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GREEN TOAD GONE:

THE DECLINE OF THE EUROPEAN GREEN TOAD (*BUFOTES VIRIDIS*) IN SWEDEN

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Abstract

Why is the European green toad (*Bufotes viridis*) threatened in Sweden while it thrives in other countries? Climate change, the overall shifts in temperature and weather events, is affecting species across the globe. The effects of climate change on species is a critical area of study, and the declining peripheral populations, especially those on the northern edges, become increasingly important. Fitting those criteria, the green toad is considered the most endangered amphibian in Sweden. Numerous sites over the past 115 years were chosen based on historical and current observations of the toads. This study uses the available climate data from the University of East Anglia CRU TS (Climatic Research Unit gridded Time Series) to determine if these factors negatively impact *B. viridis*. A species distribution model was created and used to establish the suitability of the sites based on both current climate conditions and the climate conditions from the earliest time period. The resulting differences of means indicated that climate has affected the sites, lowering the suitability over the last 100 years. However, when comparing the sites from the earliest time period, using the current climate data, that currently have a toad population with those that no longer sustain the species, the differences were minimal. There appears to be no conclusive data as to why the toads no longer exist at those sites. If climate change is the cause, reintroduction into increasingly uninhabitable areas becomes a pointless endeavor. By ruling out the effects of climate change, we can thereby focus more closely on other causes.

Key words: European green toad, climate change, Sweden, peripheral populations

Abstrakt

Varför hotas grönfläckig padda (*Bufotes viridis*) i Sverige medan den trivs i andra länder? Klimatförändringar, de övergripande förändringarna i temperatur- och väderhändelser, påverkar arter över hela världen. Effekterna av klimatförändringar på arter är ett kritiskt studieområde och minskande perifera populationerna, särskilt de i norra kanterna, blir allt viktigare. Genom att uppfylla dessa kriterier, anses grönfläckig padda vara den mest hotade amfibien i Sverige. Många platser under de senaste 115 åren valdes utifrån historiska och aktuella observationer av paddorna. Denna studie använder tillgängliga klimatdata från University of East Anglia CRU TS (Climatic Research Unit gridded Time Series) för att avgöra om dessa faktorer påverkar *B. viridis* negativt. En artsdistributionsmodell skapades och användes för att fastställa platsernas lämplighet baserat på både nuvarande klimatförhållanden och klimatförhållanden från tidigast tid. Resulterande skillnaderna i medelvärden indikerade att klimatet har påverkat platserna och minskat lämpligheten under de senaste 100 åren. Men när man jämför de platser från den tidigaste tidsperioden, med hjälp av den aktuella klimatdata, som för närvarande har en paddapopulation med de som inte längre upprätthåller arten, var skillnaderna minimala. Det verkar inte finnas några avgörande uppgifter om varför paddorna inte längre finns på dessa platser. Om klimatförändringen är orsaken blir återintroduktion till alltmer obebodliga områden en meningslös strävan. Genom att utesluta effekterna av klimatförändringar kan vi därmed fokusera närmare på andra orsaker.

Nyckelord: grönfläckig padda, klimatförändringar, Sverige, perifera populationer

Introduction

Climate change, the overall shifts in temperature and weather events, is affecting species across the globe. The most prevalent source for the rapid increase has been anthropogenic, or human caused. While the burning of fossil fuels by vehicles and industrial manufacturing, which have released untold amounts of toxic gases into the atmosphere, are mostly to blame, deforestation and increased industrial agriculture only add to the problem. Rising temperatures lead to other problems including more desertification, heat waves, and wildfires. The increase in evaporation from the heat can cause stronger storms and more extreme weather events, including harsher winters, as well as rising sea levels due to the melting of glaciers and permafrost. In addition, the ocean is becoming more acidified due to the increased reuptake of carbon dioxide that is being cycled through the atmosphere[1].

Species' ranges often cover large swathes of habitats that are similar, while local populations adapt in some ways to fit their specific environments. The groups occupying the extremes of the ranges are referred to as peripheral. Conservation biology is often about the big picture, saving species on the global scale. However, it is often easy to lose sight of this when looking at the local picture. Nonetheless, biodiversity, even in smaller ecosystems, is incredibly important. A population that disappears from one area could cause a cavalcade of unexpected changes, despite the global numbers of the species. Stopping declines before the animals becomes endangered is the best way to stop the extinction of a species[2]. There are problems with parochialism, the narrow focus on local groups, that can be detrimental to the populations both locally and globally. The groups already at the edges of their ranges, peripheral populations, are of special concern. The school of thought on peripheral populations is that they are less genetically diverse, lacking fitness and density, and often face extinction. With this as a guiding principle, conservation efforts are often discouraged on these populations[3] As we will see later, the need to preserve these groups is important. However, richer countries can allow more money to protect peripheral populations that may be locally endangered, but poor countries often spare little money to species facing global extinction. This results in a lack of proportion to the actual need of some species[2].

Returning to the topic at hand, the effect of climate change on species is a critical area of study. As the climate alters rapidly, populations have only three ways of handling the effects on their environments:

1. Adaptation to the new environment either through natural selection (genetically changing to meet the demands), or phenotypic plasticity (varying phenotypic responses to the environment, both in morphology and behavior).
2. Movement to new environments with the same basic characteristics as the old one.
3. Extirpation or extinction[4].

To fully understand the effect, and prevent widespread extinction, it is important to look at the changes currently faced by many species due to climate change. Studies have shown that alterations in phenology, the timing of reproduction, as well as range shifts towards higher altitudes and latitudes are already underway. Let us more closely examine these three points.

First, gene flow generally moves from the more populated portions of the species to the outermost groups. The local populations are often well adapted to their current range. This increase in heterogeneity can lend to the overall variation necessary to survive changing environments. The genetic makeup of peripheral populations, especially as they relate to the larger general population, is therefore important for increased diversity. The genetic adaptations to a warmer climate within the more central or southern edge populations can be passed to the peripheral populations as they migrate to more northern climes, thus increasing their fitness. A problem arises when those central groups are not adapted to the hotter temperature that is pushing the population. They become less fit[4]. By protecting the poleward populations, the global populations may be saved. However, the truth may lie more in the external causes of population reduction, including human interference, that have more direct effects than the usual evolutionary effects on smaller groups. These subpopulations often exhibit more adaptive response to their climate, which ultimately can be the way to save the entire species[5].

Second, global temperatures are rising, and there is range expansion towards the poles concurrent with range reduction at the trailing margins[5]. With climate change, the declining peripheral species, especially those on the northern edges, become increasingly important. So, focusing on these smaller, local populations does have plus sides. Movement is seen as range shifts rather than normal population fluctuations within the group and reflect only small portions of the entire range of the species. The average change in range is shown as a "latitudinal cline", where populations still exist at about a 2° northward shift from where the species are extirpated. The same may be seen in increased altitude. Although human interaction has resulted in declining populations, studies indicate that this type of decrease is seen across the entire range rather than just at the periphery. This further shows that the latitudinal cline is not the result of lower initial population density, but rather climate change[6]. However, if anthropogenic pressures are limiting the peripheral population, the entire population is at risk[5]. Changes within smaller regions or habitats are often not due to climate, but rather to anthropogenic causes[7]. Even the outermost groups are still within their historical ranges. This indicates the devastating effect of human activity as opposed to intrinsic frailties[3]. Last, extirpation and extinction happen due to a failure to adapt to the environment. That is what we are trying to avoid by understanding the processes involved.

How does this apply to amphibians? The numbers of amphibians are going down worldwide. The family Bufonidae, true toads, are showing the sharpest declines, yet the reasons for this remain undetermined[8]. Due to their contribution, as both prey and predator, to the overall global biodiversity, their loss would be devastating to all ecosystems. The very nature of amphibian skin, used as an extra breathing apparatus as well as maintaining moisture for the animal, opens them to toxins and pollutants in their environment. As such, they are a good indicator of the overall health of the ecosystem[9]. Climate change is often cited as a reason for the decrease in amphibians. Being necessarily tied to water, this class faces more problems under climate warming. One possible reason is the change in the timing of breeding, or phenology. If they come out of hibernation earlier, they may go to water that is in some ways unsuitable. The overall increase in temperature can also cause a trend in earlier reproduction. Eggs and tadpoles can die due to lack of adequate water and decreased depths or changes in the chemistry of it, with higher levels of pH or ultraviolet radiation. These problems more readily subject the animals to diseases or malformations. If an entire year's reproduction dies, the species is that much closer to extinction[10].

The amphibians at the thermal edges of their ranges are now facing climates that are too harsh for viability. With the current trends, amphibians are more likely to expand rather than retract in their ranges. During the past glacial periods, the ranges of the Holarctic species were reduced in the northern climes, followed by expansion during the interglacial periods. This is still seen with the distribution of amphibians globally, which suggests that climate warming may not have a deleterious effect on species. However, evidence does not show that it is necessarily responsible for increases either[10]. Dispersal and migration are tantamount when studying population control and fluctuations, as well as how poor aspects of habitat affect viability. Amphibians are not known for long range movements, which potentially inhibits their reactions to climate-controlled migrations[9]. Understanding the dissemination of amphibians can assist in preventing the extinction of more and more species. Movement is evolutionarily determined by the need for the best mating and feeding sites. Dispersal involves the peregrination of adult amphibians to and from breeding areas, as well as the travel around food sources and winter hibernation areas. These are often short trips within local areas. Migration is used to find the best habitats in both water and land environments[11]. Rates of expansion can be tied to the ability of the species to migrate. Amphibians tend to remain relatively close to home. Therefore, the idea that there is widespread expansion is a bit unrealistic. This can lead to population fragmentation, as well as movement being outpaced by the changing climate[10].

