A SPATIALLY EXPLICIT RISK ASSESSMENT OF SALAMANDER POPULATIONS TO *BATRACHOCHYTRIUM SALAMANDRIVORANS* IN THE UNITED STATES FOR DIRECTING CONSERVATION ACTIONS

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Abstract. Amphibian populations are threatened globally by anthropogenic environmental change (Wake and Vredenberg 2008) and *Batrachochytrium dendrobatidis (Bd)* (Skerratt et al. 2007). A closely related new fungal pathogen of salamanders, *Batrachochytrium salamandrivorans* (*Bsal*), has recently left its native range in Asia and decimated some salamander populations in Europe (Martel et al. 2014). *Bsal* has not been detected in the United States (Yap et al. 2015), but given the U.S. has the most salamander biodiversity on Earth (Richgels et al. 2016), predictive assessments of salamander risk are necessary to proactively allocate research and conservation efforts into disease mitigation. The present study mapped the risk of salamander populations to *Bsal* in the US based on the predicted environmental suitability of the *Bsal* pathogen in the US, and the distribution of potentially susceptible species to the pathogen. To predict the environmental suitability of *Bsal*, an ecological niche model was developed based on the pathogen's native range in Asia and validated on the observed invasive range in Europe using bioclimatic, land cover, elevation, soil characteristics, and human modification variables. Potentially susceptible salamander species were determined using a machine learning model that correlated known life history traits of a species with literature derived data on actual and predicted susceptibility to *Bsal* for tested and untested species. Environmental suitability, number of susceptible species, and risk to *Bsal* infection were highest in the Pacific Northwest, Gulf and Atlantic coasts, and inland states east of the plains region. The overlap of these three metrics for risk of salamander populations to *Bsal* provides direction for researchers and conservationists in efforts to protect already threatened salamanders from an additional pathogenic disturbance.

INTRODUCTION

Globally, amphibians are being threatened by *chytridiomycosis,* a disease caused by two pathogenic chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) (Yap et al. 2017). *Bd* was discovered prior to *Bsal* in the late 1990s and has since infected at least 700 amphibian species (Lips et al. 2016). Although susceptibility to both pathogens varies among species (Bancroft et al. 2011, Carter et al. 2019, Sabino-Pinto et al. 2018), *Bd* has led to the population decline of over 200 species of amphibians (Skerratt et al. 2007) and continues to threaten amphibian biodiversity over 20 years later (Wake and Vredenberg 2008). *Bsal*, which is endemic to Asia and specifically targets salamanders, spread into Europe around the late 2000s and decimated a population of fire salamanders in the Netherlands (Martel et al. 2013). Thus far, there has been no evidence for *Bsal* presence in North America, but due to the continent's high salamander biodiversity, invasion of *Bsal* poses a serious threat to North American ecosystems (Richgels et al. 2016, Yap et al. 2015). Given the fragility of amphibian populations in the face of *Bsal* and *Bd*, predictive risk assessments for the vulnerability of salamanders in the continental United States (US) to *Bsal* are necessary to allocate prevention and mitigation efforts by conservation managers.

These risk assessments should be based on both the environmental suitability for *Bsal* and the susceptibility of different species to *Bsal* infection in order to identify areas that are most at risk for salamander population declines. Past research efforts have sought to identify the areas in the US that are most vulnerable to an invasion of *Bsal*, but they considered risk to salamander populations based on how likely they are to encounter

Bsal due to trade, not how susceptible individual populations are to *Bsal*. First, the Pacific coast, southern Appalachian Mountains, and mid-Atlantic regions are predicted to have the highest risk from *Bsal* infection in the US based on the environmental suitability of each county for *Bsal*, salamander species richness, and an assessment of salamander import and trade data, which indicate where *Bsal* might first invade (Richgels et al. 2016). This risk assessment is useful because it identifies areas in which actions should be taken to prevent *Bsal* invasion through trade. However, it does not consider species differences in susceptibility to infection. Also, the risk assessment estimates environmental suitability of *Bsal* by matching county temperature data with the lab measured optimal temperature for *Bsal*, and not from an analysis of the multidimensional environmental constraints of *Bsal* in its native range. A more robust model of the environmental suitability of *Bsal* would be done using ecological niche modelling (ENM), which is a method to estimate the geographical distribution of organisms based on correlations between their occurrence and the local multidimensional environmental constraints (Johnson et al. 2019). In the case of chytrid fungi, *Bd* is constrained by both temperature and precipitation (Olson et al. 2013), both of which would be captured in an ENM along with other factors.

