Global Pattern Analysis of the Amphibian Disease Chytridiomycosis

Elizabeth Pyle

ABSTRACT

Amphibians are smooth-skinned vertebrates that utilize both aquatic and terrestrial habitats to prosper. My study analyzes the patterns in Bd and Bsal amphibian infections on a global scale. A global geographic analysis of Bd and Bsal positive cases determined that Bd is widespread around the globe, while Bsal is still relatively contained according to the limited data available. Additionally, calculation of infection rates of Bd assisted the analysis of spread and determined infection hotspots; Bd had hotspots in all continents except Antarctica. Finally, an analysis of which taxonomic groups of amphibians are most affected assisted in determining amphibian families with high disease prevalence. More research is needed to solve this disease crisis, however, legislative regulation of the global amphibian pet trade could be the key to slowing down the spread of chytrid fungi.

KEYWORDS

Amphibians, chytrid fungi, chytridiomycosis, *Batrachochytrium dendrobatidis* (Bd), *Batrachochytrium salamandrivorans* (Bsal)

INTRODUCTION

After surviving every other mass extinction, amphibians are in the midst of their own (Wake et. al, 2008). Amphibians are categorized as Anura (frogs), Caudata (salamanders), or Caecilians and are found on every continent except Antarctica (Pyron 2014). All three types of amphibians play crucial roles in balancing our ecosystems through nutrient cycling and pest control; they also act as bioindicators of an ecosystem's wellbeing. However, amphibians have been declining rapidly in recent decades (Scheele et. al 2019). Habitat loss caused by human expansion has affected some amphibian species. Climate change has caused some regions to stray outside of ideal temperatures for amphibians to thrive in, adding an additional barrier for amphibians. While several factors may be attributed to these declines, one of the most pressing threats are fungal pathogens.

Chytrid fungi is a fungal pathogen that has been causing large numbers of amphibian populations to collapse. The zoospore stage of its lifecycle is aquatic, which is when the fungi is deadliest to amphibians. Bd, which mostly affects frogs, and Bsal, which mostly affects caudata, are the two known kinds of chytrid fungi. Both fungi harm amphibians by causing an overproduction of keratin on the amphibian's skin which makes osmotic regulation more difficult, often resulting in death. Chytridiomycosis, the disease caused by chytrid, has become a threat to ecosystems globally. Over a third of amphibian species are currently threatened with extinction due to chytrid fungi (Wake and Vredenburg 2008). Amphibians use both innate and adaptive immune systems to survive (Yap et al. 2015). They are resilient creatures that have survived every mass extinction in history, so to be experiencing these widespread declines recently is alarming. The origins of the chytrid fungi are still debated due to lack of data available from the 1970's and 1980's (Scheele et al. 2017) and much about the disease's ecology remains unknown.

Amphibians have survived many mass extinctions, however, they are one of the most affected groups of our current mass extinction event. The scientific community at large has only been aware of these two specific strains of chytrid fungi for several decades, however, these fungi have already been linked to the declines of hundreds of amphibian species (Scheele et al. 2019). Luckily, Bsal, the strain that primarily affects salamanders, has not yet reached the United States; the United States has the most species richness for salamanders (Fitzpatrick et al. 2018).

One of the largest spreaders of chytrid globally is the amphibian pet trade (Fitzpatrick et al. 2018), yet it remains widely unregulated. With so much still unknown about this deadly disease, it is crucial to research more about its effects on certain species and it's trends over time. A new analysis of chytridiomycosis global patterns will be able to inform policy changes, especially those establishing regulation of the amphibian pet trade which is one of the largest chytrid spreaders. With new Bd and Bsal samples being taken almost daily, it is imperative to have up to date analyses to inform our action.

My study analyzes the patterns of Bd and Bsal infections on a global scale. My central research question is what are the patterns in the Chytrid fungi Bd and Bsal's amphibian infections globally? This question will allow for a holistic analysis of the fungal pathogens and their spread. Specifically, I will explore what are the geographic ranges of occurrences of Bd and Bsal amphibian infections. I will also analyze what areas globally have the highest infection rates of Bd and Bsal. Finally, I will identify any changes in Bd prevalence in specific amphibian families. For my data analysis, I will be working with the Museum of Vertebrate Zoology's Amphibian Disease Portal database. This database was launched to continue the disease tracking efforts of a prior project, Bd Maps, that lost its funding and was discontinued. In 2020, thousands of new Bd and Bsal samples have been entered into the portal. I utilize their database of over 30,000 entries to inform my analyses as well as conducting a literary review of previous research on the subject. While this is the best known information available, this database is not fully comprehensive of all amphibian populations globally and has some reporting biases, as this project was launched only a few years ago.