Does climate change affect the suitability of amphibian habitats? Has there been a decline in the last century that can cause this? As of yet, no direct conclusions as to the reasons for the global drop in amphibians can be drawn, but rather it is a compilation of effects of pollution, habitat reduction, increased human consumption, and climate change. While some of the loss is direct in the form of habitat destruction, populations in protected areas are also seeing a decrease. The local extinctions of marginal populations can be partially explained by random indirect events. However, this does not thoroughly elucidate the cause for the diminishing of an entire class of organisms that has rapidly progressed in the last 50 years[9].

Aim

Populations at the periphery of their ranges are seen as more fragile, less variable and more insular, thus more at risk of extinction[3]. The European green toad is at the northern most boundary of its global range (Figure 1), indicating it may not be suited to more extremes in cold and wet winters.

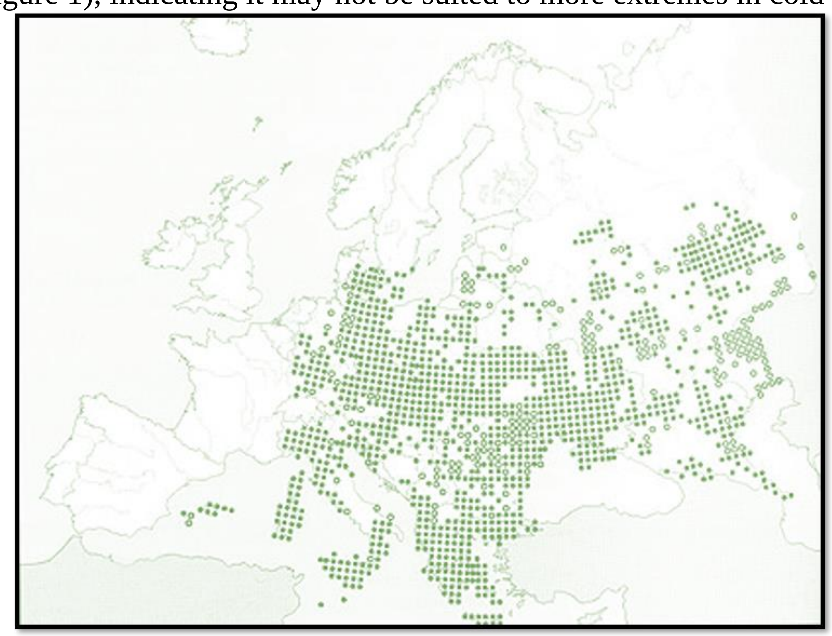

Figure 1: **The global range of *Bufotes viridis***. From Gasc, et. al., 2004.[12]

Many causes have been suggested to explain the population dwindling of *B. viridis*, including predation, habitat destruction through agricultural acquisition, as well as eutrophication. In addition, climate change has been advocated. This study will address the issue of climatic changes throughout the past 115 years to determine if the data correlates to the decline of the species. The hypothesis is that changes in climate, such as the decrease of freezing conditions, has negatively affected the habitat of the population of *Bufotes viridis* in Sweden.

Methods and materials

Initially, I had intended to return to the sites from the earliest report in order to re-evaluate the effectiveness of the conservation program for *Bufotes viridis*. However, after the initial phase of the fieldwork in late May 2020, I was unable to find any toads. This further exemplifies the problem, despite yearly releases of tadpoles. So, the focus of my research shifted to determining the effects of climatic changes over several time periods from earlier mass populations (late 1800's to early 1900's) until today, with the near extirpation of the toads in Sweden. By comparing the presence or absence of the toads at sites that have or have had populations to the climate data, I can thereby determine which has the most effect and by how much has the change affected the sites.

Historical and current studies
Laurenti presented the first official description of the European green toad, which he named *Bufo viridis.* He detailed it as having confluent green spots and warts with some red and intermediate colors. The toads' habitat was in the 'clefts and hollows of walls, enveloped in obscurity'. The picture of the toad that was with Laurenti's description (Figure 2) clearly shows the spotted pattern[13].

Figure 2: Drawing of Bufo viridis accompanying Laurenti's initial description.

The following year, Pallas described the same toad, albeit in much more detailed and flowery language. He called it *Rana variabilis*, mainly because the toad changed colors according to how much he bothered it. They found the toad in a shady area near a rock wall, and noticed that as it crawled out from its hiding spot that the toad was white or ash gray with copious blemishes a color of very beautiful green, and gold sprinkled net-like on its body[14]. Thus, began the long history of naming and renaming the European green toad[15].

The European green toad, *Bufotes viridis,* is on the IUCN Red List[16] as a separate species from the varying toad, *Bufotes variabilis*. Accordingly, the latter is the species in Sweden, Denmark and Northern Germany, and is insufficiently documented (DD-Data deficient). The *B.variabilis* was suggested as a junior synonym to the former in 2001[17]. Later, mitochondrial DNA indicated that *variabilis* was the same in Northern Europe, the Caucuses, and western Asia, despite geographical distance. Yet they were sufficiently different from *viridis*. Due to this evidence, *variabilis* was once again designated as a separate species[18]. However, new DNA evidence has shown that there is little to no difference between the species. The species are synonymous[19]. Throughout texts regarding the toads in Sweden, the names are still used interchangeably. So, for the purposes of this study, I will refer to the toad as *Bufotes viridis*, which is LC (Least concern) on the global Red List.

The first known evidence of the green toad in Sweden was a femur found in a fort on Öland. The bone is dated to the early 11th century[20]. Although it is believed that Linnaeus in 1741 and Christian Gråborg in 1733 each described the green toad, there is no direct evidence that this was the species[21]. However, Sparrman described the species for the first recorded time in Sweden when he encountered several specimens in Karlskrona in Blekinge in 1795 [22].

Over the course of the late 19th and early 20th centuries, many sightings were documented. Throughout accounts of the presence of toads, they are noted as plentiful and populous in areas as far reaching as Malmö, Lund, Karlskrona, Småland and Kalmar[23–25]. The toad was even found on Gotland, an island in the Baltic Sea. As early as 1926, the green toad was found on Utklippan, the island south of Karlskrona. The males' mating calls were loud, indicating a greater number[26]. Gislén observed the green toad in 1946, which he referred to as *Bufo viridis,* predominantly in the eastern parts of the country, in the coastal areas from Hälsingborg to Karlskrona. Many sites were documented in the areas of Kalmar and Huvudskär in southern Östergötland, as well as throughout Öland and Gotland[27]. Even as late as the 1950's, new sites were being detected with the green toads. In 1954, the toad was found in its most northern site, Hallands Väderö[28].

One interesting occurrence was the mass influx of toads into a harbor near Landskrona on September 28, 1927. Thousands of toads flocked around the town, filling the streets and even entering houses. It appeared that the toadlets were newly metamorphosed and had been driven inland by stormy weather. Toads were still found swimming in the harbor and moving about a week later before moving to their winter hibernation areas [29].

In the beginning of the 20th century, the European green toad was ubiquitous throughout southern Sweden[23]. Now, the toads are very rare to completely gone in the same areas. The Red List of Sweden points out that the species occurs in only 3 areas, Skåne, Blekinge, and on Öland, having been extirpated from Gotland and mainland Kalmar's region. The list further labels *B. variabilis (syn. B. viridis)* as VU (Vulnerable). The green toad is a protected species within Sweden[30].

Current work on saving the green toad is underway throughout the country. An extensive study was conducted in Skåne and Blekinge in 2010 to identify the current population condition of the toad, and the potential ways of reestablishing the species[21]. Despite repeated reintroductions by Nordens Ark's conservation program[31] the toad has been less successful in reestablishing.

Improved methods of raising the toads in captivity and thereby releasing tadpoles and toadlets is being undertaken, as well as captive raised adults being released in revitalized historic areas, by Nordens Ark. However, the releases are limited to Öland[32]. New research is focusing more on molecular and DNA studies of the species. Other surveys, follow-ups, and experimental studies have been undertaken in recent years to study this interesting and concerning species.

Many factors have been suggested to explain the decline of the European green toad population in Sweden. Direct human interference by means of habitat destruction, agricultural land overuse, eutrophication, road construction, and water drainage have all been put forth[21]. In addition, more natural causes (though often also as a result of humans) including increased predation and fungal infection, have been considered[33]. These factors will be taken up in more detail in the discussion section of this paper.

As the environments are altered due to temperature extremes, precipitation instability, and food source fluctuation, all species globally are affected. Therefore, the necessity of considering the effects of the crisis on the green toad in Sweden, already at the northern reaches of its domain, are tantamount to finally solving this puzzle.

Habitat and life history

The European green toad is smaller than the common toad (*Bufo bufo*). Hybrids have been seen between these species, as well as with the Natterjack toad (*Bufo calamita*). Adult females range in size from 50 to 100 mm, and males are slightly smaller. The toads live close to the shore, preferring short grass and more sandy or gravelly ground. After going into hibernation in Sweden in late September, the toads usually come out of their underground or crevasse lairs in early to late April. The males immediately head to the shallow, brackish waters with a slightly higher saline content and begin calling for females with a high-pitched trilling song, most commonly heard in the evening at dusk and later. Females arrive about a week later, and soon lay one to two gelatinous strings, 2-7 meters long, containing 8000-20000 eggs[21].

