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Predicted suitability of the invasive species, American bullfrog (*Lithobates catesbeianus)* in Central Europe based on climatic features

DIPLOMA THESIS

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In Prague on ....31.03.2023.... Breanna Emerson

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DIPLOMA THESIS ASSIGNMENT

Title

Current distribution and invasion potential of the American Bullfrog, Lithobates catesbeianus in Central Europe

Objectives of thesis

Amphibians belong to the most threatened taxa and many amphibian species are becoming increasingly vulnerable. One of the most influential causes of amphibian population declines, however, is a result of fellow invasive amphibians, particularly the American Bullfrog (Lithobates catesbeianus). The American Bullfrog is native to eastern North America and part of Mexico, but worldwide distribution nowadays including west and south Europe. Its presence has become a conservation concern since Bullfrogs are known to predate on threatened native species while also out competing for resources, as well as being a vector for deadly pathogens. Therefore, the goal of the thesis is create a literature review on current distribution of the species in Europe and its habitat requirements. Within the practical part of the thesis, an invasion potential of the species to Central Europe and the Czech Republic will be assessed.

Methodology

To understand the potential for American Bullfrogs to inhabit the Central European region, reviewing any past research on the dispersal history of this species and its habitat requirements will be performed. Understanding the most significant factors and whether the Central European ecosystem can provide a future habitat for the American Bullfrog can give insight into the possibility of invasion. This can be explained through a Species Distribution Models (SDM, specifically tool MaxEnt), a common method to explain future distribution predictions of a particular species.

ABSTRACT

Invasive species have become a growing concern worldwide, and predicting their further dispersal is crucial for developing effective management strategies. The American bullfrog (*Lithobates catesbeianus*) is a widespread invasive species known to cause substantial ecological impacts, most especially on native amphibian species. Despite the organism’s global distribution, it has yet to establish populations within the central European ecoregion. To evaluate the possible risk of invasion of this species, this study aims to analyze the potential suitability of *L. catesbeianus* in this region based on climatic conditions, primarily temperature and precipitation. This assessment was performed using a Species Distribution Model (SDM), which is a maximum entropy modeling system that incorporates known species distribution patterns and associated climatic conditions to determine suitability within central Europe. The results showed the highest suitability in Germany as well as parts of Czechia and Poland, with varying degrees. The analysis showed a higher influence from temperature in determining suitable localities for this species than precipitation. According to the study, *L. catesbeianus* favored habitats with a wider annual temperature range, showing highest suitability in regions experiencing fluctuations in temperature of up to 25^{o}C. The results from this study indicate suitable conditions within the currently uninhabited central European region for potential establishment and spread of this invasive species. By identifying areas where invasion risk is high, we can be proactive in developing essential prevention measures from this harmful organism, thus protecting the integrity of the native ecosystem.

Key Words: Species Distribution Model (SDM), American bullfrog (*L. catesbeianus*), Central Europe, Suitability Maps, invasive species

ABSTRAKT

Invazní druhy se staly celosvětově rostoucím problémem a predikcejejich dalšího šíření je zásadní pro rozvoj účinných strategií v ochraně přírody. Skokan volský (*Lithobates catesbeianus*) je široce rozšířený invazní druh, o kterém je známo, že má značné ekologické dopady, zejména na původní druhy obojživelníků. Navzdory globálnímu rozšíření organismu nejsou jeho populace ve středoevropském prostoru známy. Pro zhodnocení možného rizika invaze tohoto druhu je cílem diplomové práce analyzovat potenciální vhodnost podmínek prostředí ve střední Evropě pro *L. catesbeianus* na základě klimatických podmínek, především teploty a srážek. Toto hodnocení bylo provedeno pomocí Species Distribution Model (SDM), což je systém modelování maximální entropie, který zahrnuje známé vzorce distribuce druhů a související klimatické podmínky pro určení vhodnosti podmínek ve studovaném prostředí. Výsledky ukázaly, že v rámci střední Evropy existují vhodné podmínky pro výskyt skokana především v Německu a také v některých částech Česka a Polska. Analýza prokázala vyšší vliv teploty při určování vhodných lokalit pro tento druh než srážky. Skokan volský upřednostňoval stanoviště se značným ročním teplotním rozsahem, s nejvyšší vhodností v oblastech s výkyvy teplot až 25°C. Výsledky této studie naznačují, že existují vhodné podmínky v aktuálně skokanem volským neosídleném středoevropském regionu, tedy potenciální možnost usazení a šíření tohoto invazního druhu. Identifikace oblastí, kde je riziko invaze vysoké, je nezbytná při vývoji základních preventivních opatření proti tomuto škodlivému organismu, což umožní chránit integritu původního ekosystému.

Key Words: Species Distribution Model (SDM), American bullfrog (*L. catesbeianus*), Central Europe, Suitability Maps, invasive species

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1. Introduction

Invasive species are one of the biggest threats to biodiversity and ecosystem functioning worldwide, with 1 in 10 endangered species experiencing direct effects (*IUCN*, 2019). An estimated €28.5 billion is spent each year on management efforts for invasive species globally (Diagne *et al.*, 2021). Invasive species are characterized by efficient dispersal abilities and the capacity to adapt to variable niches. Effects from climate change are predicted to alter species distribution patterns, favoring those with these higher adaptability traits (Johovic *et al.*, 2020).

The American bullfrog (Lithobates catesbeianus) is one such species that has a worldwide distribution and is currently considered one of the world's top 100 most invasive alien species according to the IUCN (*The IUCN Red List of Threatened Species, 2020*). Although this species has not yet been observed in most of Central Europe, its potential for invasion is a serious concern given its proven ability to adapt to a wide range of habitats and its negative impacts on native fauna (Kats and Ferrer, 2003; Adriaens *et al.*, 2013).

The presence of American bullfrogs in foreign territories has been linked to the transmission of deadly viruses, such as Chytridiomycosis (Garner *et al.*, 2006; Miaud *et al.*, 2016), as well as competition pressures and predation on native fauna that have proven to possess the potential to wipe out entire fellow amphibian populations (Howell *et al.*, 2020; Adriaens *et al.*, 2013). Therefore, predicting the invasion potential of the American bullfrog in unoccupied regions, is essential to inform conservation efforts and prevent the associated negative consequences from further introductions of this destructive species. This is especially true in areas yet to be infiltrated, particularly habitat ranges that are currently in migrational proximity for this species. By determining important habitat features that contribute to this invasive organisms survival success within currently occupied areas, this information can be projected within those neighboring unoccupied spaces to identify the possibility of a future successful invasion (Andersen et al., 2021; Ficetola et al., 2007; Guisan et al., 2017).

2. Literature Review

2.1. Biological Traits of American Bullfrog

When attempting to develop regulation strategies for invasive species it is crucial to understand the unique traits leading to successful establishment within non-native ecosystems. There may be inherent traits that are genetically predetermined, or there are developed traits formed through phenotypic plasticity or rapid adaptation to the newly experienced environmental conditions (Cook et al. 2013).

2.1.1. Anatomy

The American bullfrog (*Lithobates catesbeianus*) is an amphibian species from the Ranidae family (True Frogs; Order Anura) (*The IUCN Red List of Threatened Species*, 2022). American bullfrog is a large breed, the largest true frog species in North America (*American Bullfrog | National Geographic*, 2010), reaching up to 22cm snout-vent length (SVL) and can weigh up to 500 grams or more. They are recognized to have a wide body, flat head and smooth skin with a few wrinkles or bumps (See Fig. 1) (Bruening, 2002; *National Geographic*, 2010).

Fig. 1, Photo of American bullfrog (*Lithobates catesbeianus*); (Harding, 2004)

The females are usually larger than males with an identifiable yellow coloring on their throat and black pads on their thumbs, present only during the mating season. Both males and females tend to have black or brown dots speckled all over, even during larval stage, which is the only feature that distinguishes them from the Green Frog (*Pelophylax* spp.) larvae (Adriaens *et al.*, 2013). Males and females do possess a form of sexual dimorphism that allows for identification in the adult forms. Males have a distinguishably larger tympanum, or external circular ear lobe (See Figure 1), than females do (Bruening, 2002). As an amphibious species, bullfrogs go through life stages consisting of larval (proceeding hatching of the egg), metamorph and adult stages (Adriens *et al.*, 2013). They are capable of living up to 10 years in the wild (Howell *et al.*, 2020). Adults are considered to be generalists, eating anything smaller than their own body size, including other amphibians (Kats and Ferrer, 2003) as well as having cannibalistic tendencies in which they consume members of their own brood (Alvarez & Nicieza, 2022; Bruening, 2002). Bullfrogs are considered “sit and wait” predators, in that they can stay in one place for extended periods of time, conserving energy, until any unsuspecting prey comes in close enough proximity for their long tongue to reach and grab hold of (Bruening, 2002). They tend to only be found in regions that do not reach below 15ºC, because adults will usually become inactive at lower temperatures, eggs will not hatch and metamorphosis will be unable to commence (Ficetola, Thuiller and Miaud, 2007).

2.1.2. Reproduction, Growth and Developmental Stages

A. bullfrogs typically inhabit permanent standing water where they are able to lay their eggs during the warmer summer months (Bruening, 2002). Eggs will hatch within a few days (Carlson & Langkilde, 2013) and the newly released juveniles will remain in the larval stage for typically up to 2-3 years before experiencing metamorphosis (Wang and Li, 2009; Carlson and Langkilde, 2013), although it has been observed to occur as early as 3-4 months under specific stress-induced circumstances (Cook *et. al*, 2013). Females can lay multiple clutches of more than 20,000 eggs per season, which can allow for rapid population growth (Kamoroff *et al.*, 2020; Invasive Species Council of Metro Vancouver, 2021). This is seen to be a much higher fecundity rate in comparison to other European native amphibian species, such as the common toad (*Bufo bufo*) or common frog (*Rana temporaria*) (Adriaens *et al.*, 2013).

For anurans, predation or pond depletion are two of the major causes of larval mortality in either permanent or seasonal aquatic environments (Lardner, 2000). This puts species, like A. Bullfrogs, at a much greater risk of mortality during early development, as larvae have a longer pre-metamorphosis juvenile state. More time spent confined to one ecosystem type increases that risk (Boone, Little and Semlitsch, 2004; Kamoroff *et al.*, 2020). However, studies have shown that some bullfrog populations expressed developmental plasticity in the metamorphic stages as a response to shorter hydroperiods in ephemeral water bodies (Boone, Little and Semlitsch, 2004; Cook, Heppell and Garcia, 2013).

Temperature is another abiotic factor that can significantly affect the growth pattern of these organisms, as they are ectothermic, meaning that thermoregulation is governed by external climate. This is especially true during metamorphosis (Nakajima *et al.*, 2020; Alvarez and Nicieza, 2022). Low temperatures are shown to slow down the differentiation process during metamorphosis but do not show signs of interrupting growth. Therefore, larvae experiencing metamorphosis in warmer temperatures tend to be smaller but have a shorter development period then individuals grown in cooler temperatures (Álvarez and Nicieza*,* 2002). Since bullfrogs are ectothermic vertebrates, they have developed internal mechanisms in order to deal with cooler climatic temperatures. For example, bullfrog tadpoles are able to suspend metamorphosis in the winter months and overwinter in ephemeral ponds until ideal conditions are available (Boone, Little and Semlitsch, 2004; Alvarez and Nicieza, 2022). Larvae have also shown natural variation in larval development stage, which could enhance adaptation in response to changing hydroperiods and allow them to metamorphose before desiccation. Larvae must reach full metamorphosis before their aquatic habitat dries out, or else this will lead to a population sink (Cook *et al*, 2013).

It has been observed that the optimal environmental temperature for the A. bullfrog species ranges from 15-32º C, with an average body temperature ideally between 26-30º C (Adriaens *et al.*, 2013) and a critical thermal maximum of 38.2º C (Johovic *et al.*, 2020). Temperature is highly influential on breeding processes as well, as males are seen to begin mating calls out to reproductively mature females when outside temperature reaches higher than 20º C (Johovic *et al.*, 2020). Cold temperatures can also affect the cellular processes that control the endocrine and immune systems as well as the locomotive performance. Therefore, bullfrog tadpoles are documented to have the capability of expressing different levels of plasma proteins triggering hormone responses when exposed to varying temperatures. This helps promote a consistent immune response even while overwintering to survive unsuitable thermal conditions (Nakajima *et al.*, 2020).

Locating prey during the winter season for bullfrogs can be limiting and small froglets are more prone to starvation during this time (Nakajima *et al.*, 2020; Alvarez and Nicieza, 2022). It has been proven that lower access to food can decelerate developmental stages in anuran populations. Frog species may postpone metamorphosis until quality and quantity of food improves (Álvarez and Nicieza, 2002). This issue, along with others highlighted, are greatly dependent on seasonality and other environmental conditions that may drive bullfrog populations to inhabit a more distinctive niche. Some of the examples of life history strategies that the bullfrog species have exhibited, however, help to convey this species’ high level of adaptability that can be seen in all stages of development.