METHODS

Data Collection Museum of Vertebrate Zoology Amphibian

Disease Portal

To track a global disease, thousands of samples must be collected in a globally representative manner. Though the Amphibian Disease Portal (ADP; https://amphibiandisease.org) does not have samples from every country, nor are the samplesevenly distributed, this amphibian

disease sample database is the largest of its kind; this database provides the best information for chytrid fungal infection tracking. Amphibian samples are compiled by scientists around the globe and are then uploaded to the Museum of Vertebrate Zoology's amphibian Amphibian Disease Portal database. Previously, data on Bd and Bsal were fragmented and found in papers or small databases, but the Amphibian Disease Portal is a project focused on compiling all of the data in an accessible and holistic database.

Sample Procedure

Samples of Bd or Bsal are collected by scientists around the world in the field, in captivity such as zoos and the amphibian pet trade, as well as from historical specimens in museum collections. Sample collection technique consists of swabbing the amphibians on their stomachs, backs, and legswith a sterile Q Tip like swab for about 10 rotations. Since the chytrid fungi grows on the skin of the amphibian, swabbing is only necessary on the exterior. It is important to wear sterile gloves and remove as much debris from the amphibians as possible. The swab is then put into a vial and sent to a lab for testing. Once received, the samples are analyzed via PCR in a laboratory; any laboratory cleared to run PCR tests is able to contribute to the database. The results of this test, as well as the coordinates of where the amphibian was sampled, the date, and the species are some of the collected data of interest that is uploaded to the Amphibian Disease Portal via a spreadsheet.

Data Selection

The first step of data selection is data vetting; I checked each entry for validity, typos, and completeness. The portal's data file is compared to the statistics on the Amphibian Disease Portal website to ensure the tallies of each species and their test results match. There are several reasons why a sample may be omitted from this study. Some environmental data is uploaded to the Amphibian Disease Portal; environmental data samples the surrounding areas, such as the water or soil, for the chytrid fungi, instead of an amphibian directly. Only 17 of the samples were environmental data. Since this study is solely analyzing the spread of the disease to amphibians, environmental data will not be included. Another reason for exclusion is if the sample reported is lacking crucial data such as coordinates or species name.

Data Analysis

To determine the spread and prevalence of chytrid fungi, a crucial component in minimizing its effects on amphibian populations, I analyze over 30,000 amphibian samples from the Museum of Vertebrate Zoology's Amphibian Disease Portal. Once the discrepancies and typos are corrected during data vetting, I used Quantum GIS for all subsequent analysis and data visualizations. GIS software allows for the mapping and visualization of large datasets. I also used Tableau for the organization and visualization of taxonomic spread. For the analysis of my research question and subquestions, Bd and Bsal samples are separated. Once separated, all Bd and Bsal positive cases are mapped according to their coordinates included with their sample.

Bd Spread

To look at the global spread of Bd, I used the filtered Bd data and mapped the Bd positive samples globally. Bd positive samples are those which had Bd detected whether it was fatal to the amphibian or not. First, I filter the Bd+ samples from Bd- samples, as the negative samples are not needed for this specific analysis. By selecting the specific layer in Quantum GIS that has the data points, I then filtered by disease tested (Bd) and disease detected (true). Once plotted, this visualization of data will clearly allow me to determine where Bd has spread to and infected amphibians around the globe.

Bsal Spread

To determine the global spread of Bsal, I filtered the Bsal data and plotted it in Quantum GIS. The data available for Bsal is much scarcer than for Bd because Bsal is a newly discovered disease, so there are far less samples and positive cases recorded. First, I filtered the Bsal+ data from the Bsal- data by selecting the data layer and filtering by disease tested (Bsal) and disease detected (true). I then applied those filters, and the positive Bsal samples were plotted globally.