In a second study by Yap et al. (2015), the southeastern US and Pacific coast are predicted to have the highest risk for *Bsal* based on an ENM that estimated the environmental suitability for *Bsal*. Similar to Richgels et al. (2016), risk is associated with areas of high species richness and import and trade levels for salamanders. However, the ENM used by Yap et al. (2015) has been criticized by Feldmeier et al. (2019) as biologically irrelevant because it is created for the US using potential *Bsal* salamander host occurrence data from the native range in Asia, rather than data of *Bsal* pathogen occurrences themselves, which is an analysis that may overlook places in the US where *Bsal* could exist and its hosts could not or vice versa.

While ENM is useful for discerning environmental suitability, Bsal invasion also depends on host susceptibility to infection, which varies across species. Previous risk assessments do not consider these differences in susceptibility of various salamander species to *Bsal* in risk evaluation (Richgels et al. 2016, Yap et al. 2015). Since some salamanders are asymptomatic with the infection (Carter et al. 2019, Sabino-Pinto et al. 2018), it is important to know which species are more susceptible and where they exist in the US relative to areas likely to be habitable by *Bsal* in order to properly channel prevention and mitigation efforts. A prediction based on differences in susceptibility to *Bsal* would be more accurate to population declines of salamanders, which is the ultimate threat of *Bsal* (Wake and Vredenberg 2008). Therefore, as far as we know, no study to date has created a risk model for *Bsal* invasion based on the combination of a credible ENM and species differences in susceptibility to disease, which would together give a more robust prediction of the threat to biodiversity from *Bsal* than the import and trade oriented risk assessments by Yap et al. (2015) and Richgels et al. (2016).

We identified areas in the US at risk for salamander population declines from *Bsal* by combining *Bsal* suitability with a spatial assessment of the ranges of salamander species with varying susceptibility to *Bsal*. Therefore, this study was a two part process in which we first used an ENM to assess where in the US is suitable for *Bsal* based on its known environmental constraints in its native range. Then, we used a machine learning approach to correlate life history traits with literature derived data on *Bsal* infection in salamanders, and we predicted the likelihood of infection in salamanders based on their life history traits. A study with a similar aim was conducted globally by Rödder et al. (2009) for *Bd*, in which they combined an ENM for *Bd* occurrence with the known ranges of amphibian species with life history traits that made them more susceptible to population declines from *Bsal* infection as determined by Bielby et al. (2008).

We expanded on past *Bsal* suitability models in the US and, to our knowledge, did the first assessment of life history traits that predict *Bsal* susceptibility. First, in contrast to *Bd* (Weldon et al. 2004), *Bsal* has more recently left its native range and invaded other areas (Beukema et al. 2017). As a result, we used ENM techniques that test the model, trained using the native range of *Bsal*, on data from Europe to assess its

performance in an invasive range for which pathogen occurrence data exist, similar to the methods used for predicting the range of *Bsal* in Mexico by Basanta et al. (2019). To determine the important life history traits for salamanders that determine susceptibility to *Bsal* we will use a machine learning approach similar to Bancroft et al. (2011) that will identify what the probability a species will be susceptible to *Bsal* infection. Together, robust ENM modelling of *Bsal* as an invasive pathogen coupled with machine learning techniques to determine susceptibility of salamanders to *Bsal* will give a useful model for the vulnerability of salamander populations to *Bsal* in the US. We expect to find areas that are both a suitable environment for *Bsal* and have salamanders that are highly susceptible to infection, and our results will direct conservation efforts and future research to those areas.