2.1.3. Habitat Requirements

Habitat selectivity for invasive species is important information necessary to identify the probability of future establishments and implement effective future management actions (Wang and Li, 2009). *Lithobates catesbeianus* is known to occupy various wetland habitat types (ponds, lakes, bogs, marshes, slow moving streams or rivers and swamps), however, they seem to be most successful and have a selection preference to artificial or modified aquatic habitats (Adriaens *et al.*, 2013), such as reservoirs or fish ponds. This enables this species of bullfrog to adapt well in human modified environments (Bruening, 2004). Within their native range, bullfrogs are shown to prefer larger wetland ecosystems, of $\geq$ 2ha, while only a small percent can exist in ones smaller than that (Cunningham, Calhoun and Glanz, 2007).

Bullfrogs prefer areas with a warmer average climate, but have been documented to survive successfully in places reaching minimum temperatures of -20ºC in the winter months (Adriaens *et al.*, 2013). Larvae tend to overwinter before proceeding to metamorphosis stage, which means they require a more permanent freshwater body (Cook *et. al*, 2013). Post-metamorphic bullfrogs feed, mate and hibernate in long-lasting water bodies (Wang and Li, 2009). As mentioned previously, bullfrogs have been observed breeding successfully in ephemeral, or short-lived, wetland habitats, indicating that they can adjust to habitat types with varying hydroperiods. Within permanent wetland environments, larvae are able to reach maximum growth rates before metamorphosis, which can lead to faster reproductive capability (Cook *et. al*, 2013).

Bullfrog species are reported to be greatly influenced by the presence of beavers, mostly through the alteration of stream and wetland habitats. Beavers are considered to be important keystone species that increase the existence of shallow wetlands with longer hydroperiods, an ecosystem type crucial for pond-breeding species like A. bullfrogs. With a maximum of 2-3 year larval stage, bullfrogs require longer lasting wetland types like the ones formed by beavers.

Beavers have been known to play a significant role in improving the connectivity of crucial wetland breeding and foraging sites, and bullfrogs have been known to favor active beaver sites over others throughout their native distribution (Cunningham, Calhoun and Glanz, 2007).

2.2. Distribution

The American bullfrog species is currently found in 40 different countries and all continents except Africa and Antarctica (Adriaens *et al.*, 2013; Ficetola *et al.*, 2007). They have a high dispersal rate, showing they are capable of migrating up to 8 km per year (Howell *et al.*, 2020) and a maximum 1500 m during the breeding season (Adriaens *et al.*, 2013). While A. bullfrogs can migrate efficiently through river systems (Adriaens *et al.*, 2013), this species has been subjected to many instances of human-induced translocations worldwide (Schloegel *et al.*, 2009, Andersen *et al.*, 2021).

2.2.1. Native Range

The American bullfrog (*Lithobates catesbeianus*) is an autochthonous species within the eastern region of Canada, eastern United States and northeastern part of Mexico (Ficetola, Thuiller and Miaud, 2007; *The IUCN Red List of Threatened Species*, 2022). They are considered to have a wide native range, which gives good insight into their flexible life history and extensive climatic and ecological niche. This fact makes them more likely to be a successful invasive species (Adriaens *et al.*, 2013), as they are able to adapt to varying environmental conditions (Johovic et al., 2020).

2.2.2. Invasive Distribution -- Europe

Genetic assessment indicates a total of 25 different introductions throughout the European region that have existed throughout history, from native sources starting since the 1930’s in Italy (Ficetola, Thuiller and Miaud, 2007). A total of 60% of establishments occurred within the span of a decade, between 1980-90. Within the European distribution they can be found in varying bio-geographical environments (Mediterranean, Continental, and Atlantic)

(Adriaens *et al.*, 2013). European distribution range consists of the countries Belgium, France, Germany, Greece, Holland, Italy, Spain and U.K. (Johovic *et al.*, 2020). Distribution in southwest France has increased rapidly since the early 2000's. In Italy, populations were introduced in the 1930's while French populations weren't seen until the 1960's. Italy is known to have a much larger population, but the populations found in France are more spread out (Ficetola *et al.*, 2007). This type of dispersal can limit further expansion of these established bullfrog communities as there is a lack of connectivity between wetland habitats. However, these smaller, more scattered populations suggest translocation, or movement by people (Ficetola *et al.*, 2007), as they were too distanced to be natural migratory patterns. Translocation by humans was known to be the cause of majority of the species introductions to the European ecoregion as a whole (Adriaens *et al., 2013*). Human introductions mainly through means such as breeding farms and pet trade has led to the eventual ban set forth by the EU of further introductions of this invasive species (Law of the European Council 2551/1997 -- Appendix S1; Ficetola *et al.*, 2007).

The A. bullfrog has yet to be seen within majority of the central European region (Ficetola *et al.*, 2007; Johovic *et al.*, 2020), however there has been evidence of introductions of small populations throughout parts of western Germany along the Rhine river, bordering France (Adriaens *et al.*, 2013; Laufer, 2004; Thiesmeier, Jäger and Fritz, 1994). Four known populations of A. bullfrog within western Germany were either partially or completely eradicated through means of electrofishing and fencing of the pond habitats (Reinhardt *et al.*, 2003). These populations have not been seen to resurface since. The last reported bullfrog sighting in Germany was in 2002 in Karlsruhe, where five ponds were pumped and drained, and then all adults and tadpoles were removed (Adriaens *et al.*, 2013; Thiesmeier *et al.*, 1994). Eradication measures in this region cost an average €270,000 annually (Reinhardt *et al.*, 2003).

Climate change is only expected to favor the success of organisms with effective dispersal abilities and wide geographic ranges, such as the A. bullfrog (Ficetola *et al.*, 2007).

2.3. American Bullfrog (*Lithobates catesbeianus*) as an Invasive Species

2.3.1. Impact as an Invasive Species

Native organisms that engage with invasive alien species typically suffer from a disadvantage because they lack the evolutionary background to adapt to the numerous consequences caused by this rapid encounter, which may eventually lead to a decrease or even local extinction in native populations (Kats and Ferrer, 2003). This particular bullfrog species is considered one of four of the world's worst invader anuran species. Negative impacts of this species in non-native ecosystems mainly influence native amphibian populations through competition, predation and transmission of deadly diseases (Andersen, Borzée and Jang, 2021).

Competition of A. bullfrogs with indigenous species in introduced environments can be observed in adult and juvenile stages (Scalera *et al.*, 2012). Bullfrog tadpoles are known to outcompete other tadpoles that they are found to be in proximity to, for things like resources and territory (Ficetola, Thuiller and Miaud, 2007; Cook *et al.*, 2013). They seem to also have an advantage in pre metamorphic survivorship over other native frogs found within the same aquatic habitat, since they tend to avoid predation from fish as their eggs seem to be less palatable (Bruening, 2021; Hayes & Jennings, 1986). Since Bullfrogs are capable of laying such large egg masses at once, usually larger than most other amphibian species, this can also contribute to competition and predation pressures with these other amphibian communities within the shared habitat (Ficetola, Thuiller and Miaud, 2007). As mentioned previously, bullfrogs are also considered to be a significant predator species, typically ingesting almost any other organisms smaller in size that they may come in contact with, most notably other amphibian species, as well as reptiles, mice, fish etc (Adriaens *et al.*, 2013; Kats and Ferrer, 2003). This is because the A. bullfrog is a generalist feeder and an opportunistic predator (Kats and Ferrer, 2003; Scalera *et al.*, 2012). Bullfrog diets mainly consist of crustaceans and insects, a common staple food source for many other amphibians, which makes them primary competitors as invasive species for resources with native populations as well (Wang and Li, 2009).

Many studies have shown a negative correlation with the presence of invasive A. bullfrogs and native amphibian populations (Kats and Ferrer, 2003, Hayes & Jennings, 1986, Johovic *et al.,* 2020). It was documented that in Arizona, U.S.A., outside of their native range, bullfrogs were responsible for extirpation, or local extinction, of Leopard Frogs (*L. chiricahuensis*) after introduction in 2001. This local extinction was mainly caused by habitat and resource competition (Howell *et al.*, 2020). Another example occurred in 2000, when A. bullfrogs were introduced to the island of Crete, Greece in the Agia Lake, which caused displacement and local extinction of the Cretan frog (*Pelophylax cretensis*) (Adriaens et al., 2013; *The IUCN Red List of Threatened Species*, 2022).

2.3.2. Vector for Infectious Disease

One factor in particular that makes the A. Bullfrog one of the most problematic invaders is their ability to spread deadly disease (Garner *et al.*, 2006; Miaud *et al.*, 2016). They have been discovered to be carriers of a fungus, *Batrachochytrium dendrobatidis* (Bd), an agent of Chytridiomycosis, which is a deadly infectious disease known to be the primary cause responsible for mass declines and extinctions of amphibian populations worldwide (Ficetola, Thuiller and Miaud, 2007; Miaud *et al.*, 2016). *L. catesbieanus* is able to be infected by this parasite but never fully develops the harmful disease, unlike most other amphibian species that they have come in contact with (Johovic *et al.*, 2020), making them a vector for the continued spread of the disease (Adriaens *et al.*, 2013). Due to this deadly impact, it places this species as one of the most harmful invasive species in the world and making the control of future introductions at an utmost priority (Miaud *et al.*, 2016). A study by Garner *et. al* (2006) reported a total of five populations of bullfrogs within the countries of France, Italy and the United Kingdom that were infected with the deadly fungus.

Bullfrogs are not only carriers of the infectious virus known as *B. dendrobatidis*, but they are also known to contract another deadly virus known as ranavirus, which is found only within amphibians and reptiles (Adriaens *et al.*, 2013). Both are considered emerging infectious diseases as they continue to spread to new areas. Evidence shows the introduction of this disease has increased due to influences from pet trade or laboratory animal trade, which amphibian populations, like Am. Bullfrogs, are a large contribution to (Schloegel *et al.*, 2009). Studies indicate amphibians to be the most threatened vertebrate class (Johovic *et al.*, 2020), with over a quarter of European amphibian populations considered threatened (Temple *et al.*, 2009), therefore, an additional threat of the spread of a deadly virus only enhances the dangerous of bullfrogs to surrounding amphibian populations.

2.4. Species Distribution Modeling

2.4.1. Species Occurrence Projections

Previously conducted studies on the potential for an invasion of American bullfrogs on a global scale, with consideration of climatic data, indicated that there are many vulnerable areas within the European region (Johovic *et al.*, 2020). According to a study by Johovic *et al.* (2020), some of the most influential environmental factors determining bullfrog success rate were average precipitation, annual temperature, and human interference. Highest habitat suitability for these bullfrog species was within areas with the highest annual precipitation (especially in warmer months) and with the highest maximum temperatures. Year-round, wetland ecosystems are highly susceptible to invasion for the necessity of essential habitats, most likely due to the species' long development process and the fact that adults tend to overwinter in standing water (Adriaens *et al.*, 2013, Cook *et al.*, 2013, Boone, Little and Semlitsch, 2004). For the overall physical health of these species, regions with temperatures reaching ≥15ºC were most ideal, as anything below this threshold limits most functioning capacity of the bullfrogs (Ficetola, Thuiller and Miaud, 2007).

In the study by Johovic *et al.* (2020), suitability models were conducted for this species using current environmental conditions, which showed that 3.8% of global land area was deemed suitable for invasions of this bullfrog species. Another study conducted by Andersen *et al.* (2021) attempted to determine suitability of *L. catesbeianus* worldwide by using all climatic scenarios (Fick and Hijmans, 2017), and determined a high predicted invasion rate and 99.9% potential for survival on all coinciding terrestrial biome types to their native range. In a comparison with the species' current distribution range to the calculated suitability models, it seems that potentially suitable habitats span a much wider geographic area (Andersen et al., 2021; Johovic et al., 2020). This indicates that introduced populations of this species are able to adapt to broader climatic variations across wide geographic spaces than ones found in native ecosystems. It is important to note, these models were adapted on a global scale, therefore effects from predation and/or competition from native species, microhabitats, landscape heterogeneity and human influences are not considered (Andersen et al., 2021). This justifies a more in-depth investigation on the invasion potential of this species, and has served as the inspiration for this particular study. Given that this invasive bullfrog species has not yet been identified within central Europe, focusing on this particular ecoregion could be a more useful and targeted study in this circumstance. (Andersen, Borzée and Jang, 2021).

An ecological niche factor analysis (Andersen *et al.*, 2021) is a method that can be used to determine the influence of multiple environmental factors on the distribution of A. bullfrog populations. This is a method that uses median range of outputs as general trends of climatic conditions, excluding extremes. This forecasted range is then used to identify the trends within the observed localities of the species of focus to determine other suitable habitats available outside of their known distribution (Guisan & Thuiller, 2005). As an example, some environmental conditions considered in the study by Johovic *et al.* (2020) included average and seasonal temperature ranges as well as variations in precipitation. Results indicated that current environmental conditions show 2.3% of potential suitable habitat space throughout Europe and 3.45% of most Natura 2000 areas for Am. Bullfrog populations, with Central European regions being some of the most vulnerable.

