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„Status and dynamics of a European Green Toad (*Bufotes viridis*) population in a primary habitat at the National Park Neusiedler See-Seewinkel (Burgenland, Austria) “

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Zusammenfassung

Die Salzlacken im Nationalpark Neusiedler See - Seewinkel gehören trotz anthropogen bedingter Veränderungen zu den letzten Primärhabitaten von *Bufotes viridis* in Österreich. Mithilfe eines Fang- Wiederfang – Modells und der Berechnung der individuellen Körperkonditionen wurde der Status einer *B. viridis* - Population im Gebiet um Illmitz im Jahr 2021 untersucht. Die Populationsgröße wird auf 371 Individuen geschätzt. Individuen, die im Kirchsee oder auf den Straßen rund um Illmitz gefangen wurden, hatten signifikant höhere Körperkonditions- Werte als die Individuen, welche in der Illmitzer Zicklacke gefangen wurden. Der Reproduktionserfolg war niedrig, zurückzuführen auf die Austrocknung des Kirchsees im Frühling und der Illmitzer Zicklacke Ende Juni.

Abstract

The soda pans in the National Park Neusiedler See - Seewinkel in the Pannonian plain are one of the last primary habitats of *Bufotes viridis* in Austria, although the area became more and more anthropogenically influenced in past decades. We investigated the status of a *B. viridis* population around Illmitz in 2021 by analysing population size via an open capture recapture model and calculating body condition of individuals. Total population size was estimated with 371 individuals, there were significantly higher Scaled Mass Index values of individuals from the Kirchsee and from the streets of Illmitz than that of the Illmitzer Zicklacke. The reproduction success was low, considering that the Kirchsee desiccated in spring and Illmitzer Zicklacke at the end of June.

Key words: *Bufotes viridis*, open capture - recapture model, Scaled Mass Index, population analysis, soda pans, National Park Neusiedler See - Seewinkel, amphibian conservation

Introduction

About 40% of all amphibian species worldwide are threatened or nearly extinct (IUCN Red List 2021). Main threats are habitat loss due to land sealing and building activities, habitat fragmentation and isolation of subpopulations and environmental pollution due to intensive farming and industry. Such influence factors lead to decreasing population trends, hence *Bufotes viridis,* the European Green Toad, is listed in the Annex IV of the Habitats Directive. It stipulates that all European states are responsible to take specific protective measures (Council Directive 92/43/EEC 1992). In Austria, the European Green Toad is considered as vulnerable (Gollmann 2007), as is the population in the province Burgenland (Tiedemann and Häupl 1994). Following habitat degradation, *B. viridis* became a synanthropic species that colonizes anthropogenically influenced landscapes successfully, including urban habitats such as parks in cities (Stöck et al. 2008; Wappl and Heyer 2016; Sistani et al. 2021; Kühnel and Krone 2003). Urban habitats, however, pose threats such as roads, as well as shafts and swimming pools, from which *B. viridis* cannot escape.

Primary habitats of *B. viridis* are temporary sun exposed waters with low plant cover in steppes and meadow landscapes. The National Park Neusiedler See - Seewinkel is a biodiversity hotspot for amphibians (Zulka et al. 2022a) with characteristic soda pans as one of the last remaining primary habitats for *B. viridis* in Austria. They are characterized by regular desiccation processes, high temperatures, turbid waters and high sodium concentrations. As a pioneer species, *B. viridis* can colonize such ephemeral habitats. The toads' ionic regulatory mechanisms allow it to store NaCl and urea in its plasma and therefore enables the toad to adapt to higher sodium concentrations (Gordon 1962; Hoffman and Katz 1997). Higher water temperatures cause significantly faster larval development of *B. viridis*, which increases their survival rate in spawning waters threatened by desiccation (Dastansara et al. 2017). Additionally, *B. viridis* tadpoles can deal with waters of high turbidity (Csarmann 2008).