Without any further care from the parents, the tadpoles hatch after a few days and undergo metamorphosis after 3-4 months, in late July through August. They then leave the water for new territories, which can be up to a kilometer away. Males become sexually mature at about three years, and females at four years old, and the toads have an average lifespan of around nine years[21,34].

Foods for the green toads are varied. Tadpoles are mostly herbivorous, eating the algae, grasses and detritus found in their habitats. As they age, their diet becomes more omnivorous, including insects or other animals that are in the water. Cannibalism and oophagy by tadpoles have been noted but is not a common occurrence[35]. After metamorphosis, the toads begin to eat insects, arachnids, and other small invertebrates. The toads prefer the shorter grass, so they are not in direct competition with common toads, that prefer deeper undergrowth.

Site selection

European green toads in Sweden have reduced not only in population size, but also in the extent of their domain. Identifying the sites through presence or current absence of the toad, and then comparing the effects of climatic variables on the sites can lend to the suitability or change of the habitat. These predictors can then be used to determine if the climate is a driver in the loss of toads from once suitable habitats and if these changes will cause further site declination. As all sites contained populations of *B. viridis* that have adapted to their peripheral range, the model would best be applied to other toads in similar conditions, not to *B. viridis* in general.

Sites were chosen based on documented observations. The historical data dating back to 1905 was determined by researching early site observations of old texts. The journal *Fauna och Flora* [36] was a great source, and I pored over the articles available through their website or from the library. In addition, several observations, until 1959, were found in an article by Gislén[37], which provided site names and dates. Using Google Maps on the older sites, I was able to assign each with their appropriate geographical coordinates.

Sites beginning in 1960 until 2019 were obtained from Sveriges lantbruksuniversitet (Swedish University of Agricultural Sciences) Artportalen (Species Portal) site. This site is built from reported sightings by both laypeople and professionals and include the location of the species as well as any other pertinent information the reporter wants to include [38]. In total, I found 175 sites over the span of the included years (Figure 2).

In addition, I chose the sites for all Common toads (*Bufo bufo*) that were reported in 2019 on Artportalen. These observations were used as absences for the model based on the assumption that the habitat was suitable for toads, someone was looking for and found the animals at the site, but no green toads were found there. The downside was that the coordinates were given in the scale of Swedish Grid (Riketsnät 1990, RT90), so I had to convert them to fit into R programming. Using the R function "spTransform", I successfully changed all coordinates into the World Geodetic System (WGS84) coordinate system accepted by R (as well as Google Maps). I ended up with 2748 sites for *B. bufo*. (Figure 3).

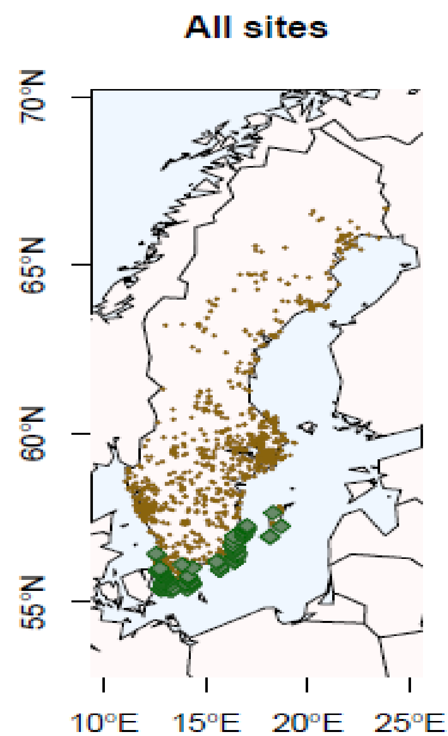

Figure 3: Sites of *Bufotes viridis* and *Bufo bufo*. These are the sites of all *B. viridis* (green diamonds) used in the study. The *B. bufo* sites (brown) are from 2019 Artportalen data.

Climate data

I chose to study the macroclimate of the southern regions of Sweden. The areas where *B. viridis* is found can maintain varying microclimates from vegetation cover (found at Limhamn's Limestone quarry) or lack thereof (Ottenby, Öland)[personal observations], or wind differences, as on the east and west coasts of the country[39]. The consideration was the overall climate because the uniqueness of each microclimate makes them too numerous to condense to a single study such as this.

Climate data from the University of East Anglia CRU TS (Climatic Research Unit gridded Time Series) [40]was used to determine conditions in Sweden throughout the years being studied. This dataset uses grids of 0.5° longitude by 0.5° latitude with stations set up nearly worldwide to record the climate. Started in 2000, the data date back to 1901, with both observed and interpolated data[41]. CRU TS data were divided into three 30-year periods with the mean of each of 10 values of the climatic conditions used as a basis for sites known to have *B. viridis* present during the time periods. These time periods were noted as: Early (1905-1935), Middle (1935-1965), and Late (1995-2019). The site locations of the toads were cross referenced with the climate information at those coordinates to obtain readings for each of the sites at the particular period of observation. Variables include mean, maximum and minimum temperature, cloud cover, precipitation, frost days, diurnal temperature, vapor pressure, wet days, and potential evapo-transpiration (Table 1)[40].

Variable	Code
Mean temperature, °C	Tmp
Minimum temperature, °C	Tmn
Maximum temperature, °C	Tmx
Diurnal temperature range, °C	Dtr
Frost days per month	Frs
Precipitation rate, mm/month	Pre
Wet days, ≥ 0.1 mm	Wet
Cloud cover, percentage	Cld
Vapor pressure, hPa (hectoPascals)	Vap
Potential evapo-transpiration, mm/day	Pet

Table 1*:* Variables from CRU TS, with their abbreviations, used in the study

Model creation

To begin the model building for the species distribution, I chose a random sample from each of the green toad sites and the common toad sites. The criteria for selection included independent sites that were not within 10 kilometers of each other. The resulting numbers were 37 and 487, respectively. All sites containing green toads were assigned a value of 1 for presence, and all common toad sites were assigned 0 for the lack of green toads at their sites. These values became the dependent variables for the model. The sites were divided into the year groups and calculated to determine the conditions at that time. They were thus Early site variables and Late site variables. The Middle time frame (1935-1965) was not used any further in the study.

Independent variables that may be too correlated with each other can have adverse effects on the regression analysis. The potential for this is ever looming. Through correlation and variance inflation factor (VIF) analyses, the multicollinearity of the climate variables was assessed in order to identify the best ones to use. Finally, multiple logistic regressions were run to create a species distribution model to indicate site suitability for green toad habitation.

Probability of presence

The logistic regression model was used to predict the probability that the green toads could be found at a particular site. That model was:

$$\ell = \log_b \frac{p}{1-p} = \beta_0 + \beta_i x_i$$

where β_0 is the intercept (constant) and $\beta_i x_i$ is the estimate of each independent variable times the value of the variable for any given site. The ℓ value is the log-odds calculation of the dependent variable of the observed trait, which in this case is the probability of presence at a site, and p is a value between 0 and 1.

Once the model was established, the formulae were used to calculate the probability of the presence of the green toad based on the concurrent climate conditions for each group of sites, from both early (from 1905-1935, 36 sites) and late (from 1995-2019, 91 sites). I then ran the model using the early sites with the climate data of the late sites, and vice versa. This enabled me to see the potential change in the suitability at a particular location over the 100-year period. The main goal, determining if the sites have decreased in suitability, was judged by subtracting the probabilities between the models to determine the degree to which the habitat has changed in fitness for the toads. A t-test was run on each of the data sets from the time periods to elucidate these differences.

In the final statistical analysis, I used the early sites as a base and determined where the toads were still found today. Then using the climate data from the period of 1995-2019, I took the basic statistical analysis of the group of existing sites compared to the group of extirpated sites. A t-test was run to determine the difference of means between the groups. The objective was to see exactly how much the change in suitability translated to actual loss of toad populations at the sites.

All statistical analyses were performed in R version 4.0.2[42].

Results

The logistic regression successfully created estimates from which I was able to build the species distribution model. Except for the constant (intercept), all estimates had low standard errors, between 0.044 and 0.497 for the early sites and between 0.050 and 0.570 in the later ones. In addition, the variation of inflation factors were well below the acceptable value of 5 (Table 2).

	Early sites estimate	VIF	Late sites estimate	VIF
(INTERCEPT)	1.780 (7.232)	---	-9.774 (8.182)	---
DTR	-0.658 (0.279) *	1.044	-1.054 (0.360) **	1.367
PRE	-0.098 (0.044) *	2.343	-0.084 (0.050) .	2.485
WET	-0.791 (0.497)	1.943	0.684 (0.570)	2.146
VAP	1.849 (0.473) ***	1.343	1.044 (0.540) .	1.310
	Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Table 2: Logistic Regression Estimates. The results of the multiple logistic regression provided the values needed for the species distribution model. Early sites are those observed from 1905-1935(n=36), and late sites are those from 1995-2019(n=86). The VIF score of each variable, being less than 5, indicates that the variables are not interfering with each other within the regression formula. The variables are: DTR=Diurnal Temperature, PRE=Precipitation, WET=Wet Days, and VAP=Vapor Pressure.