2.4.2. Introduction to Methodology

When attempting to understand an invasive alien species, like the bullfrog, and prevent future invasions, it is important to determine the environmental factors that allow these species to inhabit and reproduce successfully in their native ranges (Ficetola, Thuiller, et al., 2007; Johovic et al., 2020). A better understanding of where management actions are required can be obtained by using specific environmental features that provide habitat suitability for A. bullfrogs in their native ranges and integrating that information within other locations that are predicted to have the capability of future introductions. Using information like temperature and precipitation data within the native and invaded regions of the A. bullfrog's inhabited range can help to further identify the characteristics of this species' ideal niche (Andersen *et al.,* 2021 Ficetola, Thuiller, et al., 2007; Johovic et al., 2020) . Climatic factors like temperature and precipitation are considered limiting factors that should express a more gradual distribution over geographic gradients (Guisan & Thuiller, 2005). We can then project this information onto a larger scale outside of their present distribution, giving a better indication of invasion potential for alternative target areas (Ficetola, Thuiller and Miaud, 2007).

2.4.3. Species Distribution Models

Species Distribution Models (SDM), otherwise known as 'risk maps' (Johovic *et al.*, 2020), is a tool used to determine predictions of future population distributions based on environmental suitability for a particular species. These models use a combination of occurrence observations of a given species and various environmental factors that could influence these occurrences. Environmental factors can be either indirect or direct with a consideration of eco-physiological characteristics (temperature, precipitation, etc.), disturbances (natural or human-induced) and resources (energy source, water availability) (Andersen et al., 2021, Guisan *et al.*, 2017). When creating and analyzing an SDM it is important to determine the right data that will best help to identify the environmental predictors for the species and scale of location. It is also helpful to establish an appropriate model and statistics needed for the most accurate predictive outcome of distribution for the species of choice (Guisan and Thuiller, 2005). A distribution pattern for a species, like A. bullfrog, that has an almost cosmopolitan spread (Adriaens *et al.*, 2013) may be better interpreted by identifying the relevance of the chosen variables given scale and the species in question (Guisan and Thuiller, 2005).

2.4.4. MAXENT Introduction

An appropriate tool that can be used to estimate suitability of invasive species beyond their present distribution is the program Maxent. Maxent, which stands for maximum entropy modeling (*Maxent*, 2022), is a programming system which links inputs of presence localities of species in question to environmental characteristics of the regions in which they are found (Phillips, 2017). From these inputs, the system is able to determine a mapped projection of other possibly suitable habitat locations outside of the known distribution range of the species, based on what are ideal environmental conditions for the organism (Phillips and Research, 2017; Johovic *et al.*, 2020). Maxent can generate a map that shows habitat suitability in foreign regions for the focal species (Guisan and Thuiller, 2005) using all chosen environmental characteristics with equal consideration. The program will also produce graphic interpretations of each included environmental condition individually to see which variables might be more influential to the final predicted distribution pattern (Phillips, 2017). The map described is a niche-based model, which is an important tool to develop a proper risk analysis of invasive species (Johovic *et al.*, 2020). These niche-based models can then be utilized to better adapt conservation efforts against potential future invasions of these invasive species. The information collected can also be further manipulated to reflect future climatic conditions, especially with reference to climate change predictions, in order to calculate where these species may be suitable for a projected time in the future.

Maxent works by generating a probability distribution over pixels in a grid within the mapped area and based on the best accumulated environmental features it can then determine a percentage of suitability for each location (Johovic *et al.*, 2020; *Bioclimatic Variables - WorldClim,* 2017). All environmental factors are acknowledged in the model as an equal interpretation of what the most suitable localities are, and goodness of fit is calculated for each locality within the mapped projection (Guisan, Thuiller and Zimmermann, 2017). The program can also calculate which environmental characteristics are the most influential to this species and considers variance so that the model can be narrowed down to a more precise representation. This helps to determine which environmental factors are helping to fit the model best and how each individual environmental conditions are influencing the model when all other conditions are held constant (Phillips, Anderson and Schapire, 2006).

3. Hypothesis and Aims

The overall aim of this study is to identify invasion potential for the American bullfrog (*Lithobates catesbeianus*) within the uninhabited central European ecoregion. This analysis will be conducted by using known information about geographic observations of this invasive species within both its native and non-native distribution, as well as corresponding environmental factors found in these observed localities. These predictions will be made through the construction of a Species Distribution Model using specific bioclimatic variables most influential to the model and the necessary environmental characteristics for the species' fundamental niche. The research is meant to build an understanding of what regions in Central Europe are most vulnerable to introductions by the A. bullfrog species. This information can then be utilized to discuss possible control methods and initiatives to prevent further invasion of the species within currently unaffected territories in Europe.

I predict that, given current environmental projections, my investigation will find that successful introductions into the Central European region are feasible. This theory was adopted after conducting a more defined interpretation of the extent of traits exhibited in the American Bullfrog species that make it an effective invader. Additionally, I hypothesize that regions with higher annual rainfall, higher annual temperatures and less seasonal inconsistencies will be the most suitable localities for this invasive bullfrog species.

4. Methodology

4.1 Data Collection

4.1.1 Observation Data

In order to test the potential for establishment of the invasive species, *Lithobates catesbeianus*, within the central European region, a theoretical approach was required. This was accomplished by using observation data collected on the species along with climatic characteristics associated with the organism's survival success. Observation data of the species in question was accessed through the Global Biodiversity Information Facility (GBIF;*Lithobates Catesbeianus (Shaw, 1802)*), an open-source platform used to record observation records of various organisms on a global level. All observation data points are recorded through a community-based contribution of sightings on an international basis with associated photographic evidence. These photos can then be analyzed by experts and species confirmation is concluded (*What Is GBIF?*, 2022). The observation data points used here were focused on the European and North American continental territory, in which specific coordinates of sightings were provided. Data points outside of the study site [Central Europe] were used to represent the environmental variables found within their native range [eastern N. America] (Adriaens et al., 2013) as well as features found in locations they are considered non-native [continental region of N. America and Europe]. Limiting occurrence data was available within the GBIF European dataset that did not fully reflect the realized distribution of this species in this region. Available data for strictly European occurrences, was also highly skewed, in that, specific countries had a much higher contribution to occurrence data than others (specifically in Belguim due to a detailed study previously conducted by Adriaens *et al.* (2013)), which could have led to overestimations by the model to areas with this narrow environmental representation. This observation range was also considered in order to express the wide span of climatic variability that would be seen throughout this species' vast distribution. This was meant to represent the array of possible environmental conditions that *L. catesbieanus* can withstand.

4.1.2 Bioclimatic Variables

Bioclimatic data was also obtained, with a focus on historical climatic data, using Worldclim, a worldwide climatic recording system that estimates annual trends of temperature and precipitation on a global scale. These bioclimatic recorded trends can be more detailed in that they also consider seasonality (monthly variation) as well as limiting or extreme environmental factors over annual quarters (3 months, ¼ of year) (*Bioclimatic Variables — WorldClim*, 2017, Fick & Hijmans, 2017). Different bioclimatic variables were focused on, with the consideration of necessary environmental conditions required for the success of the species in question (Phillips, 2017) as well as their overall contribution to the model (see Table 1).

Bioclimatic Variables	**Bioclimatic Variable Details**	**Calculations**
Bio_1	Annual Mean Temperature	
Bio_12	Annual Precipitation	
Bio_7	Temperature Annual Range	(**Max Temp of Warmest Month**) – (**Min Temp of Coldest Month**)
Bio_18	Precipitation of Warmest Quarter	

Table 1, Table of Bioclimatic Variables used within the analysis of species success within the study region of C. Europe based on a climatic variables seen within the known occurrence locations throughout the Northern Hemisphere (*Bioclimatic variables — WorldClim*, 2017*; Lithobates catesbeianus, Shaw, 1802*).

This climatic data is organized as a structure of worldwide climatic grids that are formulated within geographic layers with a spatial resolution of ~1 km (Fick and Hijmans, 2017). The bioclimatic data, although historical, represents a close to current representation of the global climatic average (between 1970-2000) as a means to understand the standardized depiction of climatic patterns within the more focused study site (Fick and Hijmans, 2017, Hijmans *et al.*, 2005).

Variables regarding temperature were chosen with the attempt to indicate the degree of seasonality and climate restrictions as a means to decipher the species thermal gradient. The A. bullfrog is an ectothermic organism that has a higher thermal gradient on average, and has limited functionality at lower external temperatures (Andersen, Borzée and Jang, 2021; Alvarez and Nicieza, 2022). Therefore, temperature is estimated to be highly influential to this organism's distribution. Precipitation variables are meant to represent the potential for the availability of permanent water bodies, a necessary habitat type for developmental processes (Fuller *et al.*, 2010, Wang and Li, 2009).

Climate averages are calculated over each month throughout the year, which helps to account for seasonal variances. Each bioclimatic variable used throughout the data analysis was accessed using a 2.5 minute spatial resolution (~5km pixels) (Fick and Hijmans, 2017). This higher resolution imagery was a more focused and precise image quality in comparison to the other available data types (5m, 10m) through the Worldclim database. This resolution type was determined to be more beneficial for a smaller study site (Hijmans *et al.*, 2005), like what is observed within the Central European study site seen here. It is also important to note the study is a focus on a smaller sized organism with more narrow migratory patterns (Bruening, 2002), which would allow the model to therefore respond appropriately to a more concentrated pixel distribution (Elith *et al.*, 2011).

4.2 Species Distribution Modeling (SDM) Using MAXENT

4.2.1 Data Analysis and Model Preparation

Once all necessary data was acquired, bioclimatic variables and occurrence data of A. bullfrog, the datasets can now be modeled and analyzed to create what is known as a Species Distribution Model (SDM). All occurrence data is imported into RStudio (Version 1.3.1093) through the 'gbif' package (*Lithobates Catesbeianus (Shaw, 1802)*), which included coordinates of locations where observations of the species have been made and identification of the species was verified. Any data points obtained that did not include coordinates of sightings ('NA') or were recorded as 'ABSENT' were excluded from the resulting dataset used in the analysis. Data points were also filtered of any duplicate coordinate locations to avoid any biases or overestimates within the predicted suitability distribution (Maxent, 2022). Both minimum and maximum of longitude and latitude was calculated for all observation points within the site to note geographic extremes. All observation data is recorded onto a map of the focused study site as a representation of current distribution patterns.

Now it is possible to formulate an SDM for *L. catesbeianus*, which is done using the program Maxent Version 3.4.3. As explained previously, the Maxent program will calculate the probability of occurrence of A. bullfrog throughout the study site by using the climatic preferences found within the areas they are currently distributed (Phillips and Research, 2017) . This is done using a machine learning method to build a maximum entropy model throughout a geographic space and combining a set of climatic conditions that make up an organism's realized environmental niche to determine their fundamental niche (Phillips, Anderson and Schapire, 2006). A probability distribution of maximum entropy of the species ideal niche within central Europe will be the output.

4.2.2. Species Distribution Model Computation

In order to determine the bioclimatic variables used in the final model, a "Top-Down" statistical selection approach was used (Grace-Martin, 2014). This selection process is one in which all available bioclimatic variables are included in the model and ran through the Maxent program, which then produces the 'percent contribution' (PC) and 'permutation importance' (PI) of each variable. If any variables showed zero contribution (PC) or importance (PI), then they were excluded from the final model output. The final bioclimatic variables (See Table 1) selected were then cropped and fixed within the mapped region of the study site. The program uses the generated model to run a suitability test throughout the study site before producing a final predicted distribution model (Elith *et al.*, 2011; Merow, Smith and Silander Jr, 2013). The first output obtained from the program explains the sensitivity of the model. This is expressed through a receiver operating characteristic (ROC) analysis which indicates the performance of the model by using AUC (area under curve) (Phillips, Anderson and Schapire, 2006; Narkhede, 2021). The receiver operating characteristic (ROC) curve was constructed for the model to see how well the model performed suitability predictions across the study location. This plot indicates the predictive accuracy, or the sensitivity, of the model to choose the suitable locations for the species within the available sites and determine how sensitive it is to each bioclimatic variable that would provide the species' ideal niche (Narkhede, 2021). This is more specifically calculated through the AUC (area under curve) which explains the probability of the model to randomly choose a presence location over background localities. This can be accomplished more when there is more background data than available presence data (Merow, Smith and Silander Jr, 2013).

The next plot output is an 'Omission Rate and Predicted Area' plot. This plot explains the rate at which "test localities that fall into pixels [are] not predicted as suitable for the species" (Phillips, Anderson and Schapire, 2006), as well as the proportion of pixels within the study area that are predicted to be suitable. A low omission rate indicates the effectiveness of the model (Merow, Smith and Silander Jr, 2013). A table was also generated to measure significance of each bioclimatic variable used in the model through both 'percent contribution' (PC) and 'permutation importance' (PO). This allows for each chosen environmental characteristic to be analyzed independent from each other and show how much each variable is contributing to predicting occurrence projections (Phillips and Research, 2017).