Specific physiological adaptations to local environmental changes can lead to different life history traits across intraspecific populations, which show up by variations in size, condition, age at maturity, longevity, and fecundity (Cayuela et al. 2019; Stearns and Koella 1986). For example, faster larval development due to desiccation processes often leads to smaller body size after metamorphosis, decreasing the lifetime fitness of individuals (Sibly and Atkinson 1994; Beck and Congdon 2000). Furthermore, females can adapt their reproduction investment to their body condition (Başkale et al. 2011; Castellano et al. 2004). Although Snout-vent-length (SVL) is mostly correlated with age (Halliday and Verrell 1988; Kutrup et al. 2011), varying habitat conditions lead to variations in body size within age classes due to different resource availability at life stages. Most adult *B. viridis* individuals are in between a SVL range of 50-80 mm, based on previous investigations (Dely 1967 in Stöck et al. 2008). However, there are exceptions reaching 90 mm (Werner 1897). In Germany, the smallest sexually mature males were around 45 mm and females 55 mm (Günther and Podloucky 1996). Compared to other condition indices, the robust Scaled Mass Index (SMI) turned out to be a good method to reflect body condition of amphibians, based on body mass and body length (SVL). Although it does not reflect body composition, higher SMI values correlate with energy stores of individuals (Peig and Green 2009; MacCracken and Stebbings 2012).

There is limited information about the status and population dynamics of the European Green Toad in the National Park Neusiedler See - Seewinkel. During the 20th century, *B. viridis* occurrence in the whole area was extremely common. In the 1950s, the toads were collected because of their role in conducting pregnancy tests by medical laboratories (Eibl-Eibesfeldt 1947; Sauerzopf 1959; Sochurek 1954; Koenig 1961). In 2006 the occurrence of *B. viridis* could be verified at 23 soda pans, with the Kirchsee as one of the most important spawning waters (Csarmann 2008).

Most of the soda pans in the National Park Neusiedler See - Seewinkel are threatened to disappear. Out of 139 soda pans 59 remain, however degradation is ongoing. Wells which reduce the groundwater table, drainage systems in the surrounding meadows for agricultural purposes and the non-sustainable use of groundwater resources by cultivating plants with a high level of water consumption are threats to the groundwater table and thus to the existence of the pans (Fischer-Nagel 1977; Krachler et al. 2012; Dvorak et al. 2017; Horváth et al. 2019).

This study investigated the status of a *B. viridis* population in the National Park Neusiedler See - Seewinkel analysing population size via an open capture recapture model and calculating body condition of individuals. Steppe landscapes are known as one of the primary habitats of *B. viridis*. We chose the area to investigate population dynamics of *B. viridis* in the Austrian Pannonian steppe landscape, which has become more and more anthropogenically influenced in past decades.

Methods

Study area

The Pannonian Plain in Austria is characterized by an extended steppe landscape with special wetland formations, called soda pans. The shallow waters, not deeper than one meter, are separated from the steppe lake Neusiedler See. Their high sodium carbonate content and their alkaline pH-value inhibits sedimentation, therefore the pans are more than several thousands of years old (Krachler et al. 2000). Two of these soda pans, Illmitzer Zicklacke and Kirchsee, are adjacent to the village Illmitz.

The Illmitzer Zicklacke is characterized by structural variability, caused by groundwater inflow in the east of the pan. Occurrence of *Pelobates fuscus*, *Hyla arborea*, *Triturus dobrogicus* and *Bombina bombina* was recorded in 2012 (Krachler et al. 2012). Although the Zicklacke is highly affected by past drainage activities, it has longer lasting water levels compared to other soda pans (Krachler et al. 2012). The Kirchsee has a different chemistry due to less contact to the groundwater, caused by drainage wells in Illmitz, leading to decreased salt concentration. It counts as one of the most degraded soda pans in the region and desiccates early in spring in recent years (Krachler et al. 2012). The Kirchsee and the Illmitzer Zicklacke are separated by a paved road leading to the shore of lake Neusiedl. Fischer-Nagel 1977 recorded *B. viridis* individuals at the Krautingsee, which is located next to the Kirchsee (south-west), although no individual was recorded at the Krautingsee in 2006 (Csarmann 2008).