METHODS

The present study will combine two objectives to create a spatially explicit risk assessment for salamander population decline due to *Bsal*. First, we will create an ENM for the *Bsal* infection that determines where in the US *Bsal* is likely to occur based on its environmental preferences in its native range in Asia. Then, we will use machine learning to predict which life history traits make a salamander species susceptible to *Bsal* infection, and we will determine areas of high salamander susceptibility using range maps of each species. By combining these two methods, we can determine where in the US there is both high likelihood of *Bsal* occurrence and susceptibility of salamanders to infection.

Ecological niche model

We created an ENM of *Bsal* to predict its likelihood of occurrence in the US using the MaxEnt algorithm as implemented in the dismo package (Hijmans et al. 2017) in R version 4.0.1 (R Core Team 2020). First, we identified and collected spatially explicit datasets for variables that we deemed potentially important for predicting *Bsal* occurrence based on past research. Then, we eliminated variables that were highly correlated in the native range. We used these final variables to create an ENM of *Bsal* in its native range, and, based on this model of the native range, we predicted the occurrence of *Bsal* in the US. To assess how well our model of *Bsal* in the native range transfers to an invasive range for the pathogen, we tested how well the model predicts already observed occurrences of *Bsal* in its European invasive range.

Data collection and preparation

We searched past literature on both *Bsal* and *Bd* and found studies that describe how environmental variables predict chytrid occurrence globally or locally in order to identify potential predictors of *Bsal* for our ENM. Our literature search was structured in Google Scholar by looking at all articles in the first five pages of results using the search terms "Batrachochytrium salamandrivorans" or "Batrachochytrium dendrobatidis" and "environmental constraints." We also examined other cited papers from each article. Based on commonalities between the literature and the availability of datasets, we decided to use Global Human Modification (GHM) (Kennedy et al. 2018, Bacigalupe et al. 2019, Spitzen van der Sluijs et al. 2014), elevation (Amatulli et al. 2018, Bacigalupe et al 2019, Bielby et al. 2008, Olson et al. 2013), 12 land cover classifications (McMillan et al. 2019, Murray et al. 2011, Scheele et al. 2014, Tuanmu et al. 2014), soil pH (Hengl et al. 2017, Kärvemo et al. 2018), and the 19 bioclimatic layers from Worldclim (Fick and Hijmans 2017, Basanta et al. 2019, Beukema et al. 2018, Puschendorf et al. 2008, Rödder et al. 2009). Although it has not previously been studied, we also decided to include soil organic carbon (Hengl et al. 2017), due to the important role salamanders play in carbon cycling by increasing litter retention and carbon capture (Best and Wesh 2014), which may correlate with areas of *Bsal* salamander hosts.

For creating, evaluating, and applying the model, we defined three study extents which were in Asia, Europe, and the US respectively. The native study extent encompassed an area 20 percent greater than the range of

native *Bsal* hosts determined by Laking et al. (2017), Martel et al. (2014), and Yuan et al. (2018), whose range maps are made available by the IUCN (IUCN 2020). We used the same study extent defined in Beukema et al. (2018) for the invasive range. Finally, our study extent for the US bounded the lower 48 states. Occurrence points for *Bsal* in Asia and Europe were taken from Beukema et al. (2018) and González et al. (2019). All of our predictor variable datasets were then cropped to the three study extents and resampled to achieve a resolution of 10 kilometers, which was chosen to capture the uncertainty in *Bsal* occurrence points in a single grid cell.

Background points were sampled randomly in areas around the location of salamander occurrences in the native range to reflect where in that area would be reasonable places for *Bsal* to exist. We downloaded the occurrence of salamanders from the Global Biodiversity Information Facility (GBIF) (GBIF.org 2020) in the native range, and we eliminated entries with uncertainty higher than our study resolution, no coordinate data, duplicates, and common issues flagged by GBIF. With the remaining occurrences, we defined a buffer of 50 kilometers around each point and sampled 10,000 points randomly within the cumulative area.