With the remaining environmental variables that were used to determine distribution suitability, a response curve plot was generated for each condition in order to see how the model behaves when only considering one variable at a time. Each response curve represents how the predictability of the model adjusts depending on the variability of the presence of the environmental feature available. These response curves can also help to indicate at which thresholds each bioclimatic factor is contributing to the model (Elith *et al.*, 2011; Phillips, 2017). For example, if there is a thermal gradient that the target species is seen to be detected in as referenced through the occurrence data, then the Maxent program will identify the range of ideal temperature averages and indicate that in the response curve (Phillips *et al.*, 2006, Guisan, Thuiller and Zimmermann, 2017).

4.3. Suitability Map

4.3.1. Calculating Predicted Suitable Area Cover by Country

The Maxent program will predict regions throughout the study site that may be suitable for A. bullfrog species to inhabit. This file output can then be projected onto a map of the area within Europe that is being investigated. This will give a clearer visualization of what territories within the central European region may be most at risk to invasions.

Using the Maxent program, the ‘predict’ function was used to calculate a raster projection of predicted suitability for the species within the European study site. The raster file was uploaded into the QGIS software (version 3.28.0) and projected onto a world map where specific country locations can then be identified. While observation data was used throughout both the North American and European ecoregions, the focused study site in which the SDM is calculated for, was just within the central European area. The raster file output from Maxent is a choropleth map indicating the range of suitability for the species, between 1 and 0 (1 being most suitable) (Phillips *et al.*, 2006). Therefore there was a range of suitability throughout the study site and the binary model was built with the assumption of any sites with $\geq$ 0.5 predicted suitability (Elith *et al.*, 2011, Maxent, 2022) was a ‘yes’ and anything less was a ‘no’. This allowed for the formation of the binary projection map which indicates the areas of highest invasion risk.

From here, the raster file can be vectorized in the QGIS program to polygons, which can be used to calculate the total area in each country that the program considers suitable for this species. This was calculated for each country selected to represent the central European region, or more specifically, the currently uninhabited territories of Europe. The countries that have been chosen within central Europe, in this case, were: Germany, Czech Republic, Austria, Poland, Slovakia and Hungary. The binary map originally constructed, was then redefined by percentage ranges of expected fit.

Then, each country was clipped from the study site map and within each country being analyzed a suitability range was incorporated (20-40%, 40-60%, 60-80%, 80-100%; excluding sites with suitability range 0-20% as insignificant to the results). The suitability range was included to reference the range seen in a choropleth map of the study site, which refers to the levels of invasion risk. Then, using RStudio, the percent cover of suitable area for *L. catesbeianus* in each country within the predetermined ranges was calculated to determine a level of invasion susceptibility. Total area for each suitability range was calculated using the sum of all suitable area polygons within that range found in each country divided by the total area of the country of focus and multiplied by 100 for percentage.

As indicated previously, A. bullfrogs require long standing water bodies for various life cycles. Therefore, predicted suitable area cover within the C. European study site was projected along with permanent riverine systems found within the region, using the Copernicus EU River Network layer in QGIS (*QuickMapServices*, 2022). Although this geographic information was not considered in the calculation of predicted suitability for bullfrogs, it helps to indicate the proximity of necessary ecoregion types to areas with highest invasion risk potential. The combination of ideal climatic conditions and the presence of a necessary ecoregion type would consequently ensure successful establishment of this species (Ficetola, *et al*., 2007; Johovic *et al.*, 2020).

4.3.2. Comparison of Calculated Suitability Potential Amongst Countries

Once there is a calculation of percent cover of forecasted suitable areas for each country within the range increments, a percent cover graph can be formulated. Each country chosen contains different climatic characteristics, and therefore should provide varying suitability ranges for this species. It is important to understand the difference in probability of occurrence amongst each country in order to get a better comparison between them.

Lastly, a final graph of all predicted suitability throughout the study site can be formulated to get a visualization of the model. Using the calculated total areas within each suitability range, a stacked bar graph was formulated for each country. Each suitability range was matched with the color representing that percentile scale seen within the choropleth map of the study area to indicate where these varying suitabilities can be found.

5. Results

5.1 Model Performance

Testing the suitability potential of *Lithobates catesbieanus* outside of the species' currently inhabited localities, begins with understanding the current distribution range of the species. In this study, both native and invasive dispersal extent was considered. Occurrence points accessed from gbif.org were filtered of any duplicate coordinates to avoid any biases, as well as any 'absent' records, therefore limiting the data from 4,059 to the final 1,467 occurrence points total.

The sensitivity of the model was tested using the ROC curve and further analyzed by interpreting the AUC value. The maximum achievable AUC value is less than 1, and this model is represented by an AUC = 0.971. The Random Prediction value for an effective binary model with sufficient predictive power should have an AUC = 0.5 (Phillips, Anderson and Schapire, 2006; Narkhede, 2021), as this Maxent model does. The model was ran through a total of 500 iterations for proper training.

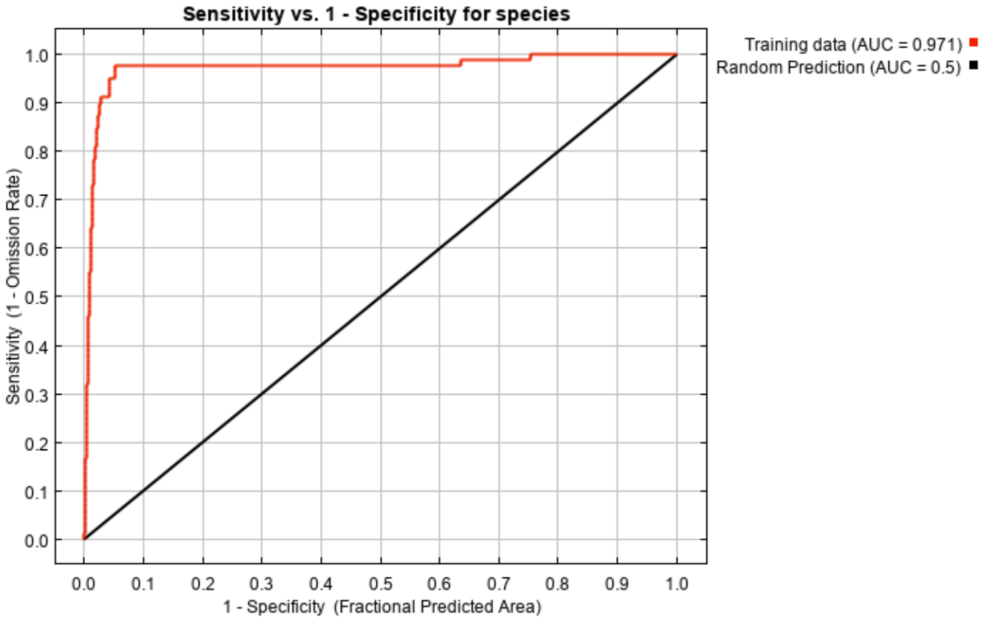


Fig. 2, Receiver Operating Characteristic (ROC) curve indicating sensitivity of the model's predictive power to identify suitable habitat locations for *L. catesbieanus*.

Another output generated from the Maxent program was an Omission and Predicted Area plot (see Figure 3). The omission rate refers to the degree at which the model excludes pixels (test localities) within the study site from being predicted as a suitable habitat. This information helps to develop distribution range boundaries within the available study site localities (Phillips and Research, 2017). This plot also shows the 'fraction of background predicted' curve, which explains the fraction of pixels predicted to be a suitable location for the species

(Phillips, Anderson and Schapire, 2006). These two curves should convey opposing trends to demonstrate the fact that the model is not too constrictive in its ability to calculate the species suitability distribution (Phillips, Anderson and Schapire, 2006). Figure 3 indicates that the model used was performing at a close to optimal predictive power.

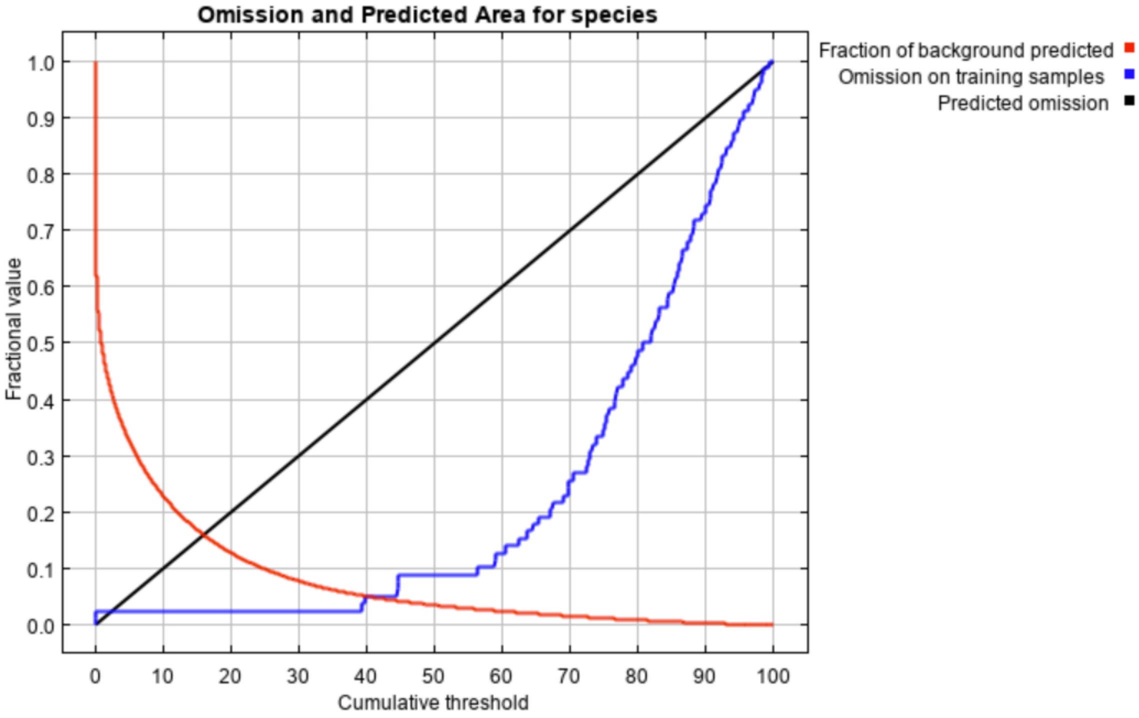
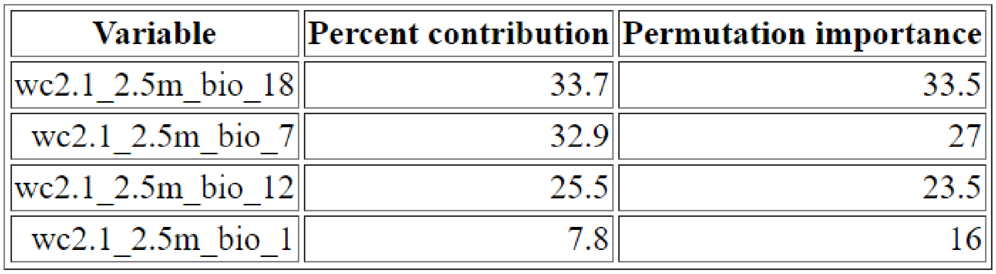


Fig. 3, Omission and Predicted Area plot produced as an output from the Maxent program in reference to the predicted suitability distribution for *L. catesbieanus* in the European region.

5.2 Contribution and Importance of Bioclimatic Variables to Suitability Model

5.2.1 Percent Contribution and Permutation Importance

While the Maxent program can describe how effectively the species distribution model (SDM) is produced overall, depending on the test and background data supplied, it can also show the relative contributions of each bioclimatic variable individually (See Table 2) (Phillips and Research, 2017). The relevance of each bioclimatic variable, therefore, was determined based on calculated values using PI and PC (see Table 2).



Variable	Percent contribution	Permutation importance
wc2.1_2.5m_bio_18	33.7	33.5
wc2.1_2.5m_bio_7	32.9	27
wc2.1_2.5m_bio_12	25.5	23.5
wc2.1_2.5m_bio_1	7.8	16

Table 2, Output of the Maxent program that calculates the percent contribution and permutation importance of each bioclimatic variable included in the model. All variables were provided with a resolution of 2.5 arc/min (~5 km pixels). {Bio_1 = Annual Mean Temp., Bio_7 = Annual Temperature Range, Bio_12 = Annual Precipitation, Bio_18 = Precipitation of Warmest Quarter}.

The difference between the percent contribution (PC) and the permutation importance (PI) for each of the environmental input variables, is that the PI is only dependent on the final Maxent model while the PC depends on the training process during the building of the final model (Phillips, Anderson and Schapire, 2006).

As seen in Table 2, the environmental characteristic ‘Bio_18’, which represents precipitation of the warmest quarter, contributed the most to predicting the occurrence of the species. However, the ‘Bio_7’ variable, annual temperature range, is considered to contribute to the prediction of occurrence for this species at a relatively similar significance as ‘Bio_18’ with slightly less permutation importance. The least contributing bioclimatic factor used in the model was ‘Bio_1’, annual mean temperature, with only a 7.8% contribution rate. It is important to note, however, that all bioclimatic variables considered in the species distribution model for *L. catesbieanus* are contributing to the model, while all others were excluded due to irrelevant contribution power.