Monitoring

All data were collected between 29th March and 3rd of June 2021. During desiccation of the Illmitzer Zicklacke around the 7th of July it was checked three times after precipitation on the 2nd and 17th of July and 7th of August. The monitoring of the sites took place in the evening hours from 9 pm to 1 am, depending on weather conditions and water status of the pans. Four main monitoring checkpoints were determined on the first day by surveying breeding calls in the whole area. During each session all checkpoints were visited, varying in temporal sequence to prevent time-bias. All individuals were captured, using at least three buckets, and were released after monitoring on each checkpoint to prevent recapturing already monitored toads. Snout-vent length (SVL), sex, body mass and location (GPS) of each toad were recorded. Snout-vent-length was measured in millimetres with a plastic vernier calliper. Body mass was measured in grams using a digital micro-scale, correct to one decimal place (Model: Brifit Feinwaage 200g/0,01g). To ensure precise body mass data the toads were put into a cup. Data about water surface of the soda pans were monitored by the scientific department of the National Park Neusiedler See - Seewinkel. QGIS was used to measure the size of the investigated area (QGIS.org 2022).

Individual identification

Pictures of the back pattern of each captured toad were used to distinguish individuals with the help of the open-source software Image Based Ecological Information System IBEIS (Crall and Parham 2020). For *B. viridis* this detection algorithm performed better than alternatives (see Burgstaller et al. 2021 for method comparison using the IBEIS precursor HotSpotter) and was also used in a previous green toad recapture study (Sistani et al. 2021). In addition, all pictures were checked visually for matches.

Population Analysis

Data analyses and graphics were made in R (R core Team 2021) using the packages “rnaturalearth”, and “ggmap” to create a map (South 2017; Kahle and Wickham 2013). Population size was estimated with a Jolly-Seber-Schwarz-Arnason capture recapture Model for open populations using “openCR” package (Schwarz and Arnason 1996; Efford 2021). The model (JSSAb in openCR) used the variables apparent survival (φ), capture probability (p), entry probability (b) and sampling occasion (Session) for calculation. Apparent survival (φ) does not distinguish between dead and emigrated individuals. We did not distinguish between sexes in the population model, as no females were recaptured. Akaike information criterion (AIC)-based model selection was used to select the best fitting model.

Scaled Mass Index was calculated to estimate body condition, every individual was counted once. Seven recapture recordings were excluded of the data analysis for body condition because of differences in SVL values over 4 mm. We used a SMI adapted from the implementation in R by Chen-Pan Liao 2018, originally formulated by Peig and Green 2009. Logarithm of mean SVL (L_i= 65.53mm) and mean body mass (M_i=30.07g) were used to calculate the scaling exponent (b_{SMA}=2.963059). Mean SVL was used as reference length for calculation (L_0 = 65.53mm). We built a multiple linear regression model with the packages "buildmer"and "glmmTMB" to test significant dependencies of SMI and body mass on variables "day", "location" (Kirchsee, Illmitzer Zicklacke, Street) and "ID" with Wald chi square test. The package "DHARMa" was used for model check (Brooks et al. 2017; Grolemund and Wickham 2011; Voeten 2021; Hartig 2021).

Sexual dimorphism shows up due to larger females (Sinsch et al. 2007), so we separated sexes for the analysis of SVL and mass values.

Results

During twelve observation nights, a total number of 110 individuals were captured on nine nights, 101 males and nine females (sex ratio 11.2:1). We did not capture any juveniles as all SVL values were above the minimum size for sexual maturity. Twenty-five of the 110 toads were recaptured, all of them males. Six males were caught three times. One male was caught on the 29th of March, 26th of May and 29th of June and thus was present for the longest time range at the Illmitzer Zicklacke. Eight individuals were not monitored in detail to prevent disturbance during amplexus. One spawn string was noted at the Illmitzer Zicklacke. The last four toads were captured on the 3rd of June. We did not confirm any individual of *B. viridis* at the Krautingsee.