ii. Model preparation

We determined which of our predictor variables were correlated with each other in the native range using a Pearson's correlation coefficient of greater than or equal to 0.8. We also did a principal coordinate analysis (PCA) of the predictor variables and found the magnitude of variation that each variable drove. We only looked at the first 90 percent of variation. For each group of correlated variables that we found, we chose the variable which drove the highest variation based on the PCA to keep in our ENM model. Based on these analyses, bioclimatic variables 2 (mean diurnal range), 3 (isothermality), 4 (temperature seasonality), 9 (mean temperature of the driest quarter), 14 (precipitation of the driest month), 15 (precipitation seasonality), 18 (precipitation of the warmest quarter), 19 (precipitation of the coldest quarter), all land cover classifications, elevation, soil pH, and soil organic carbon were included in our modelling attempts.

iii. ENM implementation and prediction

The ENM was created using MaxEnt with the ENMeval package (Muscarella et al. 2014) and dismo packages (Hijmans et al. 2017) in R (R Core Team 2020). First, we created the ENM using data from the native range. Parameters for the final MaxEnt model were found using the ENMeval package, which generates multiple MaxEnt models across multiple combinations of regularization multipliers (RM) and feature class combinations (FC). The RM values were set at increments of 0.5 and FCs were created using combinations of "linear," "quadratic," "hinge," threshold," and "product" binary settings. We extracted the values for RM and FC from the MaxEnt model generated with the lowest delta value of the corrected Akaike's information criteria (dAICc), which was equal to 0 to maximize model fitness. Based on this dAICc score, we created our final MaxEnt model with an RM of 4.0 and an FC of "linear" and "quadratic".

Then, using the MaxEnt model we created in the native range, we predicted the likelihood of *Bsal* occurrence in the native, invasive, and US ranges. We used three metrics to evaluate our model in the native range. Area under the curve (AUC) and true skills statistic (TSS) describes the relationship between the true positive rate and false positive rate or true negative rate respectively. The omission rate measures the number of omitted true positive points by the model at various thresholds. We calculated the omission rate in the European range to see how many true positive points were omitted in an invasive range and validate our model for use outside of Asia. For the TSS and omission rate we compared model performance across three thresholds, minimum presence, lowest 10% of presence, and maximum sensitivity and specificity. The thresholds calculated the model metrics using a value of suitability set by, the lowest suitability value a, to which a presence point corresponds, the suitability value of that bounds the lowest 10% of presence points, and the suitability value that maximizes the true positive rate and true negative rate, respectively. We also compared the permutation importance of each of our predictor variables in the final model, and plotted the relationship between the

values of each predictor and its prediction values for the likelihood of *Bsal* occurrence (Supplementary information). To aid conservation managers, we also show which quarter of the United States of America contains the highest abiotic suitability to *Bsal*. Our measure of suitability in each study range is a probability of *Bsal* occurrence.

Boosted regression tree analysis

To know which salamander species are most susceptible to *Bsal* infection, we correlated salamander life history traits with *Bsal* occurrence in species using a machine learning approach. First, we collected data on salamander species with positive or negative test results for *Bsal* and we combined these data with publicly available salamander trait data. Then, we used a boosted regression tree (BRT) to find multiple correlations between trait data and *Bsal* occurrence and predict the occurrence of *Bsal* in species based on their traits. Finally, we combined maps of salamander extent with *Bsal* susceptibility as determined by our model to show which areas of the US have the highest risk of salamander infection due to *Bsal*.

Data collection

We found how salamander species tested for *Bsal* from past studies that surveyed *Bsal* infection across multiple species. The first five pages of a Google Scholar search with the search terms "Batrachochytrium salamandrivorans" and "infection" or "experiment" were used to find these studies and their cited studies. For the purpose of knowing if a species can be infected by *Bsal*, we had to distinguish between surveys of *Bsal* infection in the wild from surveys of captive or experimental animals because a negative test result for a wild population of salamanders may reflect the lack of *Bsal* presence in an area rather than a species' resistance to the fungus. On the other hand, we were confident that a negative test result in captive salamanders more likely reflected a species resistance to the fungus because *Bsal* is considered to be widespread in captive populations in Europe (Sabino-Pinto et al. 2018), which is from where we collected the majority of our data (Fitzpatrick et al. 2018, Sabino-Pinto et al. 2018). We also collected data from studies that experimentally tested whether or not a species was susceptible to *Bsal* infection through inoculation with the fungus (Barnhart et al. 2019, Bates et al. 2019, Carter et al. 2019, Martel et al. 2014). We removed subspecies because life history trait data was not available for this taxonomic level, and if any subspecies were positive for *Bsal* we made the species from which they stem positive. Also, we removed multiple records for the same species. If any of the multiple records were positive for a species, we recorded the species as positive, even if there were other negative test results for the species. If any species tested positive in the wild based on our occurrence data from our ENM, we recorded them as positive here. Furthermore, if there were other species in the same location in the wild as the species that tested positive from our ENM data, and those species tested negative, we recorded those species as negative because we assumed they had high likelihood of contact with *Bsal* (e.g. González et al. 2019).