For each environmental variable considered in the building of the SDM for *L. catesbieanus* there are values permuted using presence and background data (training points) to get to the final distribution pattern which results in a decrease in training AUC for each variable. A large decrease is what informs the model of the significance in dependence that the final model has from that singular variable is calculated into the PI (as a percentage) (Phillips, Anderson and Schapire, 2006). Contribution and importance of each variable to the final suitability model should be analyzed with caution, as these variables could adjust depending on their correlation with one another. Therefore it is important to analyze each variable independently (Merow, Smith and Silander Jr, 2013).

5.2.2. Response Curves

For each of the bioclimatic variables chosen to compute the species distribution model, it is important to understand their behavior when these features are varied. This relationship can be explained through the response curves (See Figures 4-7) produced through the Maxent program that considers one variable at a time while the others remain constant (Phillips and Research, 2017).

Every one of the environmental conditions evaluated were obtained from the test data sites where observations of *L. catesbieanus* were made (both North America and Europe), in order to express the variation of temperature found where this species currently inhabits. Therefore, the bioclimatic variables used to build the final SDM for this species, indicate characteristics seen in both native (Eastern N. America) and non-native (Western/central N. America and Europe) habitats. This information was then translated into a map of the probable suitability for the species strictly throughout the chosen region within Europe.

For average yearly temperature [Bio_1], the response curve (See Figure 4) shows limited suitability for the species in locations with mean temperatures < 10º C, but shows an increase in predicted suitability when mean temperatures are > 10º C where they reach the optimal temperature ~15º C. This plot also indicates a threshold level of maximum mean average annual temperature ideal for this species to be a little over 30º C within this region.

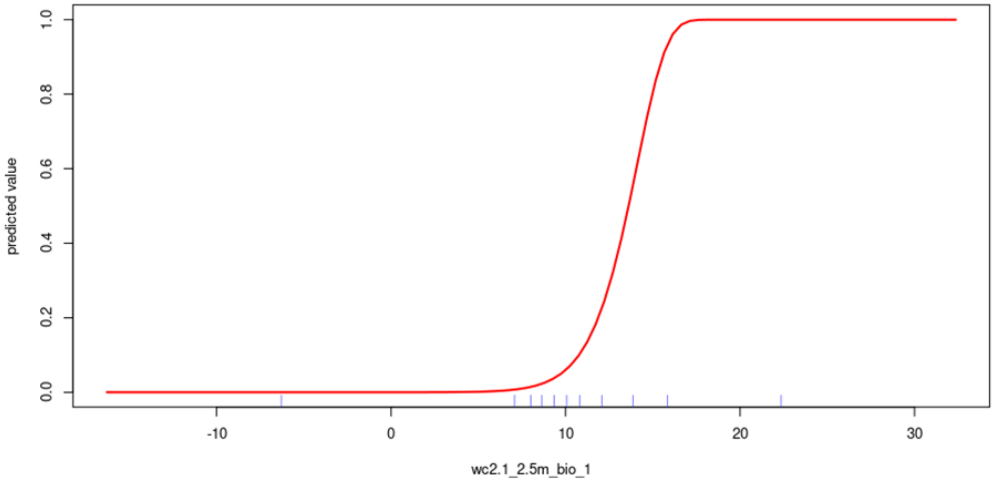


Fig. 4, Response curve for the bioclimatic variable 'Bio_1' (Mean Annual Temperature [^{o}C]), with an indicated response threshold between 0 and 20^{o} C. 'Predicted Value' (y-axis) in each response curve represents probability of suitability for the bullfrog species throughout the study site.

The Annual Temperature Range [Bio_7] helps explain seasonal variations in temperature throughout a year span. As seen in Figure 5 there is a predicted suitability of close to 60% for *L. catesbieanus* within the European study site for seasonal temperature variation ranges of ~12º C. The most suitable temperature range found within the study site that the model predicts to be suitable for the bullfrog species seems to be between 20-25º C, with a > 80% suitability. There is a maximum temperature fluctuation that does not seem to suit the species any longer, however, which occurs close to the temperature range of 25º C. With localities showing seasonal temperature changes of > ~25º C, suitability decreases dramatically. Suitability in sites with ~28º C seasonal temperature range no longer exists for this species, indicating the A. bullfrog can be successful only in regions with a maximum temperature variation of ~25º C throughout the year.

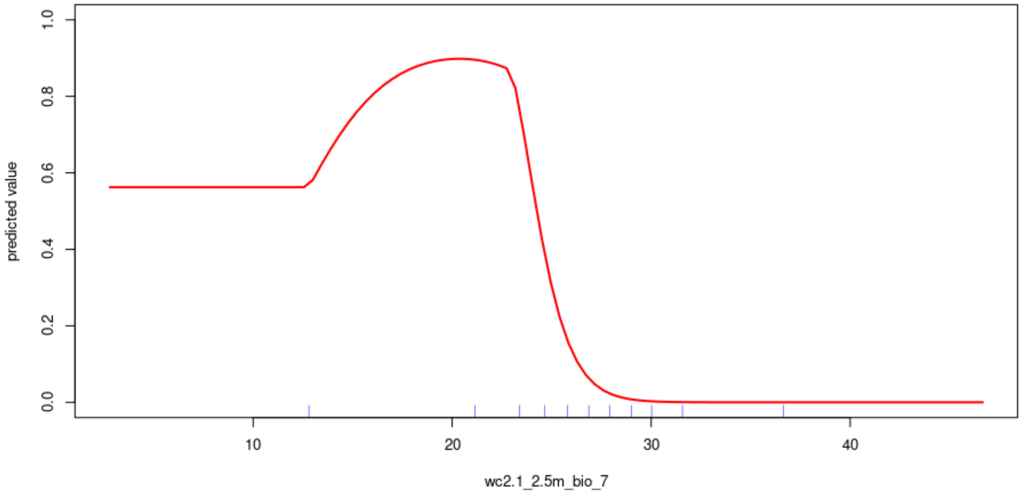


Fig. 5, Response curve for the bioclimatic variable 'Bio_7' (Annual Temperature Range [^{o}C]), indicating suitability of *L. catesbieanus* in selected study sites given variation in seasonal temperature changes.

The next environmental variable considered in this distribution model was Annual Precipitation [Bio_12]. As seen in Figure 6, the probability of the model to predict suitability based solely on annual precipitation (mm/yr) throughout the chosen study site was at its peak at the lowest range of < 500 mm/yr. When yearly precipitation levels are >500 mm/yr the model predicts a suitability level close to 50% for this species. The suitability for A. bullfrog based on annual rainfall is seen to stop once precipitation levels reach a point ≥ 1000 mm/yr within this particular region. It is not indicated from this plot what the minimum level of annual rainfall for this species is, however.

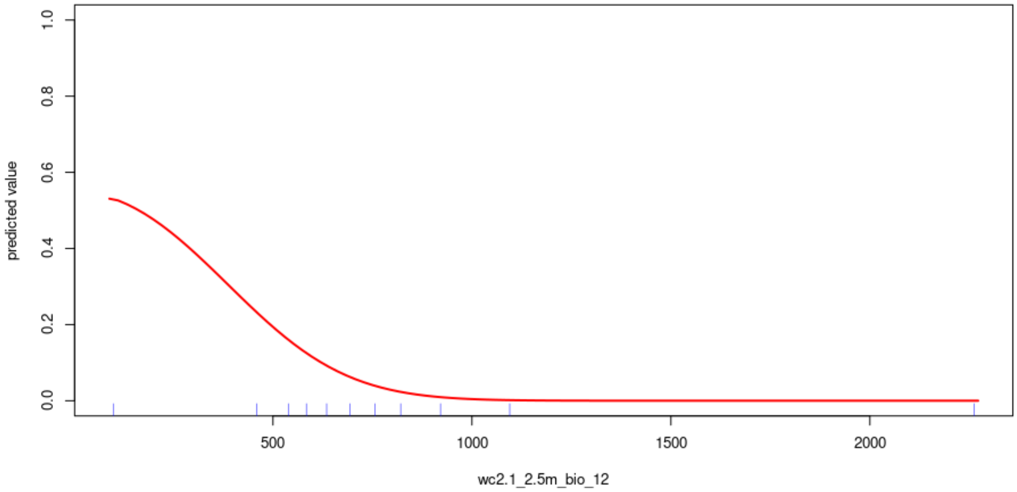


Fig. 6, Response Curve for Annual Precipitation (‘Bio_12’ variable [mm/yr]) within the chosen European region as a response for the predicted suitable area for *L. catesbieanus.* Indicates a precipitation maximum threshold of > 500 mm/yr but does not indicate a suitable precipitation minimum for this species within the study site.

The final bioclimatic variable considered in the construction of the species distribution model for *L. catesbieanus* is the Precipitation of the Warmest Quarter [Bio_18]. This variable is specifically measuring precipitation levels during the warmest three months (quarter) of the year (Fick and Hijmans, 2017). The response curve (as seen in Figure 7), representing the model’s ability to predict suitable habitats within the study site when only considering average rainfall during the warmest quarter, indicates an increase in predicted area once precipitation levels are > 500 mm. The response from this environmental characteristic in predicting suitable localities within the study area reaches a peak of > 300 mm of rainfall during the warmest season, where all areas that meet this criteria are considered suitable. This response curve contradicts the response curve for annual precipitation [Bio_12] (Figure 6), as it is strictly referring to the rainfall levels during the warm season. Referencing both of these response curves, we can infer that higher precipitation is only beneficial for this species' suitability during the months of high seasonal temperature.

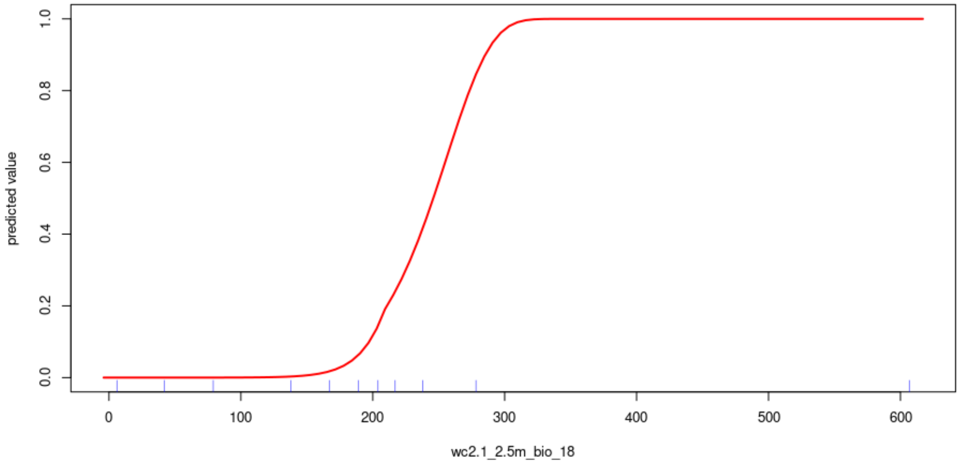


Fig. 7, Response curve for the predicted area found while considering Precipitation of the Warmest Quarter ('Bio_18 variable [mm]) while all other variables are held constant.

5.3 Mapped Projections

5.3.1 Suitable Area Cover – Central Europe

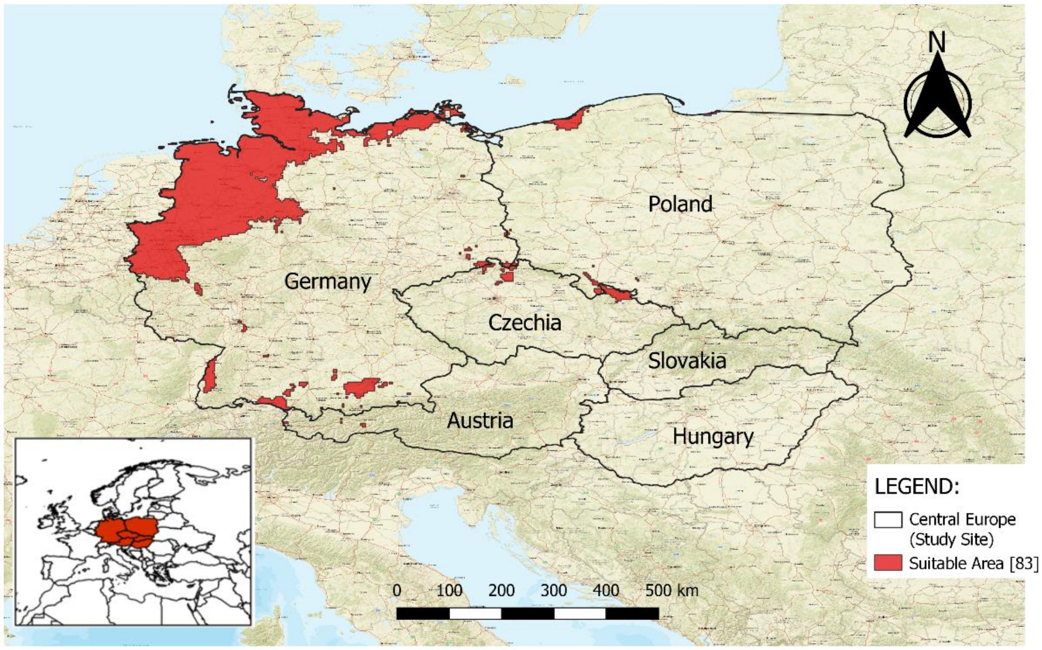


Fig. 8, Binary map of predicted suitable regions (red) within the study site of Central Europe with a suitability range of ≥50% indicating locations of highest introduction risk.