Using the values of the diurnal temperature, precipitation, number of wet days, and vapor pressure from both the historical and current climates, I used the following formula on all sites to establish the probability of the being suitable for green toad habitation.

$$\ell = \log_b \frac{p}{1-p} = \beta_{intercept} + \beta_{DTR} x_{DTR} + \beta_{PRE} x_{PRE} + \beta_{WET} x_{WET} + \beta_{VAP} x_{Vap}$$

After calculating the probability of presence of each site using its contemporaneous climate data, I then re-ran the formula using the climate data of the other time period. Thus, early sites were run with both early climate data and current climate data, and the same was done with the late sites. All original data for both early and late sites are in Supplemental Tables 1 and 2 at the end of this paper. The values of the probabilities at all sites over all time periods exceeded 50% probability of presence of *B. viridis* (Figures 4 and 5). However, the suitability did decline somewhat between the time frames, based on climate conditions. In addition, there was considerable overlap.

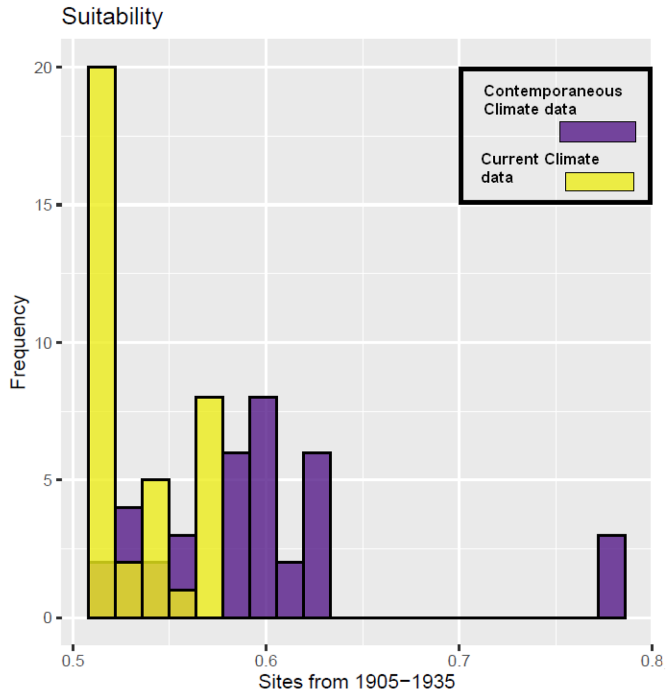

Figure 4: Suitability changes of Early sites. The probability of presence of *Bufotes viridis* at the sites was determined by the species distribution model. Using the contemporaneous climate data of from 1905-1935 (shown here in purple), the suitability of the sites gave a probability between 0.51 and 0.76 for the presence of the toads. However, suitability was higher based on historic climate data than with current, with the probability of presence ranging only from 0.51 to 0.56. There is a small amount of overlap, but only at the lowest end of the scale.

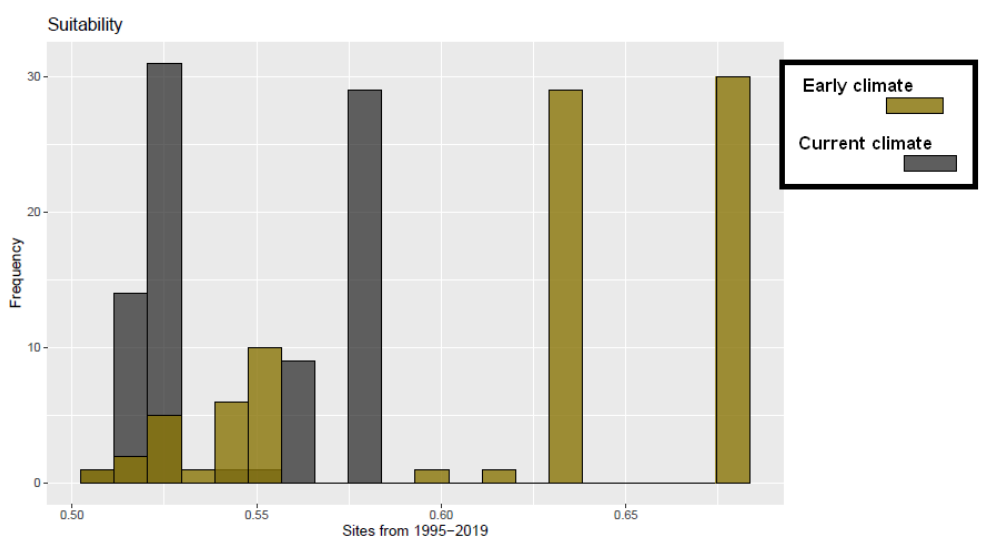

Figure 5: Suitability changes of Late sites. The probability of presence of *Bufotes viridis* at the sites was determined by the species distribution model. Using the historical (early) climate data on the sites from 1995-2019 (shown here in gold), the suitability of the sites gave a probability between 0.50 and 0.68 for the presence of the toads. However**,** suitability was higher based on historic climate data than with current, with the probability of presence ranging only from 0.50 to 0.58. There is a small amount of overlap, but only at the lowest end of the scale.

Further analysis done by subtracting the values of the historical site climate information from the current site climate information showed that the sites have worsened somewhat over the last hundred years, based on climate change alone (Figures 6-A and B). The sites showed an overall deterioration with the majority of the sites having negative values. The current sites did not show as great a downturn compared to the early sites, but the differences were not substantial. However, a few sites did show minor improvement.

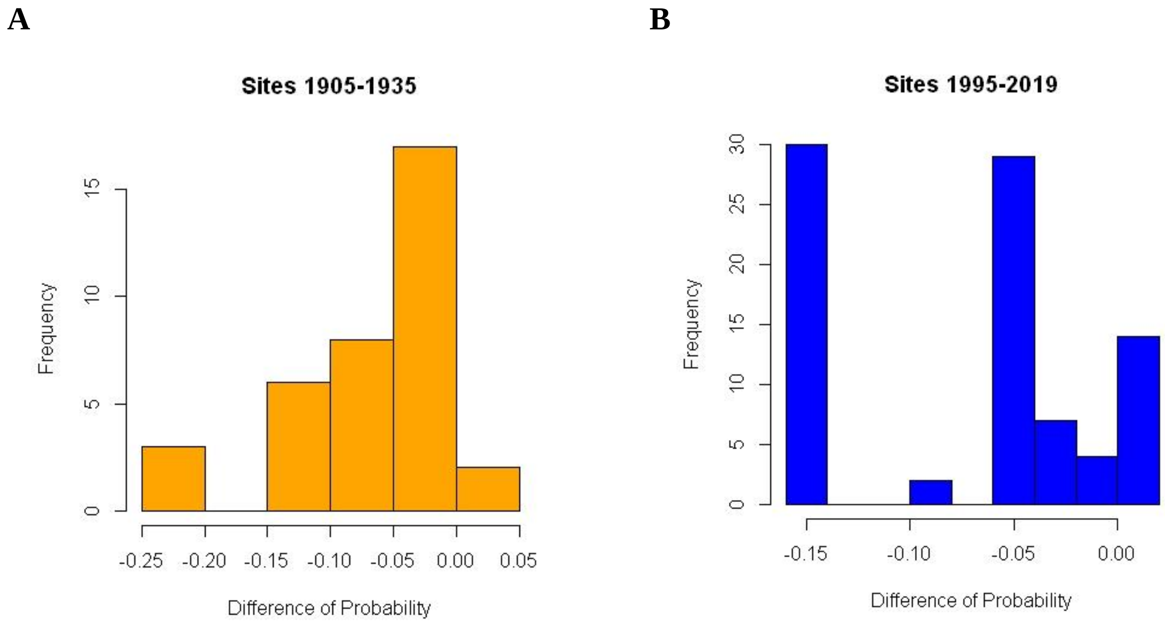

Figure 6: Difference of probability, A: Sites 1905-1935; B: Sites 1995-2019. The calculated values of the early site probability were subtracted from the late site probability on each set of sites. The differences show the amount of increase or decrease of site suitability for the habitation of the green toads. The majority fall below 0, indicating a decrease in suitability.

The t-test analysis showed a very small difference of means. The early sites are on average only 0.066 worse, while for the current sites have a decrease of 0.075. (Table 3).

T-TEST	EARLY SITES	LATE SITES
T	6.133	12.704
DF	35	85
P-VALUE	5.16e-07	<2.2e-16
95% CI	0.044 -- 0.088	0.037 -- 0.051
MEAN OF DIFFERENCE	0.066	0.044

Table 3: t-test results. These results are for the difference of probability of suitability for the green toads between the early sites and the late sites. Again, each site group was compared using current and historical climate data to see the differences over the century. T=t value, df=degrees of freedom, ci=confidence interval, and mean of differences shows the average difference between the sites using the varying climate data.

Finally, I conducted a survey of the early sites that continued to be populated today by searching the sites in 2020 on Artportalen[38]. Of those sites, nine were still viable (Figure 7).