Bd Hotspot Analysis

To determine global Bd hotspots, I plotted all samples, both with positive and negative results on a global map using Quantum. Instead of calculating the amount of positive cases in an area, I calculated the prevalence of Bd within hexagonal geographic bins by creating a new calculated field within the samples. This allows for a more accurate depiction of hotspots instead of a general heat map, especially since the samples are not collected in an organized and uniformed fashion. Prevalence is the number of positive cases divided by the number of total cases. Then this decimal is multiplied by 100 to create a percentage. This allows for a more accurate look at infections on a local level. Even with 30,000+ samples, there are still amphibian-dense regions with little to no testing. Since there is a sampling bias towards the United States, the raw data can be misleading. By looking at the data distribution, I created geographic bins to group the samples in. These geographic bins are a uniform size throughout the globe. For Bd hotspot analysis, these bins have an area of 800km. Once these bins are constructed, they are merged with the samples and shaded according to their prevalence. Bins with a higher prevalence for Bd will be shaded darker.

Bsal Hotspot Analysis

To determine Bsal global hotspots, I plotted all of the positive and negative samples in Quantum. I calculated the prevalence of Bsal within hexagonal geographic bins. Prevalence is the positive samples divided by total samples, which is then multiplied by 100 to convert it into a percentage. The Bsal geographic bins have an area of 100km. To accomplish this, I created a new calculated field to determine their prevalence in Quantum, which the geographic bins are then projected over and merged with those calculations and shaded to represent higher or lower prevalences. This analysis was only performed in the greater Germany area as that is where all of the Bsal+ cases in the Disease Portal are located. The spread of Bsal testing is scarcer than Bd, which is why Bd and Bsal have different sized bins for analysis of prevalence. Bd also has many more recorded samples than Bsal because Bsal was not discovered until 2014.

Bd Taxonomic Distribution

Patterns of infection by taxonomic groups are determined by calculating the prevalence of Bd in the samples for each amphibian family. I used Tableau to visualize these results. The best classification size to analyze and sort the amphibian data into is at the family level. The Bd samples are sorted by family, and then sorted into two groups within families: Bd+ and Bd-. Prevalence will be used instead of raw percentages to account for uneven sampling between families. Positive samples for Bd will be divided by the total number of samples, then multiplied by 100 to get the percentage of prevalence. The Amphibian Disease Portal database has a sampling bias that is taken into consideration here. Families with less than 30 samples will be omitted from this analysis due to a lack of data.

Bsal Taxonomic Distribution

I did not perform a taxonomic analysis for Bsal due to very limited Bsal Amphibian   Disease Portal.

RESULTS:

Mapped Spread of Bd

Figure 1 shows all samples in the Disease Portal. Through my analysis I show that Bd has been recorded worldwide (Figure 2). Bd is found in 41 countries; in total, samples from 48 countries were used for this analysis of Bd's spread. Figure 2 shows the spread of Bd on a global scale broken down by country. Each orange symbol represents one positive Bd sample. Countries with a darker pink shading have more positive Bd samples within their borders.
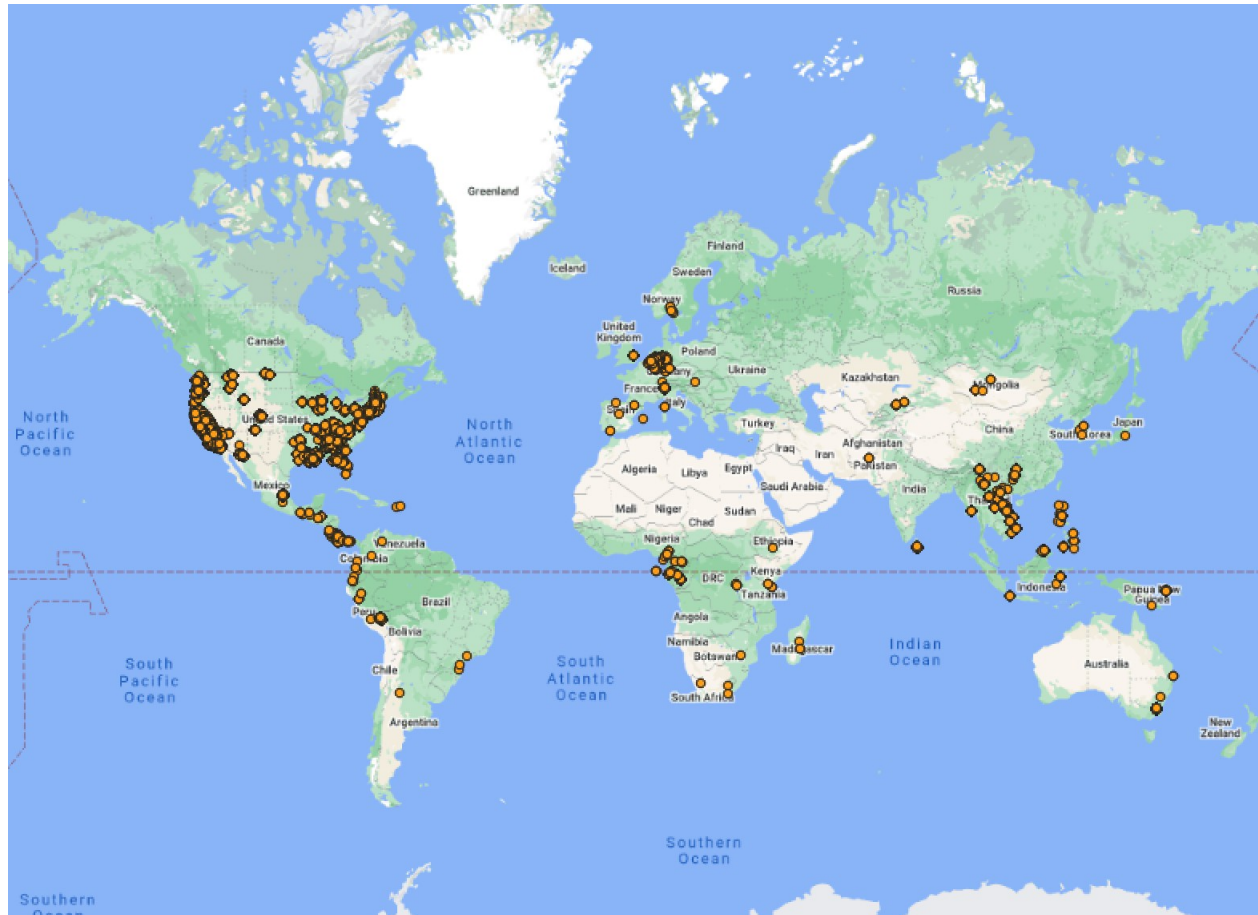
Figure 1: All Disease Portal samples from AmphibianDisease.org as of 2/7/2021 mapped on Quantum GIS
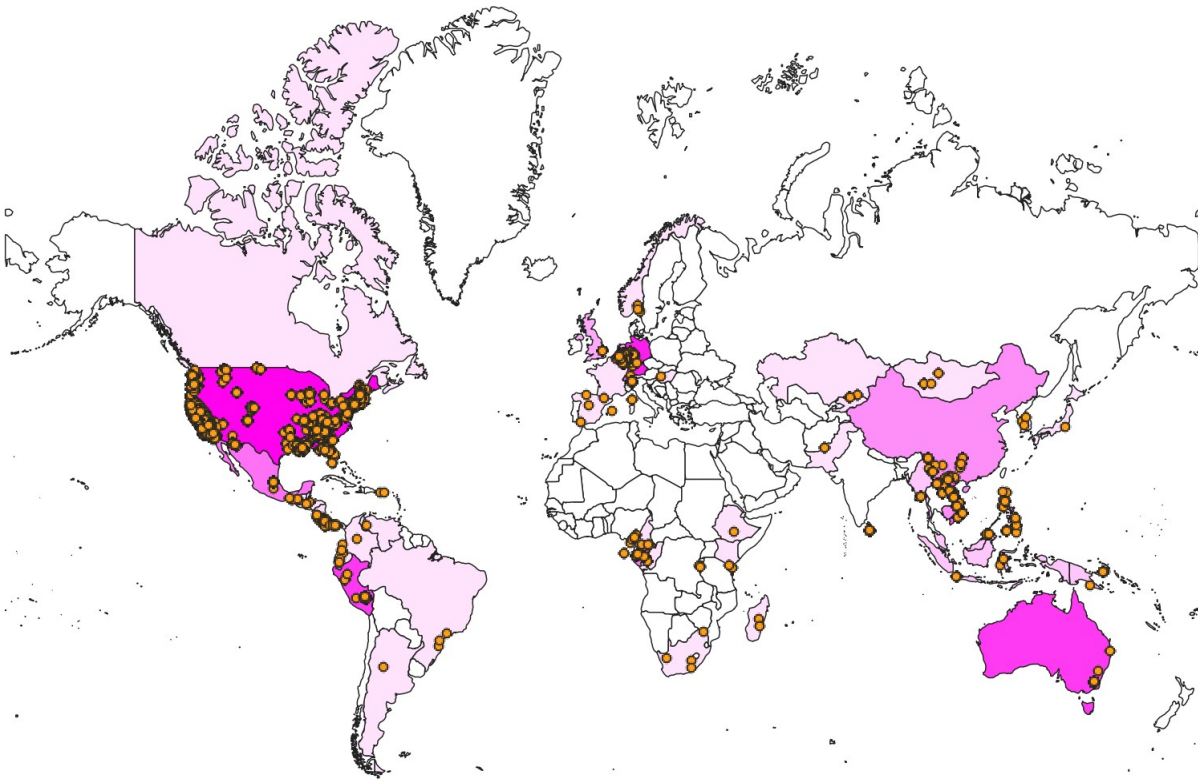
Figure 2: All Bd positive Disease Portal samples from AmphibianDisease.org as of 2/7/2021 showing the amount of positive samples per country.