The final output from the Maxent model (*Maxent model*, 2022), once all the provided data is analyzed, was the predicted total area of suitable localities within the European study site. Predicted suitable areas were first projected onto a binary map (Figure 8). Maxent assumes that prevalence is 0.5 by default (*Maxent*, 2022). Therefore, the most suitable locations explained in Figure 8 were delineated within the range of ≥ 50% suitability. This figure indicates that the majority of the most suitable regions for the potential establishment of A. bullfrog populations can be found in Germany, with some smaller regions in Czechia, Poland and Austria. No significant suitable habitats were predicted within the far eastern countries within the study site, Slovakia and Hungary.

The suitability index was then more sharply defined in order to create a choropleth map that shows a continuous distribution of predicted suitability (Figure 9). As seen in Figure 9 there is a variation of expected suitability for *L. catesbieanus* seen throughout the entire study site, as well as within each country of concern. The map appears to suggest the highest suitability, or strongest invasion potential, in the Western part of Central Europe, particularly in a significant portion of northwestern Germany and some smaller areas in the country's southwest. There are also some hot spots of suitable locations seen within parts of northern Czechia and one in northwestern Poland.

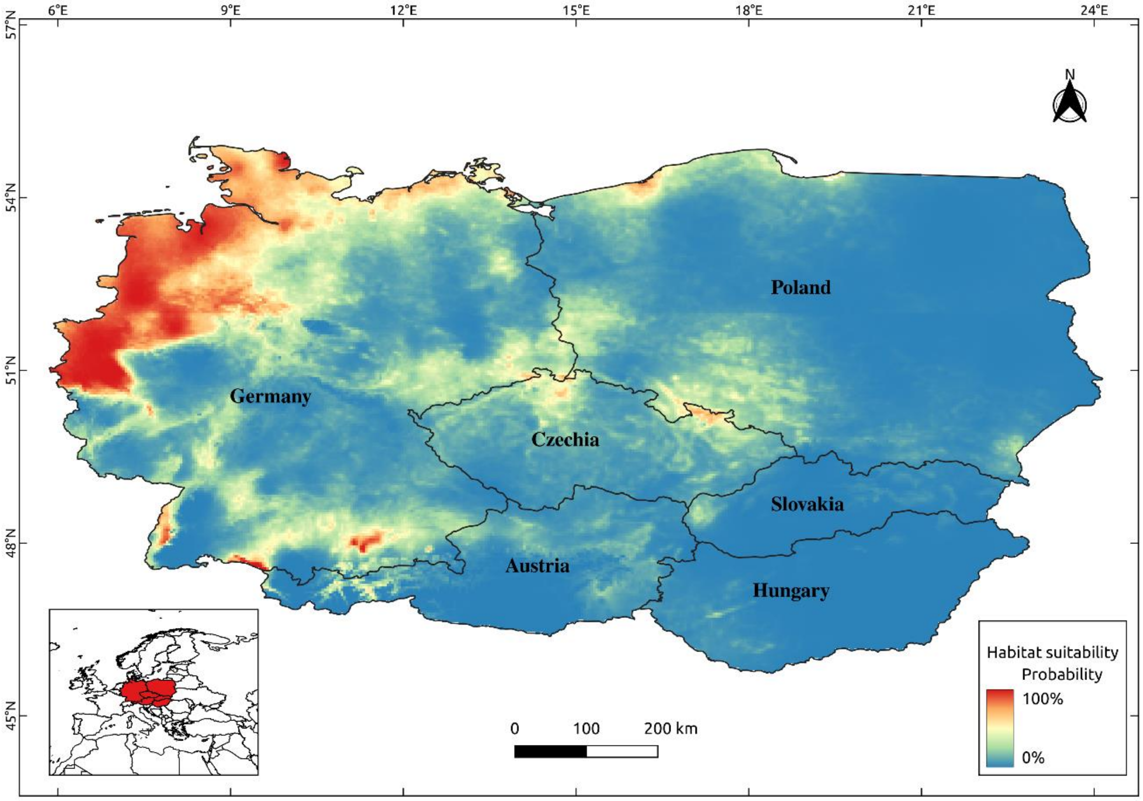


Fig. 9, Map of the Central European region showing the percent suitability (%) for *L. catesbieanus* (red = most suitable; blue = least suitable) as calculated using Maxent. Suitability is determined based on locations where ideal climatic conditions for the organism are found.

Some areas of the research site had significantly lower suitability estimates, indicating that there are likely some optimal climatic conditions for the species' success there, but not enough to make it a highly suitable niche. More specifically within parts of southwestern Poland, on the border of southeastern Germany and northern Czechia as well as far western Austria are where localities of intermediate suitability are located. This can be more clearly understood through the assessment of the percentages of suitable area cover values calculated for each country (See Table 3). This table expresses a prediction of 86.45% of the central European region to have a >20% suitability for *L. catesbeianus* given the climatic conditions considered. Germany, however, was the only country within the central European study region to have the highest risk of invasion, with ~ 10% total area cover at a 80-100 % suitability for this invasive species.

Country	**20-40%**	**40-60%**	**60-80%**	**80-100%**	**Total**
Germany	20.08	8.93	8.08	10.11	47.2
Poland	9.13	1.25	0.28	0.02	10.68
Czechia	22.96	3.55	1.01	0	27.52
Slovakia	1.00	0	0	0	1.00
Austria	3.02	0.49	0.03	0.02	3.56
Hungary	0.05	0	0	0	0.05
Total	56.24	14.22	9.4	10.15	

Table 3, Percent suitability for *L. catesbieanus* throughout a defined Central European study site, with varying levels of suitability (excluding areas with suitability range between 0-19% area coverage). Total predicted area within the study site with >20% suitability was 86.45%.

Percent suitability ranges were calculated for each country in order to represent the variability of suitable habitats found throughout the study site, from low to high suitability. These ranges are meant to represent the ranges expressed within the choropleth map seen in Figure 10. These calculated values indicate that there is a 20-40% probability of successful colonization of this invasive species throughout all countries within the Central European region, including a small portion of Hungary (0.5% area cover) (Table 3).

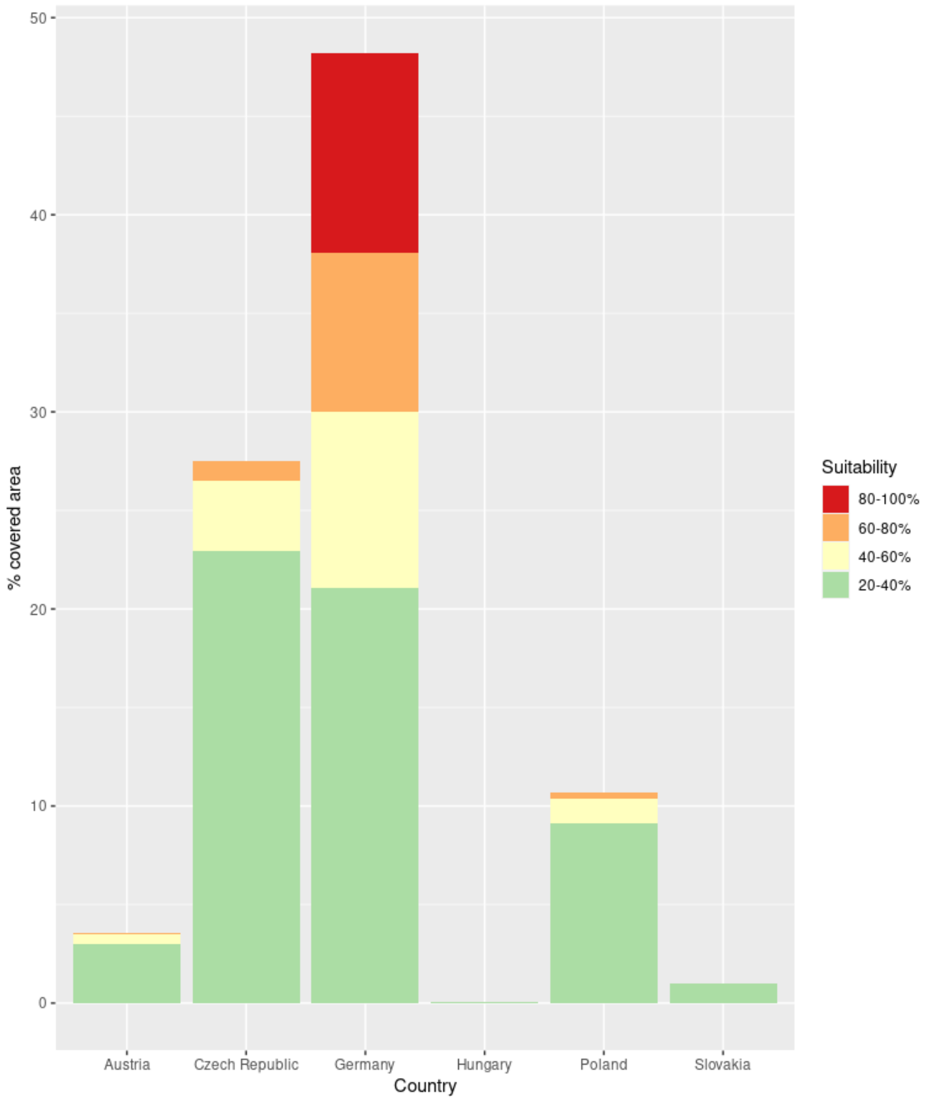


Fig. 10, Amount of total area calculated within a range of percent suitability found in predicted localities of suitable habitat types for American bullfrog. Total area of varying suitability computed for each selected country within the study site.

According to Figure 10, Germany is the only country in the Central European region, calculated by this study, that contains close to ideal environmental conditions for this species of bullfrog given current climatic conditions. This figure also helps to point out the substantial area predicted to be 20-40% suitable for this species, especially within Germany and Czechia. With an invasive species like A. bullfrog that is known to be able to adapt to an array of environmental types, localities that have environmental conditions allowing even for 20-40% suitability could be considered significant.

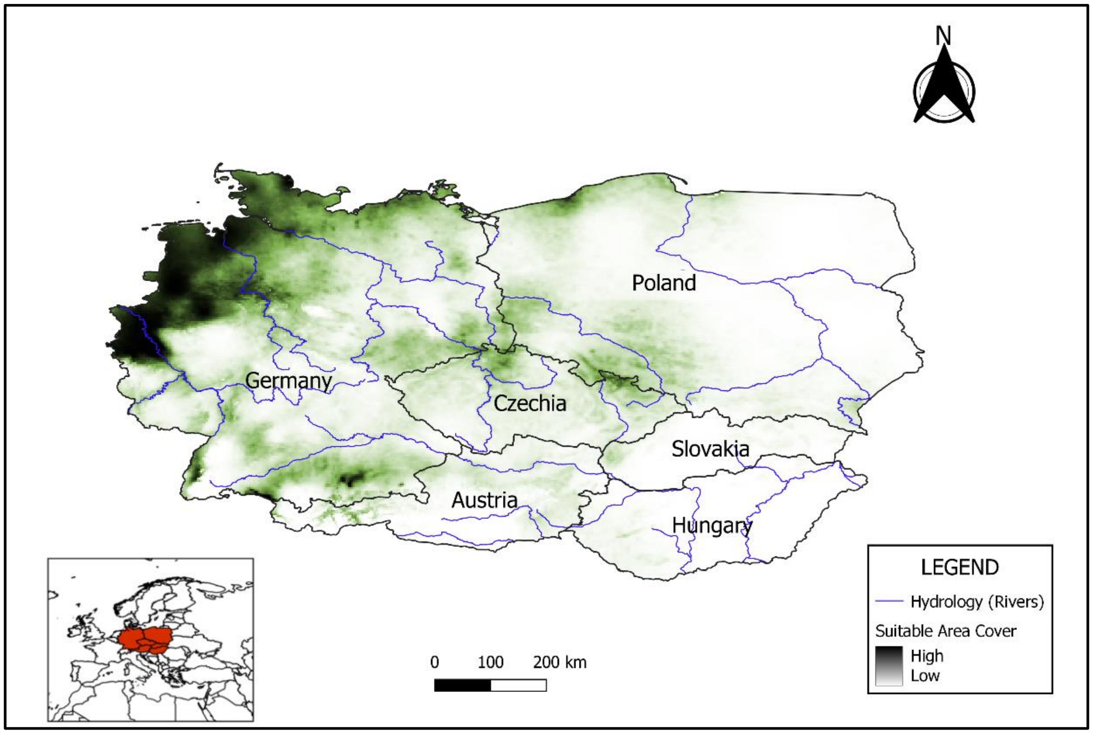


Fig. 11, Map of varying predicted suitability of the A. bullfrog within the C. European region with

indicated riverine systems found within the study site.
Indicates the proximity of predicted suitable habitats to
important freshwater habitat types.