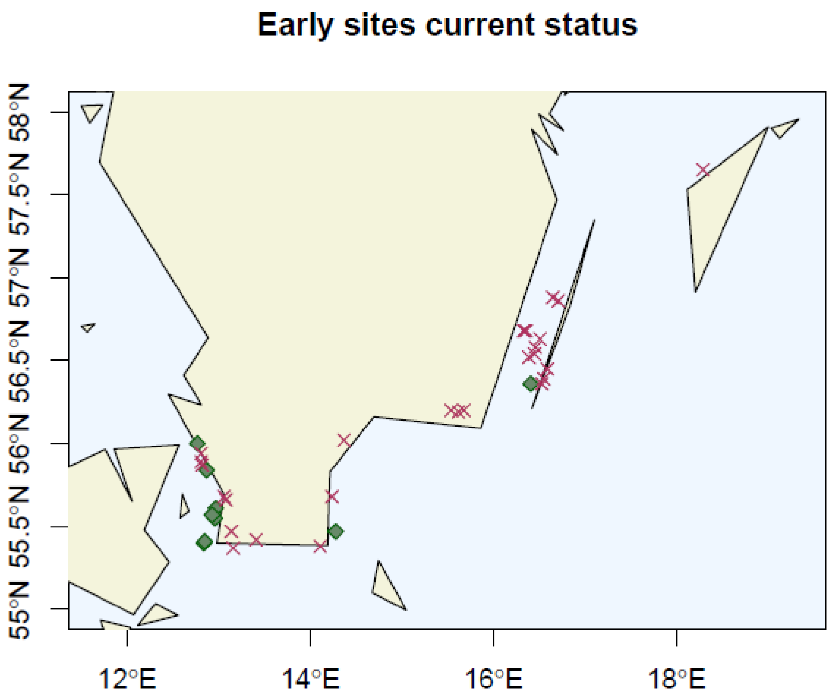

Figure 7: Early sites with their current status. These sites show the current populations of Bufotes viridis based on the sites documented in 1905-1935. The sites with toads are shown in green diamonds, and the sites where the toads no longer exist are shown with red X's.

Comparing the probability of presence at those sites to the sites where the toads have been extirpated, I found very little difference in the values. A t-test confirmed my observations (Table 4).

T-TEST	
T	0.600
DF	25.518
P-VALUE	0.554
95% CI	-0.029 0.054
MEAN OF DIFFERENCE	-0.061 -0.073

Table 4: t-test results of early site populations. This compared the probability of presence at early sites with current populations and those that are extirpated. T=t value, DF=degrees of freedom, CI=confidence interval

Some sites where the toads remained were less suitable where they had died out and vice versa (Figure 8). There is considerable overlap, with the values of the extirpated sites completely surrounding the populated ones.

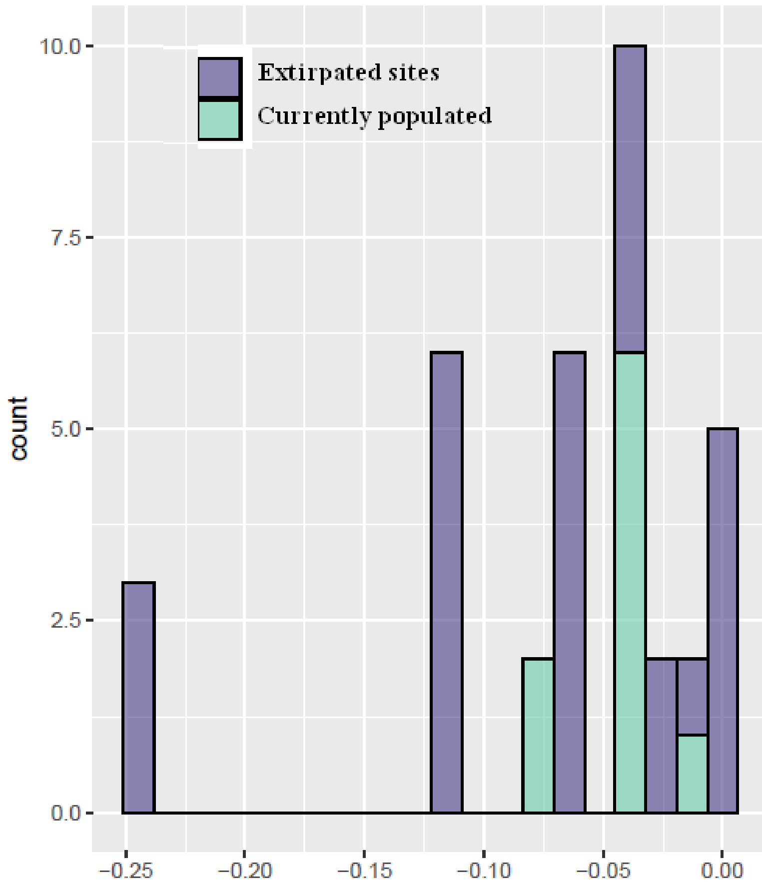

Figure 8: Differences of probability. These are the sites from 1905-1935 with the current status of inhabitation. The purple columns represent all of the sites that no longer have a population, while the turquoise columns are the sites (n=9) that are still populated.

Mention must be made that there is a viable population of *Bufotes viridis* on an island, Utklippan, about 25 kilometers southwest of the town of Karlskrona. Because the climate data were not available for this site, it was not included in the study. However, historical records place the toad on at the site as far back as the 1920's[26]. It is still present today, and even shows an increase[43].

Discussion

The late sites that had the most decline were on the southwest coast of Sweden, but interestingly, they are the majority of sites where the toads live even today. The least degradation was seen on the east coast. Surprisingly, some of the sites that showed improvement, especially Hallands Väderö, no longer maintain populations of green toads. Even sites that have the same degree of decrease have both kept and lost the toads. So there seems to be no real pattern to how the suitability of the sites from the climate data alone can explain why the toads are disappearing.

A hypothetical, but sadly plausible, explanation for the seemingly random extirpation is that we are just beginning to see the extinction. Most sites are worsening, but in the window of time in this study, the chaotic loss of sites seems arbitrary. Maybe this will end in total extinction due to climate change.

Bufotes viridis in Sweden is at the very edge of the species' global range. The hypothesis regarding peripheral populations migrating as a result of climate change creates a disconnect when considering the green toad. This population seems to be most affected by the vapor pressure, which may have more to do with moisture than heat. However, the hypothesis still indicates that we should see an increase, rather than a decrease in the populations in Sweden. We aren't. Therefore, we need to consider other causes outside of the decreasing climate conditions.

Disease and predation

The fungus *Batrachochytrium dendrobatidis* causes the disease chytridiomycosis, which has been the culprit in the decrease, and in some cases, decimation of amphibians throughout the world. Originating most likely in Africa, the organism spread widely through the trade of *Xenopus laevis*, which is popular in both biological research and the pet trade. In addition, the American bullfrog (*Lithobates catesbeianus*), sold worldwide as a food source, is also an unaffected carrier of the fungus[44].

Specifically, the fungus affects the skin, causing problems with hydration, breathing, and protection of the animals it infects. The fungus survives best at temperatures lower than 28° but are affected by desiccation. That is one reason chytrid is so prevalent in water-dependent species. Salinity seems to also affect the rate of infection, with higher levels of salt concentration negatively affecting the progression of the fungus[45].

In several studies, *Bufotes viridis* has been shown to have high levels of the fungus[46–48]. Rates of infection or fungal load do not translate to death rates because different species have different physiological responses to the parasite[48]. However, the death rate is often not as high as seen in other species. Some of the adult animals exhibit little to no outward effects of the infection. The reason for this is unknown, nor is it known to what extent the animals die as a direct result[49]. This could also be attributed to the fact that *B. viridis* is found most often close to brackish water, where the salinity is around 0.5-0.7%.

Another contributing factor to the decrease in the toads could be due to increased predation. One of the most ubiquitous predators is signal crayfish (*Pacifastacus leniusculus*)[50]. This species was introduced in the 1960's to help bolster the population of the native noble crayfish (*Astacus astacus*), which fell victim to the *Aphanomyces astaci* pathogen[51]. A form of brown algae, *A. astaci* is benign in its native North America, but is highly lethal in European crayfish species[52]. Unbeknownst to the well-meaning people (biologists?) who imported the signal crayfish, they also carried the parasite. Thus, *A. astaci* spread, eventually eliminating nearly 97% of the native noble crayfish[45]. The problem came with the proliferation of *P. leniusculus*. As omnivorous species, signal crayfish eat or kill tadpoles and eggs of amphibians[53]. They are present throughout Sweden, including all sites where *B. viridis* is found[50,51,54]. Thus, the potential, if yet inclusive, assumption is that the crustacean is a direct threat to the toad.

Bird predation is not seen as a large problem. While the toads are being re-released in bird sanctuaries[32], return of species there do not appear to be a problem. The majority of footprints found around pools with released tadpoles were determined to be geese and/or swans (*Anserinae*), which are not known to be carnivorous [personal observation]. In addition, throughout Europe, where the green toads are more common, the same species of birds also thrive personal communication, Donald Blomqvist, October 5, 2020.

Mammal predation is definitely an area that needs further investigation. Natural predators that have lived for centuries with the toads have had to change their own behavior in order to survive because of the factors affecting their environment. Foxes (*Vulpes vulpes*) and assorted species from *Mustelidae*, including martens, otters[55], and badgers, are known to eat toads, but the degree to which they are a larger threat than in the past is unknown. American mink (*Neovison vison*), brought to Sweden in the 1920's for the fur industry, have become a serious invasive threat through escape and deliberate release. They are now spread throughout the country and are known to feed on many native species[56].

Direct human interference
Humans affect the environment, even without any sort of malice. Every aspect of our lives changes, in some small or large way, the lives of all living organisms on Earth. Habitat destruction[57] is the main threat by humans to toads in Sweden. The decimation of living and breeding habitats is the result of infrastructure, agriculture, and eutrophication.

Despite the spread of the human population within the country, areas where the green toad were most prevalent are also areas where humans most want to live. Waterfront living and summer houses are in great demand. The necessary infrastructure to meet these demands, such as roads, sewage, and water, destroy biodiversity through habitat fragmentation. This results in a lower carrying capacity for the populations, as well as higher competition for resources and mates. The long-term effects can be seen in lack of genetic diversity due to smaller subpopulations[58].