We collected trait data for 619 salamander species from the Amphibio dataset, which is the number of salamander species discovered by the year 2011 (Oliveira et al. 2017). Binary traits came in the following groups, habitat, diet, diel, seasonality, and breeding strategy. A species was a combination of the traits in those categories, for example, either nocturnal, diurnal, or crepuscular or some combination of the three. Where data on a trait was not recorded in past literature for a species, the Amphibio dataset includes NA values (Oliveira et al. 2017). As a result, binary traits had either an NA or one value. However, BRTs require zero values as well to properly function. We decided to make all NA values zero for binary traits, where at least one binary trait within its grouping was not NA for a species. This assumption is reasonable because we consider the binary traits that are equal to one for a species to be the primary trait of a specific grouping since they were found first.

All 619 species were used to create our BRT model, and the infection status of each species for *Bsal* fell into one of three categories, which were either a positive record, a negative record from the literature, or a negative

record for species in the AmphiBio dataset but had not been tested for *Bsal*. We applied weights for each of these categories calculated as the multiplication product of the confidence we had in the accuracy of the infection status for showing *Bsal* susceptibility for each species and the proportional representation of each category in the dataset. We had the least confidence in test results for species with no data on *Bsal,* which was a confidence value of one. Species that were negative based on the literature were assigned a confidence value of two. Finally, we were twice as confident in the positive records as we were for the negative records based on the literature, so we assigned positive records a confidence value of four. Within each category, confidence values were multiplied by values for representation of each category in the dataset. Since, negatives based on no data far outnumbered the other two categories, the representation value was equal to how much less frequently the positive and negative based on a test result categories appear in the data in comparison to the negatives based on no data. Therefore, the representation value was equal to the proportion of the data that was negative based on no data divided by the proportion of the data in each of the other categories.

ii. Model preparation and execution

We used the gbm package (Greenwell et al. 2020) in R (R Core Team 2020) to implement our BRT model. To make predictions, the BRT uses a subset of the provided data to combine multiple regression models and then tests the accuracy of the model on the remaining data (Elith et al. 2008). Model hyperparameters, including the learning rate and variable interaction complexity, were maximized by testing multiple hyperparameter combinations and finding the combination of parameters which gave the best testing data AUC, using a fivefold cross validation, because there was low variation in training data AUC across hyperparameter combinations. A learning rate of 0.001 and an interaction complexity of three were the optimal model parameters for our model. Given the stochastic nature of model predictions, we found the average model metrics, including variable importance, AUC, optimal trees, and marginal effect, with these parameters by bootstrapping the model 5 times. After the one round of bootstrapping, we removed the variables with zero importance from the rest of the analysis and redid the bootstrapping for final model metrics. We also bootstrapped a null model with separately randomized *Bsal* test results and their corresponding weights for each species to determine a corrected test AUC and ensure that our model predicted better than random. Each bootstrapping run included 5 bootstraps, and 80% of the data was used for training and 20% of the data was used for testing.