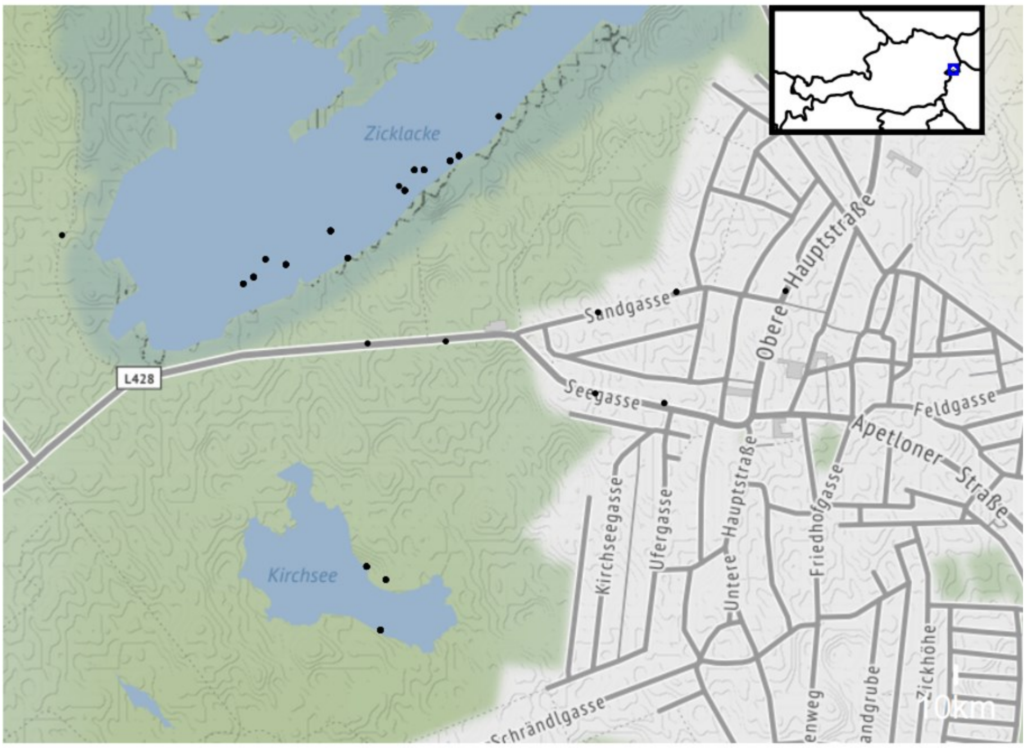

Fig.1: Map of the investigated area (400 hectares) including Illmitzer Zicklacke, Kirchsee and Krautingsee (southwest below the Kirchsee). All monitoring points are marked with black dots. The inset shows the location of the investigated area in Austria.

On 21st of June the water surface of the Illmitzer Zicklacke was at 10% and fell dry at the 8th of July. The Kirchsee desiccated first around 10th of May and filled up again for a short period at the end of May. After first desiccation no toad could be recorded at the Kirchsee anymore. The Krautingsee desiccated at 7th of June.

Demographic estimates

Estimated population size (SuperN) of the Illmitzer Zicklacke and the Kirchsee combined was 371 individuals (CI= 224-615). The best model used recapture probability as non-continuous time dependent variable and apparent survival dependent on sampling occasion. Entry probability and superpopulation size were calculated as independent variables (Tab.1). Apparent survival (φ) slightly decreased between sessions from 0.9 to 0.7.

Tab. 1 shows the best ten JSSAb-models with the calculated interactions of the parameters Apparent survival (φ), Capture probability (p) Entry probability (b), population size (SuperN) and sampling occasion (Session). SD= Standard deviation, CI= Confidence interval, AICc= Akaike information criterion modified for small sample size.

	Model	***SuperN***	***SD***	***CI***	***AICc***
1	p~ t, phi ~ Session, b~1, superN~1	371	95.5	224-615	677.74
2	p~ t, phi~ Session, b~ Session, superN~1	337	72.7	221-514	678.97
3	p~ Session + t, phi~ Session, b~ Session, superN~1	334	70.9	220-506	681.57
4	p~ t, phi~1, b~ Session, superN~1	350	87.5	215-572	682.31
5	p~ t, phi~1, b~1, superN~1	602	189.6	325-1116	682.79
6	p~ Session, phi~ t, b~1, superN~1	427	149.98	214-850	684.58
7	p~ Session + t, phi~1, b~ Session, superN~1	350	87.6	214-572	684.93
8	p~ Session + t, phi~1, b~1, superN~1	602	189.6	325-1116	685.36
9	p~ Session, phi~ t, b~ Session, superN~1	443	156.5	221-885	687.46
10	p~ t, phi ~ t, b~1, superN~1	333	56.0	240-463	689.83

Body condition

Mean body mass of the Kirchsee population was 33.3 g (SD= 6.6) and therefore heavier than the toads of the Zicklacke which were 28.7 g (SD= 6.7) on average (Fig.2). The average mass of female toads was 37.2 g (SD= 9.1) ranging from 26.7 g to 54.6 g (Fig.2). Body mass was significantly dependent on location ($p= 0.035$) and time ($p < 0.0001$) (Tab.2). Our results show a decreasing trend of mass values over time of all three locations.