Since the early 1930's, Sweden has experienced a significant upturn in industrial agriculture. Aside from the habitat destruction as a result of land clearing for planting, the problems of industrial fertilizers and concurrent eutrophication have had a powerful effect on amphibians in the country. Commercial fertilizers, full of nitrogen, potassium, and phosphorus, have affected the waters on both the West Coast and the Baltic Sea. Plankton overgrowth is a result of the fertilizer draining into the waters. When they die, plankton sink to the sea floor, where they decompose, consuming oxygen. The lack of oxygen leads to the death of aquatic species.

Storage and use of the fertilizers are also fraught with hazards. Nitrous oxide is released into the atmosphere where it becomes a potent greenhouse gas. Storage creates ammonia fluids which can lead to toxification, acidification, and eutrophication. Precision cultivation, increasingly popular in Sweden, can reduce the damage created by nitrogen fertilizers[59].

Further problems with eutrophication are the changes to the organism itself. Alterations of the water chemistry from industrial runoff can not only affect the eggs as they develop, but also the DNA of the parents. Deformations of the developing frogs lead to more fatalities from inability to sustain life or avoid predation[60].

The interaction of the forms of disturbance is important to consider. Changes due to pollutants in the water come from not only farming, but also industrial air pollution and ozone depletion. Often the eggs or tadpoles die from the lack of oxygen, chemical toxicity, reduced sunlight, or even excess ultraviolet-B radiation[61]. These factors advance climate change, which causes more problems[62].

Aquaculture has also had a popular stint throughout the country. Some of the farming of aquatic species is for consumption, while a portion is for release and further farm stocking[63]. The problem with aquaculture was the production of the signal crayfish, discussed earlier. Luckily, the number of aquaculture farms have decreased over the last decade[64].

So, is climate change an issue? Simple answer, yes. However, nothing is so simple. The fact is all areas have shown declination. There are only thirteen areas left in Sweden with *Bufotes viridis,* as of 2020. The hypothesis that peripheral populations will see increases due to warming temperatures has failed to show any backing. As a matter of fact, toads have disappeared from some areas more than others. There is no pattern to the disappearances. There are always other factors to consider.

Conclusion

Climate change appears not to have played as significant a role as previously thought, and the loss of toads in sites seem to have no relationship to the overall climate conditions. So, there is no single cause, thus no single solution to species loss. The complexity of the conservation issue makes it more difficult to come to conclusions about how best to address it. All need to be examined. All need to be reckoned with if Sweden is to have any hope of saving *Bufotes viridis*.

Acknowledgments

I dedicate this work to the memory of my parents, Alan and Betsy Ballard, who taught me the value of learning. I would like to thank my supervisors: Søren Faurby, for keeping me scientifically grounded and teaching me way more about R than I cared to know; Mats Niklasson, for steering me towards the toads in the first place; and Ewa Wikberg, for helping and supporting me along the way. In addition, I would like to thank Carina Johansson for her amazing editorial skills, Tala Mohimani for being there, and last, my wonderful husband, Magnus Johansson, for asking every day for 2 years if I was done yet. Now I can say, “Yes. I am!”

References

1. Stocker, T. F. *et al.* Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (2013).
2. Hunter Jnr, M. L. & Hutchinson, A. The virtues and shortcomings of parochialism: Conserving species that are locally rare, but globally common. *Conservation Biology* **8**, 1163–1165 (1994).
3. Channell, R. & Lomolino, M. v. Dynamic biogeography and conservation of endangered species. *Nature* **403**, 84–86 (2000).
4. Allendorf, F., Luikart, G. & Aitken, S. *Conservation and the Genetics of Populations, 2nd. Edition*. (Wiley-Blackwell, 2013).
5. Gibson, S. Y., van der Marel, R. C. & Starzomski, B. M. Climate change and conservation of leading-edge peripheral populations. *Conservation Biology* **23**, 1369–1373 (2009).
6. Parmesan, C. Climate and species' range. *Nature* **382**, 765–766 (1996).
7. Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42 (2003).
8. Stuart, S. N. *et al.* Status and trends of amphibian declines and extinctions worldwide. *Science* **306**, 1783–1785 (2004).
9. Blaustein, A. R. & Wake, D. B. Declining amphibian populations: A global phenomenon? *Trends in Ecology and Evolution* **5**, 203–204 (1990).
10. Araújo, M. B., Thuiller, W. & Pearson, R. G. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* **33**, 1712–1728 (2006).
11. Semlitsch, R. D. Differentiating Migration and Dispersal Processes for Pond-Breeding Amphibians. *Journal of Wildlife Management* **72**, 260–267 (2008).
12. Gasc, J.-P., Cabela, A., Crnobrnja-Isailovic, J. & Dolmen, D. *Atlas of Amphibians and Reptiles in Europe*. (Muséum national d'Histoire naturelle, 1997).
13. Laurenti, J. N. *Specimen Medicum, exhibens synopsin reptilium*. (1768).
14. Pallas, P. S. *Spicilegia zoologica : Tomus I*. (Gottlieb, August Lange, 1774).
15. Frost, D. Bufotes @ amphibiansoftheworld.amnh.org. https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Bufonidae/Bufotes (2019).
16. IUCN. IUCN Red List of Threatened Species. Version 2012.1. *IUCN 2012* vol. 2010 www.iucnredlist.org (2012).
17. Stöck, M. Progress towards a taxonomic revision of the Asian Bufo viridis group: Current status of nominal taxa and unsolved problems (Amphibia: Anura: Bufonidae). *Zoologische Abhandlungen* **51**, 253–319 (2001).
18. Stöck, M. *et al.* Evolution of mitochondrial relationships and biogeography of Palearctic green toads (Bufo viridis subgroup) with insights in their genomic plasticity. *Molecular Phylogenetics and Evolution* **41**, 663–689 (2006).
19. Dufresnes, C. *et al.* Fifteen shades of green: The evolution of Bufotes toads revisited. *Molecular Phylogenetics and Evolution* **141**, (2019).
20. Ratnikov, V. Y. On the finds of green toads (Bufo viridis complex) in the late cenozoic of the East-European Platform. *Paleontological Journal* **30**, 225–231 (1996).
21. Wirén, M. *Åtgärdsprogram För Bevarande Av Grönfläckig Padda Åtgärdsprogram För Bevarande Av Grönfläckig Padda*. vol. 6406 (2010).
22. Sparrman, A. En grönfläckad Groda, funnen i Carlscrona och beskrifven. *Kongl. Vetenskaps Academiens Nya Handlingar* **1795**, 183–185, 1 plate (1795).

23. Heintze, A. Studier öfver groddjurens utbredning i östra Småland och på Öland. *Fauna och Flora* **4**, 236–249 (1909).
24. Lundblad, O. Om Bufo viridis. *Fauna och Flora* **66**, 37–39 (1913).
25. Erikson, J. Ett par ord om den grönfläckiga paddan i Blekinge och på Öland m.m. *Fauna och Flora* **66**, 37–39 (1913).
26. Strömberg, G. Krönika över groddjuren på Utklippan. *Blekinges Natur* 114–120 (1988).
27. Gislén, T. Svenska amfibiers och reptilers utbredning. *Fauna och Flora* **41**, 127–133 (1946).
28. Vallin, H. Grönfläckiga paddan funnen på Hallands Väderö. *Fauna och Flora* **50**, 1955 (1955).
29. Hanström, B. Marint massuppträdande av Bufo viridis i Öresund. *Fauna och Flora* **22**, 249–257 (1927).
30. SLU ArtDatabanken. *Rödlistade arter i Sverige 2020*. (2020).
31. Nordens Ark. European green toad. https://en.nordensark.se/conservation/green-toad/ (2020).
32. Försäter, K. Reintroducing Green Toads to historical sites; a collaborative effort between Nordens Ark and the County Administrative Board of Kalmar, Sweden. *AARk Newsletter* **48**, 5–6 (2019).
33. Rosquist, G. *Coordinated Actions Against Chytridiomycosis in the Nordic Countries*. (2020).
34. Trochet, A. *et al.* A database of life-history traits of European amphibians. *Biodiversity Data Journal* **2**, (2014).
35. Vlcek, P., Kudlácek, M. & Jablonski, D. First record of the egg cannibalism in tadpoles of Bufotes viridis complex (Anura: Bufonidae) from Croatia. *Biharean Biologist* **7**, 106–107 (2013).
36. Naturcentrum. *Fauna och flora*. https://www.artdatabanken.se/publikationer/tidskriften-fauna-och-flora/.
37. Gislen, T. & Kauri, H. *Zoogeography of the Swedish Amphibians and Reptiles with Notes on Their Growth and Ecology*. (JSTOR, 1959). doi:10.2307/1439910.
38. SLU. Artportalen. https://www.artportalen.se/ (2020).
39. Hasager, C. B., Badger, M., Peña, A., Larsén, X. G. & Bingöl, F. SAR-Based Wind Resource Statistics in the Baltic Sea. *Remote Sensing* **3**, 117–144 (2011).
40. Harris, I., Osborn, T. J., Jones, P. & Lister, D. Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Scientific Data* **7**, 1–18 (2020).
41. Climatic Research Unit (University of East Anglia) and Met Office. https://sites.uea.ac.uk/cru/cruhome.
42. R Core Team. R: A Language and Environment for Statistical Computing. (2020).
43. Strömberg, G. *Grönfläckig padda--åtgärdsprogram för Blekinge*. (2004).
44. Weldon, C., du Preez, L. H., Hyatt, A. D., Muller, R. & Speare, R. Origin of the Amphibian Chytrid Fungus. *Emerging Infectious Diseases* **10**, 2100–2105 (2004).
45. Stockwell, M. P., Clulow, J. & Mahony, M. J. Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia* **177**, 901–910 (2015).
46. Kärvemo, S., Meurling, S., Berger, D., Höglund, J. & Laurila, A. Effects of host species and environmental factors on the prevalence of Batrachochytrium dendrobatidis in northern Europe. *PLoS ONE* **13**, 1–16 (2018).
47. Meurling, S. The response in native wildlife to an invading pathogen: Swedish amphibians and Batrachochytrium dendrobatidis. (2019).