Using the optimal model parameters, we then predicted the probability of *Bsal* infection for each species based on our trait variables. To create a binary prediction result, any species that was at least 50% likely to be susceptible to *Bsal* we labeled as susceptible, and any species less than 50% likely to be susceptible to *Bsal* was not susceptible. We evaluated our model at this stage by comparing the prediction for each species to its literature derived *Bsal* test result and finding the number of false positives and negatives and true positives and negatives. Using this assessment of prediction, we tried two other weighting schemes by applying no weights and only weights for confidence. The aforementioned scheme gave the best results by minimizing false positives and negatives and maximizing true positives and negatives.

iii. Spatial analysis

We found the number of salamanders at risk across the US as the percentage of salamanders susceptible to *Bsal* relative to salamander richness in an area. All salamander range maps for the US were downloaded from IUCN Red List (IUCN 2020). At a resolution of 100 kilometers, we found the total number of ranges that overlapped a grid cell to calculate species richness. The same method of summation was used to calculate the total number of species susceptible to *Bsal* in each grid cell. Finally, the percentage of salamanders susceptible to *Bsal* in each grid cell was the quotient of the number of species susceptible divided by species richness.

iv. Risk analysis

The product of the percentage of salamanders susceptible to *Bsal* and the ENM prediction gave the final estimate of risk of salamanders to *Bsal* in a specific location in the US. We normalized the value for risk in every grid cell in the US by diving by the maximum predicted risk value in the US. Also, we show which quarter of the country is most at risk from *Bsal* by highlighting areas in the top quartile of risk factor values.

RESULTS

In the native range the ENM had an AUC of 0.90 (Table 1). The TSS was lowest for the minimum presence threshold and increased by about half at the lowest 10% of presence points threshold. Similar to omission rate, it was highest using the maximum sensitivity and specificity threshold. Omission rate remained below 20% for all thresholds and was below 5% for the minimum presence threshold. The omission rates in the invasive range remained below 15%. They approximately doubled from the minimum presence and maximum sensitivity and specificity to the lowest 10% threshold.

The most important variables for prediction were bioclimatic variables two (mean diurnal range) and four (temperature seasonality), presence of evergreen deciduous needleleaf trees, and presence of herbaceous vegetation (Supplementary information). These variables were at least five times more important than all other variables. All of these variables were inversely related to the presence of *Bsal* (Supplementary information).

In both the native and invasive range, the most suitable places predicted for *Bsal* tend to be close to coastlines or on islands (Figure 1). Coastlines with suitability above 0.75 in both of these ranges include Vietnam, Norway, and Taiwan. The same trend applies to the US where places such as the Rocky mountain and plains regions have low suitability for *Bsal* while areas such as the Pacific Northwest, Florida, and the Gulf and Atlantic coasts (Figure 1). An exception is the Appalachian mountain region and other areas in the eastern half of the country which are all included in the quarter most suitable areas for *Bsal* in the US (Figure 2). However, based on the ENM the US is generally less suitable for *Bsal* than Europe and Asia (Figures 1 and 2).

The BRT model metrics were a training AUC of 0.99, a testing AUC of 0.90, a corrected AUC of 0.85, and an optimal number of trees of 1601. The model predicted less than 10 false results, which corresponds to accurate predictions greater than 91% of the time (Table 2). Maximum longevity and mean body size were both at least two times more influential for prediction than the other variables in the model (Figure 3). Both of these variables predict a maximum susceptibility around their median values (Figure 4). Furthermore, as the maximum litter size increases, the third most important variable (Figure 3), susceptibility to *Bsal* infection also increases (Figure 4). Finally, inclusion in the family Salamandridae is more than twice as important for predicting *Bsal* susceptibility as inclusion in any other Caudata group (Figure 3), and inclusion in this family means increased susceptibility to *Bsal* (Figure 4).

The highest number of susceptible salamanders occurs in the southern Appalachian region, while moderate amounts occur throughout the states east of the plains region and the northern Pacific coast, and these areas roughly correspond with areas of the moderate to high salamander species richness in the country (Figure 5). The product of the percent susceptible species and pathogen suitability in each location shows that the Gulf coast of Texas and Louisiana, the Pacific Northwest, and Florida appear to have the highest risk for infection of *Bsal* (Figure 5). However, most areas in states east of the plains region have risk of species infection by *Bsal* (Figure 5), and all of these states are at least partially included in the quarter of the country most at risk (Figure 6).