Tab.2 shows the calculated p-values for body mass with Type II Wald chi square test for “location” and “day” (DF= degrees of freedom).

	VARIABLE	CHI SQUARE	DF	P-VALUE
BODY MASS	location	6.7024	2	0.035
	day	143.1673	1	< 0.0001

Snout-vent-length of the toads from Kirchsee was 66.4 mm (SD= 4.7) on average and 65.3 mm (SD= 5.3) on average of the population from Illmitzer Zicklacke. The SVL of female toads ranged from 56.0mm to 80.0 mm with an average of 67.4 mm (SD= 6.9) (Fig.2).

Mean SMI of the toads of the Kirchsee was 32.06 g (SD= 4.18), of the Illmitzer Zicklacke 28.91 g (SD= 3.94) and of individuals found on the streets of Illmitz 32.70 g on average (SD= 6.39). SMI values were significantly dependent on location (SMI $p < 0.0001$) and significantly dependent on day related to location ($p < 0.0001$) (Tab.3; Fig.3).

Tab.3 shows the calculated p-values for SMI with Type II Wald chi square test for “location”, “day” and “location related to day” (DF= degrees of freedom).

	VARIABLE	CHI SQUARE	DF	P-VALUE
SMI	location	14.4273	2	<0.0001
	day	1.5724	1	0.209
	location:day	18.7561	2	<0.0001

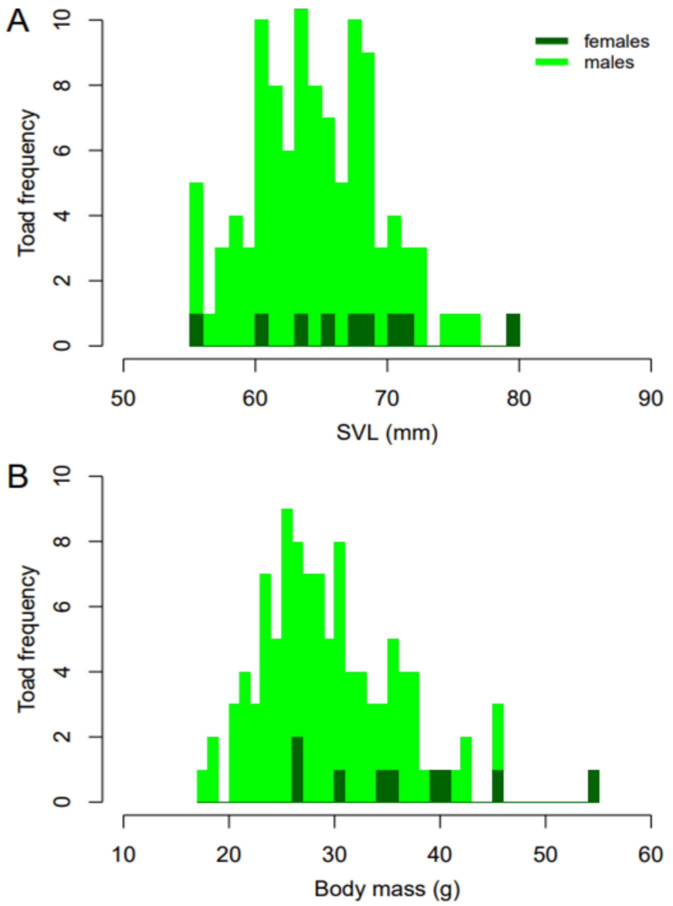

Fig.2: SVL (A) and body mass (B) of all captured individuals, recaptured individuals were counted once.