Figure 11 indicates areas within the study site predicted to have high suitability and their proximity to permanent river channels within Central Europe. The areas deemed most significant in this map seem to be within western Germany, the northeastern part of Czechia bordering Poland and a small portion of western Austria, as mentioned previously. Figure 11 expresses that these regions show high invasion risk due to their calculated ideal climatic conditions for this species, while also indicating the closeness of these suitable areas to the preferred aquatic habitats.

6. Discussion

6.1. Review of Key Findings

6.1.1. Suitability in Central Europe

The spread of invasive species into non-native environments remains an ongoing issue, and the possibility of future introductions continues to grow, especially in light of concerns surrounding climate change (Johovic *et al.*, 2020). Upon interpretation of prior research collected on the American bullfrog species, it was apparent that there was limited information on the susceptibility of invasion in the C. European area (Ficetola et al., 2007; Johovic et al., 2020), which further confirmed the need for the analysis at hand. Our understanding of the regions in central Europe that are most at risk from an oncoming invasion by the invasive A. bullfrog has improved as a result of this study's findings.

According to the suitability map calculated in the study (see Figures 8 and 9), it would indicate a sizable number of uninhabited regions within the defined areas of central Europe that would qualify as ideal habitats for the organism, based on climatic conditions present there. Current climatic conditions suggest that every country within the chosen central European study site had some level (minimum 20% suitability or higher) of suitable habitats for the invasive bullfrog species (Hungary >1 % at 20-40% suitability). More specifically, the Western region of Germany, and parts of Czechia and Poland seem to contain the most suitable climatic conditions for A. bullfrog populations, according to the results. This was further explained as these countries were also calculated to have the highest percentage of suitable area cover (47.2%, 27.2%,10.68% respectively, of total area cover with ≥ 20% predicted suitability). However, there is a range of suitability seen throughout most of the central European study region, which explains varying degrees of invasion risk potential. This information proves the theory that most countries studied in this analysis show some level of vulnerability to invasion from *L. catesbieanus.*

6.1.2. Climatic Variables Most Influential to Suitability

The results calculated from the SDM were obtained with the consideration of climatic variables associated with the species' survival success, as well as their statistical contribution to the model. Climate conditions were considered in both the species native and non-native distributions which helped to demonstrate the equivalent localities within central Europe that coincides with the habitat qualities necessary for this species' life history strategies. With the chosen bioclimatic variables used to build the final suitability model, almost all were considered to be significant characteristics for the bullfrog species. More specifically the most significant habitat characteristics for this species were precipitation of the warmest quarter of the year and annual temperature range, along with annual mean temperature. These findings particularly suggest that temperature is a defining environmental feature, especially higher temperatures, which is essential for an ideal habitat for the A. bullfrog's survival success (Álvarez and Nicieza*,* 2002). Optimal mean annual temperature for the most suitable regions in the study site reached ≥ 10ºC. On the other hand, precipitation as an independent environmental factor has less of an impact on creating a favorable habitat for this particular species, unless corresponding with a warmer climate. From these findings, it can be deduced that A. bullfrogs prefer locations with lower average annual rainfall of >600 mm/yr.

It is interesting to note the significance of the bioclimatic condition of annual temperature range in the construction of the model (see Figure 5), which refers to the seasonal temperature variations throughout the year. According to the model output, temperature variability was favored, with a >80% suitability in regions with a climatic fluctuation range of ~25ºC (Figure 5). This finding really highlights the adaptability that this particular species possesses and can help to further explain why this species may have been successful within many different ecosystem types worldwide (Ficetola *et al.*, 2007; Johovic *et al.*, 2020).

6.2. Interpretation of Results

6.2.1 Validation of Hypothesis

Predicted suitability for the A. bullfrog in the central European ecoregion was measured by deciphering what areas contain the climatic characteristics required for this species' success, based on what is found throughout their current distribution. One hypothesis proposed, predicted that the unoccupied region of central Europe would in fact contain areas with climatic conditions similar to the habitats found within their current distribution, indicating the presence of an ideal niche for *L. catesbeianus*. From my results, I can conclude that parts of the central European region houses suitable environmental conditions for this species, signifying the potential vulnerability to future introductions, and therefore supporting this hypothesis. It was also hypothesized that higher annual temperatures would be more favorable for this species' success, which was proven a valid inquiry. However, the assumption made for the preference of higher annual rainfall and less seasonal temperature variability was proven invalid. According to the model outcome it seems that annual rainfall did not contribute much to the species' success unless higher rainfall was coupled with warmer climate. Temperature fluctuation throughout the year seemed advantageous for the bullfrog species as well, which may be associated with specific life strategies for this species.

It is important to determine the significance of the distribution model with the known biology of the species in question, in order to determine whether or not my predictions are in fact logistically sound. Based on the suitability map produced for this species, it can be observed that the most suitable localities were found closer to the areas in which populations of bullfrogs are or have been in existence. For example, the most suitable areas seen in parts of western Germany have been verified as former and/or current occupied territory (Thiesmeier, Jäger and Fritz, 1994; Laufer, 2004; Ficetola *et al.*, 2007). The observation points used in this analysis did not include any of the populations within Germany, however, so there was no influence of these occurrences in the final constructed SDM, but we can infer that these predictions appear valid.

After Germany, Czechia is seen to have the highest predicted area with 40-80% suitability. These regions are mostly concentrated around the northern borders with Germany and Poland. This region is known to have a transitional climate, experiencing influences from all varying climates of the surrounding areas of Europe. With more abundant lowlands and substantial rainfall during the warmer months (*Europe - Climate | Britannica, 2023*), it makes sense that there are some suitable habitats found here for A. bullfrogs. Czechia and Austria are countries documented to have some of the highest number of native amphibian species for all of Europe (21 and 20, respectively) (Temple *et al.*, 2009), which suggests that climatic conditions essential for amphibious organisms, like A. bullfrog, exist here. Moving eastward, towards the Danube basin, including parts of Hungary, Slovakia and eastern Austria, there is a moderate amount of annual rainfall (~600 mm/yr in Budapest, for example). While less annual rainfall is favored for this species, higher average rain during the spring and summer is important, which is what would ultimately define whether or not this constitutes the preferred ecological niche (Wang and Li, 2009; Andersen *et al.*, 2021; *Europe - Climate | Britannica, 2023*). As it was calculated here, temperature is more influential to bullfrog dispersal rate and it is, therefore, important to consider the climatic variable averages used were representations of current conditions but only calculated from 1970-2000 (*Bioclimatic variables — WorldClim 1,* 2017). This means that certain climatic anomalies were not considered here. For example, recent reports determined that Europe has experienced an increase in annual temperature averages of ~1ºC in the last two decades (*European temperature* | *Copernicus*, 2018). These trends may have only increased due to climate change impacts.

Another interesting result is that there are little to no suitable spaces projected in Hungary for this species based on environmental factors found there. Hungary is not void of amphibian species with similar life processes, and is even known to house some of the largest populations of endangered amphibian species in Europe, like the Danube

Crested Newt (*Triturus dobrogicus*) (Béla, 2018). This could be interpreted as a more optimistic finding, however, since the adverse effects of this species may not be as pressing in this area if less suitable habitats are available for effective establishment.

Previous research using species distribution modeling indicated that over 99% of comparable ecosystem types found outside of their native range is considered to be suitable for A. bullfrog (Andersen et al., 2021). Although the results from this particular study show a similar prediction ratio (86.45% total) for this species, it is important to note that this particular study emphasizes varying suitability. Climatic conditions fluctuate throughout the C. European ecoregion (*Europe - Climate | Britannica, 2023*), therefore the entire study site can not be regarded as uniform. Giving a gradient of suitability across the area provides more information about the likelihood that the area will serve as a viable habitat for this invasive species. It allows for a more accurate analysis of the severity of potential introductions in each predicted site.

6.2.2 Clarity of the Model

In an attempt to determine the logistical clarity of the suitability model, it is necessary to recognize whether the results correlate with the ecological functionality of the focal organism. Due to the fact that bullfrogs are ectothermic amphibians that spend some of their time on land and some in the water, the climate that contributes to these environmental circumstances will result in the most suitable habitat types (Bruening, 2002; Howell et al., 2020). Precipitation during the warmer season would, therefore, make sense as being one of the most contributing variables to the model in determining the most suitable habitats for this species. Localities that have higher precipitation during the warmer seasons may help to provide better access to freshwater bodies during the breeding seasons, for example, which are necessary ecosystems for bullfrogs to lay their egg masses (Cunningham, Calhoun and Glanz, 2007; Adriaens *et al.*, 2013).

The fluctuation of temperature throughout the year was calculated to be another influential environmental factor in determining suitable habitats for this species. This influence is to be expected with the limited thermal range in which ectothermic organisms can live within. It is crucial to maintain a warmer temperature range for the bullfrog growth process in order to undergo full development, particularly for a species with a prolonged metamorphosis period (Cunningham, Calhoun and Glanz, 2007; Cook *et. al*, 2013). The A. bullfrog has a metamorphosis process that can last up to 3 years (Carlson and Langkilde, 2013), which means temperature must remain fairly consistent during this developmental phase. Egg development for *L. catesbieanus* is impaired when external temperatures are >31ºC or <15ºC (Cook, Heppell and Garcia, 2013; Johovic *et al.*, 2020).

On another note, A. bullfrogs are known to be relatively resilient to temperature flux compared to other amphibian species (Wang and Li, 2009) . This explains the stronger statistical impact that the bioclimatic condition of wider seasonal temperature variability has on forecasts of this organism's suitability. Due to life strategies that allow them to hibernate in winter (Adriaens *et al.*, 2013), for example, American bullfrogs are able to adapt to lower temperatures during the colder seasons much easier.

6.3. Impact of Results

6.3.1. Analyzing Vulnerability in Central Europe

Understanding the capability of an invasive species to infiltrate new environments is crucial in determining how to properly formulate necessary control methods. With the exception of a few isolated regions of Germany, the majority of the countries considered in this analysis have yet to show any confirmed sightings of this invasive bullfrog species. Taking into account a changing climate providing more favorable conditions and their known presence in south and western regions of the continent, (Ficetola *et al.*, 2007; Howell *et al.*, 2020) this species absence within the Central European ecoregion may not be for long. To identify vulnerable areas, it is essential to have a better understanding of which regions within this uninhabited territory may be most susceptible to successful invasions, if translocation or migration patterns permit it. The suitability percentages shown throughout the predicted regions in this study may only increase due to climate change, making the need for management measures imperative.

6.3.2. Building Blocks for Future Impact Management

Inspiration for this analysis came from a previous study conducted by Johovic et al. (2020), in which suitability was calculated for *L.catesbieanus* on a global perspective. The main difference in the results obtained from this particular analysis was the inclusion of the gradient of suitability distributed within the study site, allowing for interpretation of the level of vulnerability that each location possesses. This understanding can point out which regions require a focus of defense measures the most, and what areas may become more susceptible to invasion given future climate change projections. Specific precautions can be adapted and applied for endemic or endangered organisms as well, especially other amphibians, that are present in places predicted to be most suitable for this invasive species. This information can also lead to further eradication of the spread of emerging infectious diseases, like Chytridiomycosis, one that is spread directly through close proximity to infected American bullfrog specimens (Miaud *et al.*, 2016).

While prior removal attempts against this species in invaded environments were successful, they tended to concentrate on small, isolated populations (Thiesmeier, Jäger and Fritz, 1994; Laufer, 2004; Kamoroff *et al.*, 2020). According to the findings found here, Germany contains the most suitable areas for this invasive species, but there has also been evidence of prior introductions of *L. catesbieanus* throughout parts of Western Germany (Adriaens *et al.*, 2013). It is still unclear if the communities previously documented near the city of Bonn, in northwestern Germany, are still in existence or have spread since their original sightings (Laufer, 2004). These facts imply the probability of reintroduction or continued dispersal of these invasive populations, especially in the localities deemed most suitable, which makes immediate protection of these areas of utmost importance. Previous eradication procedures applied in five invaded ponds in Karlsruhe, Germany in which habitats were fenced and all inhabitants executed, resulting in costs of €53,000 per pond/yr. These particular populations were considered small and fragmented, making control procedures manageable. However, it is estimated that if spread of this species is not contained, with 8,500 lakes of > 0.01 km^2, costs of control and/or eradication measures could reach €4.4 billion just for Germany alone (Reinhardt *et al.*, 2003; Bundesumweltministerium, 2021).