48. Searle, C. L. *et al.* Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. *Conservation Biology* **25**, 965–974 (2011).
49. Baláž, V., Kubečková, M., Civiš, P., Rozínek, R. & Vojar, J. Fatal chytridiomycosis and infection loss observed in captive toads infected in the wild. *Acta Veterinaria Brno* **82**, 351–355 (2014).
50. Kouba, A., Petrusek, A. & Kozák, P. Continental-wide distribution of crayfish species in Europe: Update and maps. *Knowledge and Management of Aquatic Ecosystems* (2014).
51. Diemer, M. Signalkräfta. *Havs och vatten Myndigheten* https://www.havochvatten.se/arter-och-livsmiljoer/arter-och-naturtyper/signalkrafta.html (2015).
52. Oidtmann, B. Aphanomyces astaci. https://www.cabi.org/isc/datasheet/93177 (2017).
53. Axelsson, E., Nyström, P., Sidenmark, J. & Brönmark, C. Crayfish predation on amphibian eggs and larvae. *Amphibia Reptilia* **18**, 217–228 (1997).
54. Edsman, L. *Flodkräftan*. (2018).
55. Smiroldo, G. *et al.* Amphibians in Eurasian otter Lutra lutra diet: osteological identification unveils hidden prey richness and male-biased predation on anurans. *Mammal Review* **49**, 240–255 (2019).
56. Strand, M., Aronsson, M. & Svensson, M. *Klassificering av främmande arters effekter på biologisk mångfald i Sverige – ArtDatabankens risklista*. (2018).
57. Pino del Carpio, A., Sánchez, S., Nieto, A. & Bilz, M. *Sweden's biodiversity at risk*. (2013).
58. Beebee, T. J. C. & Griffiths, R. A. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* vol. 125 271–285 (2005).
59. Khan, M. N. & Mohammad, F. Eutrophication: Challenges and Solutions. in *Eutrophication: Causes, Consequences and Control.* (eds. Ansari, A. & Gill, S.) 1–15 (Springer, 2014).
60. Egea-Serrano, A., Relyea, R. A., Tejedo, M. & Torralva, M. Understanding of the impact of chemicals on amphibians: a meta-analytic review. *Ecology and Evolution* **2**, 1382–1397 (2012).
61. Blaustein, A. R. & Wake, D. B. The Puzzle of Declining Amphibian Populations. *Scientific American* **272**, 52–57 (1995).
62. Blaustein, A. R. & Kiesecker, J. M. Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters* **5**, 597–608 (2002).
63. Jordbruksverket. *Swedish aquaculture – a green industry in blue fields*. (2012).
64. Jordbruksverket. *Aquaculture in Sweden in 2018*. (2019).

Green Toad Gone:
The past and future of the European green toad in Sweden
Mary Ballard-Johansson

It was a dark and stormy night…. In 1927, green toads swarmed into a harbor near Landskrona, in Southeastern Sweden. Thousands of toads flocked around the town, filling the streets and even entering houses. It appeared that the toadlets had recently undergone metamorphosis and had been driven inland by stormy weather. In the beginning of the 20th century, the European green toad was ubiquitous throughout southern Sweden. Now, however, the toads are very rare to completely gone in the same areas. Why is the European green toad (*Bufotes viridis*) threatened in Sweden while it continues to thrive in other countries? Is it the habitat? The climate? Predators? This study addresses the questions of the status and fate of the toad in Northern Europe.

Climate change, the overall shifts in temperature and weather events, is affecting species across the globe. As the environments are altered due to temperature extremes, precipitation instability, and food source fluctuation, all species globally are affected. Species' ranges often cover large swathes of habitats that are similar, while local populations adapt in some ways to fit their specific environments. The groups occupying the extremes of the ranges are referred to as peripheral. Global temperatures are rising, and there is range expansion towards the poles concurrent with range reduction at the trailing edges.

European green toads in Sweden are at the edge of the green toads' global range. They have reduced not only in population size, but also in the extent of their domain. Many factors have been suggested for this decline, such as human interference by means of habitat destruction and agricultural land overuse, among others. In addition, more direct causes including increased predation, fungal infection, and genetic incongruencies have also been considered. However, these factors can not properly be assessed until the effects of climate change have been examined. Therefore, the necessity of considering the effects of the crisis on these toads, already at the northern reaches of its range, is tantamount to finally solving this puzzle.

This study addresses the issue of climatic changes throughout the past 115 years to determine if these factors negatively impact *B. viridis* and if the data correlate to the decline of the species. The hypothesis is that changes in climate, such as the decrease of freezing conditions, has negatively affected the population of *Bufotes viridis* in Sweden. Sites were identified through presence of the toad, and then a model was built to determine the probability of toads remaining at the sites based on those climatic factors. The predictors were used to determine if the climate was a driver in the loss of toads from once suitable habitats and if these changes will cause further site loss. As it turned out, sites that had the most decline were the majority of sites where the toads live even today. Surprisingly, some of the sites that showed improvement no longer maintain populations of green toads. Even sites that have the same degree of decrease have both kept and lost the toads. So there seems to be no real pattern to how the suitability of the sites from the climate data alone can explain why *Bufotes viridis* are disappearing. The loss of toads from sites did not follow any specific climatic trends. Therefore, we need to consider other causes outside of the decreasing climate conditions.

Name	year	Latitude	Longitude	dtr	pre	wet	vap	GFPP	PredresL	xGFPP	Probability	xPredresL	Probability	Eresults
Trelleborg	1929	13.156349	55.372665	7.2	52.0	13.6	9.6	-1.1	-2.46	0.333	0.582	0.085	0.5213	-0.0611
Löderup	1929	14.114477	55.384374	7.2	52.2	13.8	9.5	-1.436	-2.479	0.238	0.559	0.084	0.5209	-0.0382
Falsterbo	1910	12.836611	55.404361	7.1	52.2	13.6	9.7	-0.826	-2.272	0.438	0.608	0.103	0.5258	-0.082
Skanör	1911	12.846430	55.410526	7.1	52.2	13.6	9.7	-0.826	-2.272	0.438	0.608	0.103	0.5258	-0.082
Jordberga	1908	13.414090	55.416236	7.2	52.0	13.6	9.6	-1.1	-2.46	0.333	0.582	0.085	0.5213	-0.0611
Östra_Grevie	1908	13.139429	55.466180	7.2	52.0	13.6	9.6	-1.1	-2.46	0.333	0.582	0.085	0.5213	-0.0611
Skillinge	1913	14.279547	55.470381	7.2	52.2	13.8	9.5	-1.436	-2.479	0.238	0.559	0.084	0.5209	-0.0382
Vintrie	1911	12.962112	55.552917	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Limhamn	1911:1926:1929	12.930078	55.566785	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Malmö	1911:1926	12.974634	55.607105	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Alnarp	1911	13.082899	55.656077	6.6	52.7	14.2	9.3	-1.739	-1.763	0.176	0.544	0.172	0.5428	-0.001
Lomma	1911:1926:1929	13.058791	55.675997	6.6	52.7	14.2	9.3	-1.739	-1.763	0.176	0.544	0.172	0.5428	-0.001
Kivik_Äsperöd	1908	14.240426	55.678391	6.6	48.2	14.2	9.1	-1.547	-1.572	0.213	0.553	0.208	0.5517	-0.0013
Lundåkrabukten	1913	12.871346	55.842717	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Landskrona	1927	12.823852	55.867034	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Borstahusen	1927	12.806288	55.892894	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Glumslöv	1929	12.8104863	55.9416984	6.3	50.3	13.9	9.4	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Raus	1929	12.7653426	56.0004256	7.2	57.8	14.3	9.2	-0.856	-1.315	0.425	0.605	0.268	0.5667	-0.0379
Trolle_Ljungby	1929	14.372935	56.015638	7.2	51.7	14.3	8.9	-2.954	-2.667	0.052	0.513	0.069	0.5174	0.0043
Karlskrona_Sunna	1913	15.617913	56.192109	7.1	46.0	13.2	9.1	-1.082	-2.703	0.339	0.584	0.067	0.5167	-0.0672
Lösen	1914	15.684196	56.200549	7.1	46.0	13.2	9.1	-1.082	-2.703	0.339	0.584	0.067	0.5167	-0.0672
Nättraby	1913	15.536869	56.200697	7.1	46.0	13.2	9.1	-1.082	-2.703	0.339	0.584	0.067	0.5167	-0.0672
Södra_Möckleby	1913	16.408804	56.355241	7.0	51.2	13.9	9.1	-1.972	-2.485	0.139	0.535	0.083	0.5208	-0.0139
Segerstad	1909	16.533217	56.362985	7.0	51.1	13.9	9.0	-2.029	-2.528	0.131	0.533	0.08	0.5199	-0.0129
Mellby_Ör	1909	16.547077	56.391966	7.0	51.1	13.9	9.0	-2.029	-2.528	0.131	0.533	0.08	0.5199	-0.0129
Hulterstad	1909	16.588107	56.448969	7.0	51.1	13.9	9.0	-2.029	-2.528	0.131	0.533	0.08	0.5199	-0.0129
Mörbylånga	1909	16.386555	56.522332	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Resmo	1909	16.449514	56.540570	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Vickleby	1915	16.459557	56.576972	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Torslunda	1909	16.507529	56.633454	6.7	39.5	12.8	9.1	0.217	-1.941	1.242	0.776	0.144	0.5358	-0.2401
Kalmar	1909	16.362070	56.676946	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Törneby	1909	16.328320	56.678770	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Skälby	1909	16.336381	56.680764	7.6	41.0	12.8	9.0	-0.685	-3.072	0.504	0.623	0.046	0.5116	-0.1118
Köpingsvik_Ramsätra	1909	16.710347	56.855480	6.7	39.5	12.8	9.1	0.217	-1.941	1.242	0.776	0.144	0.5358	-0.2401
Borgholm	1910:1913	16.652589	56.884748	6.7	39.5	12.8	9.1	0.217	-1.941	1.242	0.776	0.144	0.5358	-0.2401
Visby	1918	18.294973	57.646429	6.8	51.0	13.9	8.5	-2.913	-2.836	0.054	0.514	0.059	0.5147	0.0011