Mapped Spread of Bsal

The Amphibian Disease Portal only has Bsal positive data from one country, which is Germany (Figure 3). Bsal is a much newer disease than Bd, so the fungi remains contained to 40 countries less than Bd as of now. This statistic may be misleading, however, because the database that donated this information does not have access to bordering countries potential samples. Several samples are extremely close to Germany's borders, which suggests that the fungi could very well be in Belgium and the Netherlands as well (Martel et al., 2013). Bsal samples from 6 countries were used in this analysis of Bsal's spread. All other countries had no Bsal data available, so they were excluded from my analysis.
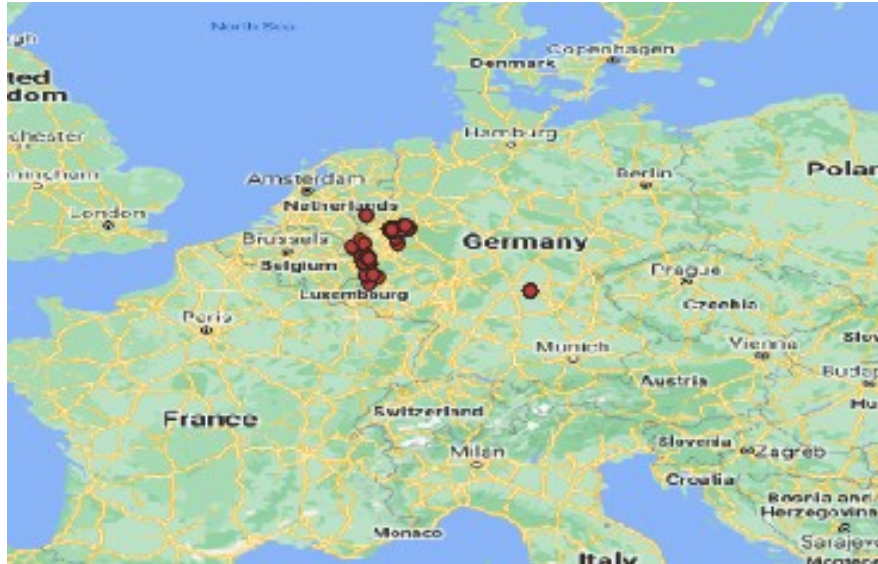
Figure 3: Bsal positive samples in Germany from global analysis from AmphibianDisease.org as of 2/7/2021

Bd Global Data Hotspots

I have found that Bd has several prominent hotspots around the globe, as seen below in figure 4. All continents except Antarctica have strong Bd hotspots present. As one can see in figure 4, there are many more dark geographic bins, which have higher prevalences of Bd, than white geographic bins, which have no positive samples in their 800km range. Many spots on the globe that have amphibian populations are not sampled yet, or are not yet included in the Disease Portal database. With so many Bd hotspots spread around the globe, untested amphibian populations could be just as infected with Bd as what we are seeing below.