6.4. Acknowledgement of Limitations

6.4.1. Limitations in Data

It is crucial to recognize shortcomings in the current analysis in order to enhance future research and better comprehend how to prevent additional harm from invasive species like the American bullfrog. For this particular study, enhancement in the data used to build the species distribution model (SDM) is recommended. To start, the observation data used to build the model did not fully reflect all known occurrences of the species, like the ones seen in Germany, for example. The community-based sighting application used, gbif.org, is restricted by the contributions made to the overall sightings. The quantity of available data is dependent on individual participation, which means observation information may not be consistent with the literature. In particular, occurrence points recorded within the European habitat for *L. catesbeianus* was very limited, which was the reasoning for including occurrence data from N. American sightings. Including this additional information would only enhance the accuracy of the proper representation of the species current distribution, and therefore, provide the full spectrum of climatic variability of this species' ideal niche. Including more occurrence points of known habitats within the European ecoregion could improve the model in predicting suitable habitats for future prevention.

Climatic data is another limiting factor in the production of this species' suitability model. The bioclimatic variables used for this research analysis was extracted from worldclim.org, a web source that provides climate data meant to represent current environmental conditions. The bioclimatic figures available for present conditions are only recorded between 1970-2000, as mentioned previously, which would not reflect the conditions of the last more than 20 years. As climate change is continuously progressing, this missing information is crucial in order to get a complete interpretation of the current climatic conditions existing today, and therefore, how this information will impact the final results.

6.4.2. Limits in the Model

The SDM of *L. catesbeianus* developed here is intended to illustrate the probable invasion risk that the countries of Central Europe face from this species.

Therefore, it is important that the components used to build the model are a complete representation of the conditions most influential to the species distribution. The climatic variables chosen to emphasize A. bullfrog habitat characteristics were only conditions that would foster species success, while limiting factors were excluded. The limiting elements that could be considered for this particular species would be things like 'Mean Temperature of Driest Quarter' or 'Minimum Temperature of the Coldest Month' (*Bioclimatic variables — WorldClim 1, 2017*), for example. Including both positive and negative influences to distribution for this species in future studies may give an alternative perspective of the existing niches. However, it is important to note, that adding more variables to the model may not always result in a more accurate reading and some variables may correlate with others used and could result in less contribution to the model or an overestimation (Merow, Smith and Silander Jr, 2013).

Another consideration for future interpretation of A. bullfrog's suitability throughout the European region, would be to include further ecoregion data into the model. While climate conditions can give a good insight into the variables contributing to an ideal habitat throughout the study site (Andersen *et al.*, 2021, Guisan & Thuiller, 2005), identifying habitat types found in the most suitable areas may give a more complete story. Although Figure 11 gives a good indication of suitable habitat's closeness to river systems in the area, it is important to know exactly how far these riparian and wetland ecosystems extend out in the landscape to get a better indication of the potential distribution and migration pathways that could lead to further spread of this invasive species (Béla, 2018). As mentioned previously, there has been evidence of introductions of A. bullfrog populations throughout parts of western Germany along the Rhine river, bordering France, indicating that bullfrogs use the river systems as invasion corridors (Adriaens *et al.*, 2013; Laufer, 2004; Thiesmeier, Jäger and Fritz, 1994) A. bullfrogs are also known to have the capacity to adapt to anthropogenic aquatic habitats (Wang and Li, 2009; Adriaens *et al.*, 2013). Therefore, it can be beneficial to know whether the areas predicted to have the most suitable climate conditions for this species also contain habitat types that could further benefit this species within these introduced areas. This more in depth analysis will allow conservation efforts to concentrate on any threatened regions that meet all requirements for the ideal habitat that is best suited for this invasive species.

6.5. Recommendations for Future Research

Future research is necessary in order to effectively determine methods to combat further impacts from this invasive amphibian species, *L. catesbeianus.* As effects from climate change become increasingly apparent (Jacob *et al.,*2014), the most susceptible areas of Central Europe may become more suitable for invasions from this species given future environmental conditions. Therefore, determining how future climate projections alter these findings is essential. According to Johovic *et al.* (2020), in accordance with climate change projections of an increase of CO_2 emissions by 2050, suitability for bullfrogs increased by 5% globally. Climate change predictions also claim a projected increase of annual temperatures in central Europe to increase by 3-4.5°C by 2070 (Jacob *et al.,*2014), which would only benefit the spread of bullfrogs in this area (Álvarez and Nicieza*,* 2002). Annual rainfall is also projected to increase by about 25% by 2070 (Jacob *et al.,*2014), which as our results would indicate, might only hinder the further dispersal of bullfrogs in central Europe.

Introductions of American bullfrogs are one of the leading causes of the spread of deadly infectious diseases, like Chytridiomycosis, which has been a large contributor of amphibian declines worldwide (Garner *et al.*, 2006; Adriaens *et al.*, 2013; Miaud *et al.*, 2016). The implementation of more effective conservation policies can be aided by using the findings of this study and comparing this knowledge with the sites of endangered and endemic amphibian species. As mentioned previously, reports of multiple populations of bullfrogs within their European range have been identified to be a carrier of the deadly fungus Bd, which causes the chytrid virus (Garner *et al.*, 2006). Therefore, if conditions allow, further dispersal of the A. bullfrog within the European territory will most likely advance the spread of this deadly virus, which would devastate local amphibian populations (Kats & Ferrer, 2003). Overall, determining how best to control the further spread and associated impacts of this invasive species is most beneficial for the Central European ecoregion.

7. Conclusion

In order to combat negative consequences associated with the introductions of invasive A. bullfrog populations, it is essential to determine environmental traits that contribute to their success. Although control measures can not necessarily be applied to climatic conditions, it is necessary to understand these basic habitat features that can lend to invasion potential. This study helps to define the areas within central Europe that are most vulnerable to future introductions of A. bullfrogs as well as identify locations of less concern, given the state of current environmental conditions. By acknowledging this information, further analyses of impacts that this species may cause in the most susceptible areas can be addressed. As discussed, this invasive species can be detrimental to native amphibian populations (Kats & Ferrer, 2003), so determining which species could be most impacted by these introductions and focussing prevention measures in these areas of concern would be the next step.

Combating negative consequences due to the introduction of this invasive amphibian species is much more difficult once populations have been established (Adriaens et al., 2013, Kats & Ferrer, 2003). While successful eradication procedures have occurred for invasive A. bullfrog populations in the past, for example in California U.S.A. (Kamoroff et al., 2020) and in Gemrany (Laufer, 2004), this process was long and costly. Therefore it is best to try to limit the spread of this invasive species before the detrimental effects of their introduction are able to occur (Adriaens et al., 2013, Kats & Ferrer, 2003). The A. bullfrog is known to have very few natural predators (Adriaens *et al*., Bruening, 2004), therefore effective control measures would be best through legislative actions. Since human translocations through the pet trade or food production industry is one of the most common means of introductions of the A. bullfrog to non-native habitats (Adriaens *et al.*, 2013; Johovic *et al.*, 2020; Miaud *et al.*, 2016; Schloegel *et al.*, 2009), limiting this dispersal method is crucial. Currently, the European Union (EU) has invested strong nature protection legislation plans implemented through the Horizon 2020, in which important management efforts are being focussed on the control of invasive species in Europe (Johovic *et al.*, 2020). With the help of these initiatives, research such as this one can help to bring attention to the species of most concern and the ecoregions that may be most impacted.

Additional research is essential to understand the full scope of the current and future threats that the A. bullfrog poses on the presently unaffected central European region. However, this study is a valuable first step in identifying the areas in which we can mitigate future expansion and associated impacts of the invasive bullfrog species. Our understanding of the regions in central Europe that are most at risk from an oncoming invasion by the *L. catesbeianus* has improved as a result of the study's findings.

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9. Appendix

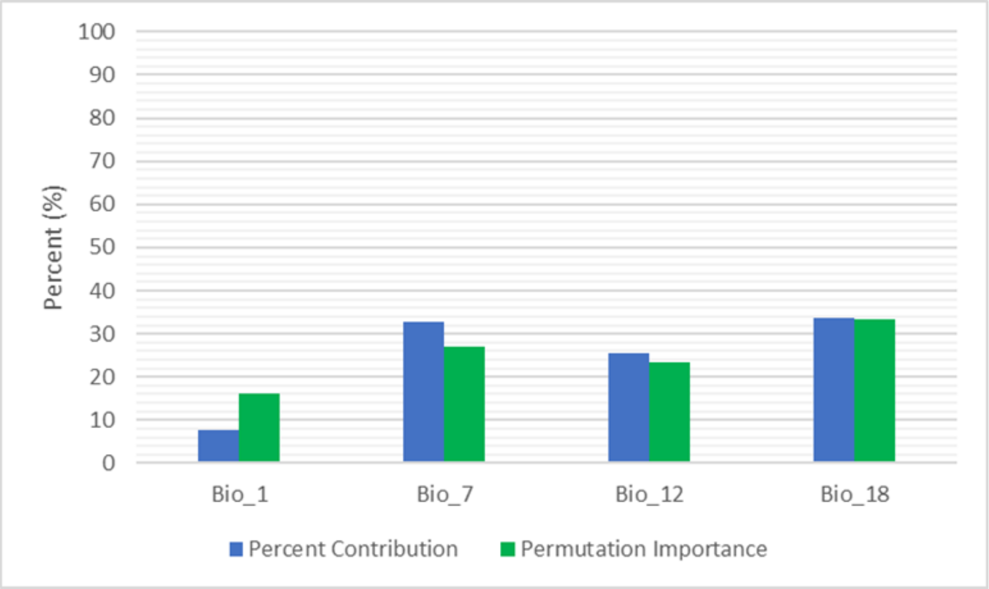

Fig. 12 , Comparison of contribution percentage to permutation importance calculated for each bioclimatic variable used to build the suitability model for *L. catesbieanus*; {Bio_1 = Annual Mean Temp., Bio_7 = Annual Temperature Range, Bio_12 = Annual Precipitation, Bio_18 = Precipitation of Warmest Quarter}.

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Fig. 1, Photo of American bullfrog (*Lithobates catesbeianus*); (Harding, 2004)

Fig. 2, Receiver Operating Characteristic (ROC) curve indicating sensitivity of the model's predictive power to identify suitable habitat locations for *L. catesbieanus*.

Fig. 3, Omission and Predicted Area plot produced as an output from the Maxent program in reference to the predicted suitability distribution for *L. catesbieanus* in the European region.

Fig. 4, Response curve for the bioclimatic variable 'Bio_1' (Mean Annual Temperature [ºC]), with an indicated response threshold between 0 and 20º C. 'Predicted Value' (y-axis) in each response curve represents probability of suitability for the bullfrog species throughout the study site.

Fig. 5, Response curve for the bioclimatic variable 'Bio_7' (Annual Temperature Range [ºC]), indicating suitability of *L. catesbieanus* in selected study sites given variation in seasonal temperature changes.

Fig. 6, Response Curve for Annual Precipitation ('Bio_12' variable [mm/yr]) within the chosen European region as a response for the predicted suitable area for *L. catesbieanus.* Indicates a precipitation maximum threshold of > 500 mm/yr but does not indicate a suitable precipitation minimum for this species within the study site.

Figure 7, Response curve for the predicted area found while considering Precipitation of the Warmest Quarter ('Bio_18 variable [mm]) while all other variables are held constant.

Fig. 8, Binary map of predicted suitable regions (red) within the study site of Central Europe with a suitability range of ≥50% indicating locations of highest introduction risk.

Fig. 9, Map of the Central European region showing the percent suitability (%) for *L. catesbieanus* (red = most suitable; blue = least suitable) as calculated using Maxent. Suitability is determined based on locations where ideal climatic conditions for the organism are found.

Figure 10, Amount of total area calculated within a range of percent suitability found in predicted localities of suitable habitat types for American bullfrog. Total area of varying suitability computed for each selected country within the study site.

Fig. 11, Map of varying predicted suitability of the A. bullfrog within the C. European region with indicated riverine systems found within the study site. Indicates the proximity of predicted suitable habitats to important freshwater habitat types.

Fig. 12, Comparison of contribution percentage to permutation importance calculated for each bioclimatic variable used to build the suitability model for *L. catesbieanus*; {Bio_1 = Annual Mean Temp., Bio_7 = Annual Temperature Range, Bio_12 = Annual Precipitation, Bio_18 = Precipitation of Warmest Quarter}.

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Table 1, Table of Bioclimatic Variables used within the analysis of species success within the study region of C. Europe based on a climatic variables seen within the known occurrence locations throughout the Northern Hemisphere (*Bioclimatic variables — WorldClim*, 2017*; Lithobates catesbeianus, Shaw, 1802*).

Table 2, Output of the Maxent program that calculates the percent contribution and permutation importance of each bioclimatic variable included in the model. All variables were provided with a resolution of 2.5 arc/min (~5 km pixels). {Bio_1 = Annual Mean Temp., Bio_7 = Annual Temperature Range, Bio_12 = Annual Precipitation, Bio_18 = Precipitation of Warmest Quarter}.

Table 3, Percent suitability for *L. catesbieanus* throughout a defined Central European study site, with varying levels of suitability (excluding areas with suitability range between 0-19% area coverage). Total predicted area within the study site with ≥20% suitability was 86.45%.