Supplemental Table 1: Sites from 1905-1935. Including all variables. Dtr=Diurnal temperature; Pre=Precipitation; Wet=Number of days with more than 0.1 mm precipitation; Vap=Vapor pressure. All numbers are the mean average for that latitude and longitude between the years 1995 and 2019. The results of the statistical analyses are indicated by the labels: EarlyP=the predictions of the presence at that site using estimates from1905-1935; PredresL= the predictions of the presence at that site using estimates from1995-2019; xEarlyP and xPredresL are the exponential functions of each variable. Probabilities are the results of the formula exp(x)/1+exp(x). Eresults is the remainder of EarlyP-PredresL.

Name	Year	Longitude	Latitude	Dtr	Pre	Wet	Vap	NowP	PredresE	xNowP	Probability	xPredresE	Probability	Lresults
Smygehamn	2007	13.379143	55.345682	7.39	54.78	13.7	10.0	-2.324	-0.748	0.098	0.524	0.473	0.616	-0.092
Falsterbo_Nabben	1997:1998:2019	12.810925	55.378108	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Flommen/Falsterbo_Fyr	1998:2007:2008:2010:2013:2015:2016:2018	12.817407	55.384607	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Falsterbo	1999:2000:2008:2009:2016:2017	12.820279	55.388262	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Höllviken	2017	12.935526	55.393458	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Flommen	2008:2009:2011:2014:2015	12.831462	55.404346	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Skanörs_ljung_NR	1999	12.891304	55.404946	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Kungsvik	2014	12.981687	55.438043	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Kungstorps_ängar	2010	12.972618	55.441515	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Marklunda	2015	12.984090	55.441673	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Vellinge_södra	2009:2010	12.971460	55.447577	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Hammars_näs	2010	12.94732	55.449659	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Vellingeblomman	2014	12.992570	55.458043	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Vellinge_västra	2009:2010:2011:2018	12.971396	55.459700	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Vellinge_västra_trädgård	2008	12.992089	55.461373	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Kronan	2012:2013:2015	12.989255	55.467824	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Eskilstorps_ängar	1996:2000	12.956884	55.475614	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Vellinge_norra	2009:2010:2011:2018	12.958423	55.476107	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Eskiltorps_ängar	2001-2019	12.956508	55.478122	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Gessie_ängar	2009-2012:2018	12.942447	55.497423	7.33	54.22	13.6	10.2	-2.173	-0.303	0.114	0.528	0.739	0.677	-0.149
Tygelsjö_ängar	2008:2010-2012:2018	12.933949	55.510795	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Brantevik	2011-2019	14.348211	55.511339	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Tygelsjö_bollplatsen	2010	12.924647	55.519075	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Klagshamn	2001:2008:2010:2011:2019	12.914569	55.521948	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Högaborg	2007	14.007663	55.534085	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Ullstorp	2008	13.975823	55.538140	6.96	57.5	14.8	9.6	-1.824	-2.303	0.161	0.54	0.1	0.525	0.015
Högaborg_NR	2001:2003:2005-2009:2012	14.009039	55.540028	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Bunkeflostrand	2017:2018	12.921874	55.540896	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Lunnarp	2008:2009	14.070315	55.548900	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Bunkeflostrandängar	2010:2015-2018	12.906654	55.555784	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Stenören_Norra_Bunkeflostrand	2007:2016	12.900851	55.561270	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Lernacken	2007-2011:2013-2017:2019	12.891506	55.565045	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Linhamns_kalkbrott	2000:2001:2003-2019	12.932828	55.567592	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Bäckhalladalen	2001	14.307448	55.577529	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Prästens_badkar	2000	14.297510	55.612611	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Norra_hamnen	2012:2013:2014:2016	13.011685	55.632909	6.84	57.38	14.6	9.8	-1.65	-1.767	0.192	0.548	0.171	0.543	0.005
Ravlundafället_Plattan	2001:2006	14.173916	55.748066	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Jordbroskogen	2010	12.977934	55.751602	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Lyckehusen	2011	12.974304	55.751659	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Bodeborns_korra	2001:2004:2008	14.160278	55.755618	6.83	52.56	14.5	9.6	-1.413	-1.509	0.243	0.561	0.221	0.555	0.006
Landskrona_soptipp	1997:2014-2018	12.861283	55.859083	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Lundåkrahamnen	2017:2018:2019	12.848793	55.859484	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Gråen	2001	12.821912	55.863771	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Landskrona_citadellet	2011	12.822467	55.873012	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Borstahusen_golfbanan	2011	12.809000	55.903676	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Tuna	2007	12.707009	55.904933	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Ven	2009	12.684448	55.911694	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Hildesborg	2001:2003:2007-2014	12.804865	55.914777	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Sundvik	2013:2014:2015:2017	12.789824	55.933169	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Örby_ängar	2013:2014:2015:2016:2017:2018	12.753889	55.984103	6.56	53.64	14.2	9.9	-1.187	-0.618	0.305	0.576	0.539	0.632	-0.056
Flakskär	2011:2012:2013	15.729484	56.061561	7.47	49.84	13.4	9.6	-2.738	-0.922	0.065	0.516	0.398	0.598	-0.082
Ignaberga	2007	13.854598	56.111764	7.71	62.49	15.1	9.4	-3.009	-3.957	0.049	0.512	0.019	0.505	0.007
Ottenby_Södra_udde	2009-2019	16.399161	56.195932	7.33	55.37	14.0	9.6	-2.606	-1.77	0.074	0.518	0.17	0.542	-0.024
Kyrkhamn	2017	16.403465	56.205497	7.33	55.37	14.0	9.6	-2.606	-1.77	0.074	0.518	0.17	0.542	-0.024
Ottenby	2013	16.419513	56.210081	7.33	55.37	14.0	9.6	-2.606	-1.77	0.074	0.518	0.17	0.542	-0.024
Andfänget	2017	16.442016	56.21169	7.33	55.37	14.0	9.6	-2.606	-1.77	0.074	0.518	0.17	0.542	-0.024
Torekov	2007	12.624630	56.401226	7.43	63.29	14.6	9.7	-2.859	-2.967	0.057	0.514	0.051	0.513	0.001
Hallands_Väderö	2007	12.581184	56.436227	7.43	63.29	14.6	9.7	-2.859	-2.967	0.057	0.514	0.051	0.513	0.001
Kättlevik_Husrygg	2008	18.142536	56.936013	7.32	52.71	13.6	9.4	-2.832	-1.581	0.059	0.515	0.206	0.551	-0.036
Högby_hamn	2012:2013:2014:2015:2017	17.034303	57.168936	7.34	54.34	14.1	9.3	-2.784	-2.267	0.062	0.515	0.104	0.526	-0.011
Räveslätt_Horns	2004:2005	16.909645	57.199118	7.37	54.93	14.1	9.3	-2.828	-2.358	0.059	0.515	0.095	0.524	-0.009
Horns_Kungsgård2	2017	16.912665	57.202300	7.37	54.93	14.1	9.3	-2.828	-2.358	0.059	0.515	0.095	0.524	-0.009
Mensalvaret	2014	16.959175	57.225062	7.37	54.93	14.1	9.3	-2.828	-2.358	0.059	0.515	0.095	0.524	-0.009
Djaupdy_Stånga	2010	18.708141	57.249886	7.29	52.99	13.5	9.2	-3.088	-1.895	0.046	0.511	0.15	0.537	-0.026

Supplemental Table 2: Sites from 1995-2019. Including all variables. Dtr=Diurnal temperature; Pre=Precipitation; Wet=Number of days with more than 0.1 mm precipitation; Vap=Vapor pressure. All numbers are the mean average for that latitude and longitude between the years 1995 and 2019. The results of the statistical analyses are indicated by the labels: NowP=the predictions of the presence at that site using estimates from 1995-2019; PredresE= the predictions of the presence at that site using estimates from 1905-1935; xNowP and xPredresE are the exponential functions of each variable. Probabilities are the results of the formula exp(x)/1+exp(x). Lresults is the remainder of NowP-PredresE.