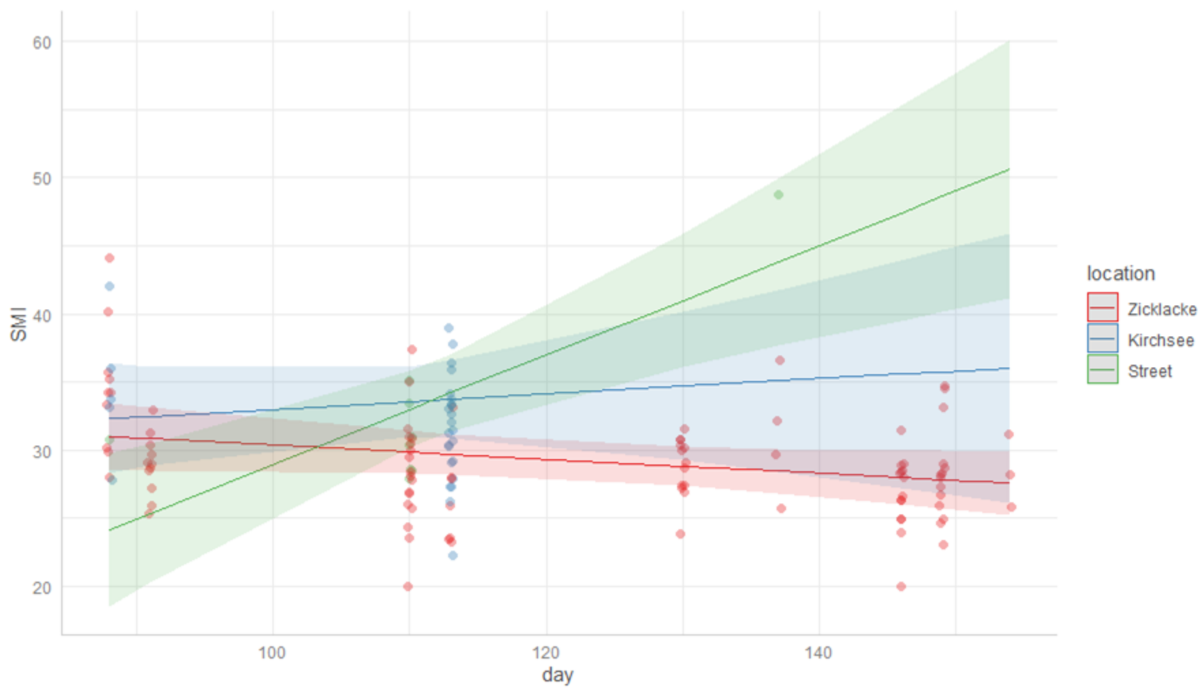

Fig.3 shows the predicted SMI values for all three locations over time.

Discussion

In our study we expect that the *B. viridis* population consists of 371 individuals within a subarea of the National Park Neusiedler See - Seewinkel, covering 400 hectares. Compared to urban habitats in Austria, the Donaufeld population consisted of 137 individuals, and the population in the Rudolf Bednar Park in Vienna more than 200 individuals (Sistani et al. 2021; Wappl and Heyer 2016). In the north of Berlin, the largest *B. viridis* population counted more than 500 individuals around the sewage irrigation fields in 1992, whereas Kühnel and Krone described the largest population in west Berlin with 250 individuals (Günther and Podloucky 1996; Kühnel and Krone 2003). Heckes and Gruber confirmed one population with more than 350 individuals and two populations with 101-350 individuals in Munich, Germany (Heckes and Gruber 1994). The authors pointed out that most of the investigated populations show a decreasing trend. Large populations with 1.000 individuals or more are rare but were confirmed on the island Sprogø in Denmark and on the Rheinschanzinsel in Germany (Hesselsøe 1997; Günther and Podloucky 1996). Sinsch et al. 1999 described a metapopulation of *B. viridis* with an estimated total population size of about 1.000 individuals in a gravel pit (Sinsch et al. 1999). Considering that the soda pans are one of the last primary habitats of *B. viridis* in Austria, population size within an area covering 400 hectares is smaller than we expected.

Bufotes viridis tadpoles hatch after three to five days and need 72-84 days for their development until metamorphosis, depending on water temperature, oxygen concentration and nutrient availability of spawning waters (Schweiger and Grillitsch 2013; Darrow et al. 2004; Blaustein et al. 2010). The reproduction success seemed to be low in the study year, considering that the Kirchsee desiccated completely in April, and the Illmitzer Zicklacke at the beginning of July. Warmer winters and more frequent winds cause stronger evaporation of the pans, longer dry periods and less winter precipitation are threats to the water level of the pans due to climate change (Zulka et al. 2022b). Because of shrinking groundwater levels, the pans loose contact to the groundwater, which leads to a decreased salinity and affects their ecological functioning (Zulka et al. 2022b). Both climate change factors and habitat degradation cause faster desiccation processes of the pans, hence, the population is at risk to decrease. Low reproduction success over years in combination with other additive effects like extreme drought in summer may lead to fast extinction processes of populations within a few years (Zahn et al. 2020; Zylstra et al. 2019).

