, and how could it's effects on the future of amphibian biodiversity are unknown, but threatening. In the past forty years, more has been learned about the rapid decline of amphibian populations than ever before in history. After years of searching, we finally are beginning to understand the deadly fungus known as BD that has ravaged amphibian populations worldwide. We are slowly understanding how chytridiomycosis affects frogs and toads on an individual and on a species level. But work is far from finished. Chytrid is still spreading, and the general public knows next to nothing about the mass extinction that our amphibian friends are undergoing. Herpetologists, ecologists, and groups of scientists and researchers are doing the best they can to stop this deadly epidemic. But knowledge alone is never enough. Action must be taken before it is too late. We must step up and listen to scientists, teach ourselves and our friends, and make an effort to preserve the species we have left.

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