Figure 4: Bd prevalence hotspots worldwide based on data from AmphibianDisease.org in 800km hexagon bins. The key is not converted to percentages.

Bsal Global Hotspots

I found that with limited Bsal samples, the areas most resembling hotspots were mainly in western Germany with one outlier in central Germany as seen in figure 5 below. Bsal positive data is limited and reflects only what has been shared by the Bsal Consortium of Germany (Lötters, 2020). As stated above, it is likely that Bsal has spread to Belgium and the Netherlands and that the data is not yet available to the Disease Portal.
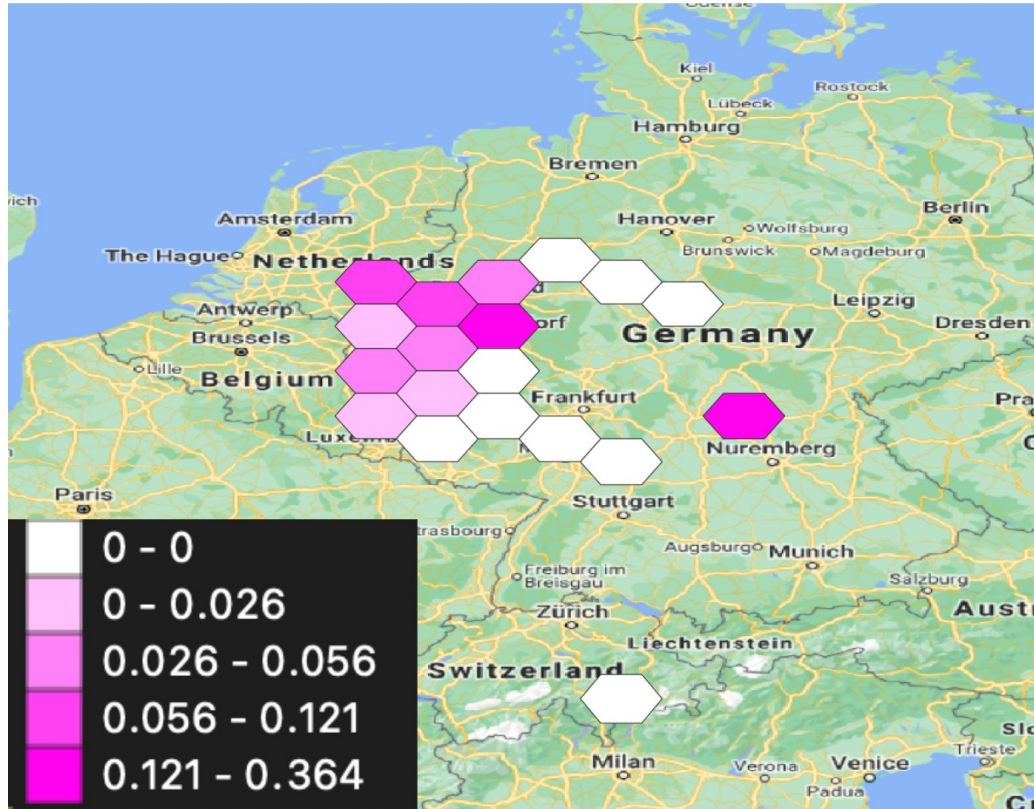
Figure 5: Bsal prevalence hotspots worldwide based on data from AmphibianDisease.org in 100km hexagon bins. The key is not converted to percentages.

Patterns of Bd Distribution in Taxonomy

I found several amphibian families have high prevalence for Bd. As seen below in Table 1, 17 families met the criteria for taxonomic analysis with more than 30 samples for Bd. These families are Alytidae, Arthroleptidae, Bufonidae, Ceratobatrachidae, Craugastoridae, Dicroglossidae, Hemiphractidae, Hylidae, Hyperoliidae, Megophryidae, Microhylidae, Myobatrachidae, Plethodontidae, Ranidae, Rhacophoridae, Salamandridae, and Strabomantidae. The three families with the highest Bd prevalence are Myobatrachidae with 97.9%, Strabomantidae with 41.5% and Hylidae with 38.2%.