DISCUSSION

The United States has the highest biodiversity of salamanders on Earth (Yap et al. 2015). We show that areas of the most *Bsal* suitability and salamander susceptibility to infection overlap in the US, a finding that demonstrates the both demonstrates the need for and gives direction for future conservation actions. An ENM was used to predict the suitability of the environment in the US for the *Bsal* pathogen, which was combined with predictions of species susceptibility to infection from *Bsal* to estimate risk to salamanders across the country.

First, the ENM model gives a strong prediction for areas in the country that would be suitable for the *Bsal* pathogen because of its strong model metrics. In the native range, the model's AUC was high, the omission rate remained below 20% at all thresholds, and the TSS was above 0.5 for two thresholds. The places of highest pathogen suitability in the US are relatively close to coastlines, which is likely driven by the model's reliance on high mean diurnal range and temperature seasonality that exist near coastlines (Fick and Hijmans 2017).

One potential issue with this ENM model is the inherent difficulty in accurately predicting suitability into an invasive range in the US with no records of *Bsal* there to test the prediction (Beukema et al. 2018). We address this issue of model transference by seeing how well the model performs on a different invasive range in Europe for which there are *Bsal* occurrence records, similar to the workflow of Basanta et al. (2019). There was a low possibility of overfit by our model prediction as signaled by the omission of only five to ten percent of *Bsal* records in the European invasive range depending on the omission rate threshold. Therefore, we conclude that the ENM created in the native range projects well into an invasive range. This result is in accordance with the finding from Beukema et al. (2018) that *Bsal* in Europe only occupies a subset of the natively occupied environmental space, which proves both good model performance in an invasive range and the likely expansion of *Bsal* throughout Europe. Although strong model performance in the invasive range proves the ENM works well outside of the pathogen's native range, future work should see to what extent the environmental space in the US overlaps that of the native range to specifically know how well ENM predictions will transfer from the native to US range. Even though the model transfers well into an invasive range, the US range might have a much different environmental space than other invasive ranges and the native range.

One reason to suspect misaligned environmental spaces and poor model transference between the US and southeast Asia would be that the main drivers of *Bsal* occurrence, temperature seasonality and mean diurnal range, are generally inversely related in these two ranges (Fick and Hijmans 2017). However, a demonstrated preference of *Bsal* for coastal areas supports our predictions for pathogen suitability in the US. Both bioclimatic variables are high in the native range, they are low across the US. If there were misaligned environmental spaces, environmental constraints in the US that are similar to low temperature seasonality and mean diurnal range but not present in the native range may permit *Bsal* survival that our model does not predict. In contrast, one mechanism for this trend in bioclimatic variables may be the proximity to coastal areas (Fick and Hijmans 2017), and most *Bsal* occurrences in the native range are close to the Pacific Ocean. Likewise, the most suitable areas for *Bsal* in the US are close to the coastlines, and the same is generally true in the invasive range. Basanta et al. (2019), Beukema et al. (2018), and Puschendorf et al. (2009) found similar trends in their work where the southern Gulf coast of Mexico, northwestern coast of Europe, and Costa Rica showed high suitability for *Bsal* respectively. In fact, a widespread sampling within the native range in China found no occurrences of *Bsal*, and those sampling locations occur mostly inland (Zhu et al. 2014). Furthermore, Olson et al. (2013) found that the other chytrid fungus, *Bd*, occurred less with increasing temperature range, which aligns well with our findings in the native range. Therefore, the fact that *Bsal* suitability is generally limited to the coastlines of the US may be due to its preference for these areas, rather than poor model transference due to misaligned environmental spaces.