Family Name	Prevalence
Alytidae	26.3% (15/57)
Arthroleptidae	27.0% (33/122)
Bufonidae	11.5% (45/390)
Ceratobatrachidae	5.3% (2/38)
Craugastoridae	7.5% (33/439)
Dicroglossidae	2.4% (10/418)
Hemiphractidae	31.5% (17/54)
Hylidae	38.2% (642/1680)
Hyperoliidae	26.1% (59/226)
Megophryidae	1.1% (5/471)
Microhylidae	3.7% (3/82)
Myobatrachidae	97.9% (94/96)
Plethodontidae	5.7% (64/1114)
Ranidae	12.7% (191/1500)
Rhacophoridae	5.4% (19/351)
Salamandridae	3.7% (83/2217)
Strabomantidae	41.5% (199/480)

Table 1: Amphibian families listed alphabetically with overall Bd prevalence percentages, followed in parentheses by positive Bd samples divided by total samples for the respective family.

DISCUSSION

I analyzed over 30,000 amphibian samples to determine the spread of Bd and Bsal, two aquatic fungal pathogens that cause the deadly amphibian disease chytridiomycosis. These samples are from the Museum of Vertebrate Zoology's Amphibian Disease Portal and are contributed by scientists around the globe. I show that Bd is recorded around the world and this has been supported elsewhere (Olson et al., 2013). Currently, the Amphibian Disease Portal shows that 41 of the 48 countries sampled have returned Bd positive results. Much less data is available for Bsal since it was discovered much more recently. Only recently have the German scientists agreed to contribute their data, which contained the only positive samples in the database. After a taxonomic analysis of Bd, I found that seven of the 17 families that met the criteria for taxonomic analysis had prevalences over 25%. There was not enough data available to complete a taxonomic analysis of Bsal.

Bd Geographic Range

As of February 7, 2021, 41 of the 48 countries sampled had positive Bd results. This confirms that Bd is currently very widespread across the globe and is a massive threat to amphibian populations (Scheele et al., 2017). Not all countries are included in this dataset, but authors have reported Bd from countries worldwide (Olson et al., 2013). However, the vast majority of countries around the globe have at least some amphibians (Amphbiaweb.org, 2021).

Because amphibians are globally spread out and species can seasonally acclimate to handle a variety of climates (Winterová & Gvoždík, 2020), the habits of an amphibian are more responsible for the spread of chytridiomycosis more so than solely climate. The amphibian pet trade causes ecosystems from across the globe to interact in ways that were previously impossible. The amphibian pet trade is a largely unregulated that has played a large part in spreading chytrid fungi (Fitzpatrick et. al 2018). A recent study found that over 17% of amphibian species are being traded around the globe as pets (Hughes et al., 2021). This leads to a

large disruption of wild amphibian populations, such as over-harvesting and increased predators, which in turn greatly increases the potential for global spread of amphibian pathogens.

Bsal Geographic Range

In comparison to Bd, the known range of Bsal is limited. The data on Bsal is also limited, which is hindering the ability to track the disease. Out of the six countries with Bsal data, only one has positive results; Germany returned 343 Bsal+ samples. A reason for this limited sampling could be because only 9% of amphibians are salamanders (Amphibiaweb.org, 2021), and Bsal only induces Chyt clinical signals in salamanders (Romero-Zambrano et al., 2021). These signals are what cause the disease chytridiomycosis to develop in salamanders, and if these signals are only able to develop in the 9% of amphibians that are salamanders, it will be harder to detect Bsal in sampling in comparison to Bd, which affects many more amphibian species.

Since Bsal was recently discovered in 2014 (Wake and Vredenburg 2008), this is another explanation for the lack of sampling. Many scientists have yet to share their information with the Disease Portal; this is one of the main reasons that the spread of Bsal is so difficult to track. It is known that Bsal originated in Asia and has caused an outbreak in Belgium, Netherlands, and Germany (Pyron et al., 2014).

Bd Infection Rate

Since 41 countries returned Bd+ samples, Bd hotspots were found all over the globe, with the highest prevalence being 100%; there were only a small number of samples in this geographic bin, however. Bd is widespread, and action must be taken to reduce its further spread as much as possible. Amphibians inhabit all continents except Australia. One possible reason that hotspots are so widespread around the globe is due to Bd's ideal temperature range being very large; the Bd strain of the chytrid fungi can optimally grow from 17° C and 25° C (Johnson et. al., 2003). The recent increase in the amphibian pet trade in recent years is also likely a contributing factor.