Although *Bsal* suitability is generally low across the country, we show the quarter of the country by area that is most suitable for *Bsal*, including the Appalachian mountain range, the southeastern Gulf and Atlantic coasts, and the Pacific Northwest. In order to discern appropriate conservation actions in these regions, however, it is important to know if the areas suitable for the *Bsal* pathogen are also inhabited by salamanders that are susceptible to *Bsal* infection. We used a machine learning approach (BRT) to predict the susceptibility of salamanders to *Bsal* based on their life history traits. Our BRT model was able to predict salamander susceptibility to Bsal with 90% accuracy, and also showed low false positives and low false negatives in predictions. The most important variables in these predictions were max longevity, body size, and max litter size. Compared to other salamander species, we find that *Bsal* hosts tend to have intermediate body sizes and life spans while still achieving high fecundity. We postulate that susceptible species with intermediate body size of between 250 and 750 millimeters and max longevity of around 20 years might represent a tradeoff between increased contact rate with the pathogen over the body's surface area and throughout life (Kuris et al. 1980), and increased investment in immune function by longer lived (Johnson et al. 2012) and larger individuals (Downs et al. 2020). Similarly, species with larger litter sizes tend to have lower investment in immune function (Johnson et al. 2012), and we show they are more likely to be susceptible to *Bsal*. These results are in accordance with trait based modeling done for *Bd* susceptibility by Bancroft et al. (2011) that showed species with large body sizes and clutch sizes are more susceptible to *Bd* infection. Bancroft et al. (2011) also propose that large clutch sizes indicate species spend a longer time in amplexus which increases contact between individuals as another mechanism for driving chytrid infection. In contrast, Bielby et al. (2008) found that small clutch sizes lead to species declines in species with *Bd*, which considers both species susceptibility to disease and population dynamics. However, as rationalized by Bancroft et al. (2011), the finding in Bielby et al. (2008) may be confounded by the fact that smaller population sizes driven by small clutch sizes are more at risk of population declines, which outweighs the decreased susceptibility of these species with small clutch sizes to *Bd*.

In the present study our definition of susceptibility defines the first level of possible response of salamanders to the *Bsal* pathogen, which is to be infected or not. However, among the salamanders that are susceptible to *Bsal* there are differences in the degree to which *Bsal* affects species. For example, some salamanders are asymptomatic to infection, while other salamanders show clinical signs of infection but limited mortality (Martel et al. 2014). Future work should examine traits predicting to what degree salamanders respond to *Bsal* infection, which would expand on the present definition of susceptibility and provide a more focused view of susceptibility across species. Such work would require more experimental data to be available, such as the data in Carter et al. (2019) and Martel et al. (2014), that determines the level of susceptibility of some species. The main issue with this data is that an individual's response to infection varies significantly across experimental conditions, such as temperature (Sauer et al. 2020). Therefore, a standardized experimental approach is also necessary.

Nevertheless, the present study begins to elucidate which areas in the country are inhabited by species that are at all susceptible to *Bsal* infection. We show that the areas with the most susceptible salamanders correspond to areas of high salamander species richness, *Bsal* suitability, and risk for *Bsal* infection, which are the Appalachian mountain region, Gulf and southeastern Atlantic coasts, and the Pacific Northwest. Other studies estimating *Bsal* risk in the US focused on the impact of trade on *Bsal* spread (Richgels et al. 2016, Yap et al. 2015). Our results, which include both species traits driving susceptibility and *Bsal* environmental suitability, largely corroborate these findings, highlighting these same areas plus the coast of California as potential hotspots for *Bsal* infection. Given widespread agreement among multiple spatial and biological risk assessments of *Bsal*, there is growing consensus that conservation efforts should be prioritized to particular areas with both high invasion risk from trade and high susceptibility of salamander species to *Bsal* infection. To this end, we provide all of our final datasets, including raster data for *Bsal* suitability, predictions for which species are most susceptible around the globe, and final raster risk data (Supplementary information), which may generate tailored data visualizations most relevant to inform local conservation and

research efforts. With this information, conservationists and researchers can use this study as a guide for directing their efforts in the future.

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APPENDIX

TABLE 1. Model evaluation metrics for the ENM in the native and invasive ranges.

	Model evaluation metric			
	Native range			Invasive range
Threshold	AUC	TSS	Omission rate	Omission rate
Minimum presence	0.90*	0.38	0.03	0.05
Lowest 10% of presence		0.52	0.12	0.11
Maximum specificity and sensitivity		0.69	0.18	0.05

*AUC not defined for a threshold

TABLE 2. A comparison of the observed infected status for species with available test data in the literature and the predicted infection status of those species by the BRT model.

		Observed infection status	
		Positive	Negative
Predicted infection status	Positive	40	6
	Negative	2	47