Bsal Infection Rate

Because only Germany had Bsal+ samples in the Disease Portal database, the hotspot analysis for Bsal was limited to Germany's borders. Within Germany, four main hotspots were identified. Their prevalences ranged from 0%-36.4%. Several of these hotspots were located near Germany's borders. Since Bsal data is lacking from surrounding countries in the Disease Portal, it is likely that Bsal has spread to surrounding countries and is not represented in this analysis.

Taxonomic Analysis of Bd Infections

Out of 74 amphibian families, only 17 met the requirements for taxonomic analysis. Myobatrachidae had the highest reported Bd prevalence with 97.9% of samples submitted being Bd+. This family was an anomaly in comparison to other families, as the next highest reported prevalences were Strabomantidae with 41.5% and Hylidae with 38.2%. Since the samples submitted to the Amphibian Disease Portal are done on a volunteer basis, it is likely that there is a sampling bias towards positive samples. The prevalences reported here may be inflated if the scientists reporting their data were omitting negative samples.

Seven of the 17 amphibian families that were included in this taxonomic analysis had prevalences over 25%. It is apparent that behavioral differences among species seem to have larger impacts on Bd infections. A research study found that amphibian species whose larvae are aquatic-born have higher infection rates early in life compared to direct-developing amphibians; however, these aquatic-born larvae tend to have better resistance to Bd later in their lifespan if they survive,as they are initially exposed to the aquatic zoospores of chytrid fungi (Mesquita et al., 2017).

Only one amphibian family met the requirements for a taxonomic analysis of Bsal spread, Salamandridae, so no taxonomic analysis was conducted. There is not enough data to make accurate predictions of taxonomic influences in the spread of Bsal.

LIMITATIONS

AmphibiaWeb's Disease Portal submissions come from researchers around the world, who volunteer to share their data. Because of this, the sampling of amphibians is unevenly distributed, which leads to large sampling biases. For example, the United States makes up a large portion of the Disease Portals samples, however, some countries that are also species rich in amphibians are completely unrepresented in the database. Amphibiaweb's database is expanding daily and they are working to acquire samples from all around the world. Another consideration for my analysis is that these samples are self reported and scientists may not be reporting all of their samples. There should be emphasis on reporting negative data as well as positive test results.

For my taxonomic analysis, the testing of Bd and Bsal within amphibian families is uneven. Again, the lack of systematic disease testing will always limit this analysis. However, by calculating the prevalences of Bd and Bsal within amphibian families rather than purely counting positive samples we can see where the data is available and understand the bias that may have led smaller amphibian families to have different results than larger ones.

FUTURE IMPLICATIONS

The ongoing amphibian crisis makes it clear that action must be taken to limit the spread of the chytrid fungi Bd and Bsal. One of the most direct ways to combat the spread of Bsal is through the regulation of the amphibian pet trade. Anthropogenic trade of amphibians has greatly increased their dispersal and changed interactions between species; this has greatly increased the occurrences of disease spread (Scheele et al., 2019). International regulation of the amphibian pet trade could greatly reduce the spread of chytrid fungi around the globe. Several salamander species that are popular in the amphibian pet trade are those who have already been affected by Bsal (Yap et al. 2015); this makes the regulation of the amphibian pet trade even more of a pressing issue. One plausible regulation would be to test amphibians for Bd, Bsal, and other fungal diseases before their shipment. It would also be critical to test the amphibians they are exposed to. Amphibians provide crucial ecosystem services, and it is important to not let the global pet trade negatively affect wild populations.

Though my analysis was thorough with respect to the available public data in the Amphibian Disease Portal, it is clear that we still lack enough data to best track the spread of chytrid fungi. The decline of amphibians populations is a complex issue that does not have a one-size fits all solution. One research team studied 832 peer reviewed papers and found no general consensus on factors driving epidemiological patterns of pathogen transmission among amphibians (Bienentreu et al., 2020). With so much that still remains unknown about Bd and Bsal, there needs to be more research and funding devoted to solving this complex yet critical problem, and sharing data is essential to that endeavor.

ACKNOWLEDGEMENTS

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