According to our observations and personal communication information of H. Grabenhofer 2021, a few *B. viridis* individuals seemed to hibernate in the centre of Illmitz. Urban habitats store heat in winter, gardens provide more moisture in summer due to irrigation and thus may provide better winter and summer habitats, which can lead to different SMI values depending on locations. Significantly higher SMI values of individuals found on the street were dependent on the day they were captured, which underlines the assumption that they migrated from Illmitz to the pans for reproduction. However, many *B. viridis* individuals can be found on the streets of Illmitz in spring, and some of them die due to roadkill. In 2013, 100 individuals were monitored on the streets of Illmitz, most of them in April (herpetofaunistic database of the Natural History Museum Vienna). Apparent survival calculated in our model is focused on the toads which were captured inside the pans, so the slight decreasing trend can be explained by emigrating individuals after spawning.

Furthermore, variances in body condition (SMI) between intraspecific populations can point out the development of divergent life history strategies, depending on local environmental conditions. The Kirchsee is a strongly stochastic, degraded habitat, which desiccates earlier compared to other pans (Krachler et al. 2012; personal communication information of H. Grabenhofer 2021). Stochastic habitats can lead to a slowing down effect on life history traits of populations, resulting in individuals with better conditions, higher survival rates and longer lifespans (Cayuela et al. 2019). Therefore, higher SMI values may be caused by skipping reproduction in years of bad environmental conditions, and rather investing them to somatic condition (Castellano et al. 2004).

Compared to two populations in Vienna, body mass and SVL of Kirchsee males in the National Park Neusiedler See - Seewinkel were larger than males in the Rudolf Bednar Park (mean SVL=64.9 mm, mean body mass= 29.8 g), and smaller than Donaufeld males in Vienna (mean SVL=70.8 mm, mean body mass=42.0 g) (Wappl and Heyer 2016; Sistani et al. 2021). Zicklacke males had the lowest body mass with 28.6 g on average. The average SVL of females was 1.4 mm smaller than Rudolf Bednar -Park females and 10.8 mm smaller than those of the Donaufeld population (Wappl and Heyer 2016; Sistani et al. 2021). Both sites are strongly anthropogenically influenced. Males of the sewage irrigation fields in the north of Berlin were larger and heavier than Kirchsee and Zicklacke males (mean SVL=66.9 mm, mean body mass=36.6 mm) (Kühnel and Krone 2003). Females of the sewage irrigation fields were 5.6 mm larger and 5.8 g heavier (mean SVL=73.0 mm, mean body mass=43.0 mm) (Kühnel and Krone 2003). All in all, Illmitzer Zicklacke toads had the lowest body mass values while Kirchsee toads fit in the normal range, compared to other populations.

As sex ratio of *B. viridis* populations is assumed to be 1:1 females were underrepresented (sex ratio 11.2:1) (Stöck et al. 2008). We justify the imbalance of sexes by the higher possibility to locate males in our study area due to their breeding calls and their longer inhabitation of spawning waters.

Conclusion

Considering that the soda pans are one of the last primary habitats of *B. viridis* in Austria, population size of an area covering 400 hectares is smaller than we expected. The pans desiccated early, and reproduction success seemed to be low in the study year, as it could have been in recent years. Additionally, the impact of roadkill on apparent survival of the population is not yet investigated and would be an important topic for future studies and management plans, especially in combination with climate change threats like dry periods. Therefore, conservation measures in the centre of Illmitz can counteract declining population trends.

Significantly lower body condition values of Illmitzer Zicklacke individuals can point to varying quality of summer and winter habitats as well as to the development of divergent life history traits between locations as an adaptation to water resources of the pans. However, our study forms the basis for the investigation of population dynamics in the National Park Neusiedler See - Seewinkel by enabling the further investigation of apparent survival and demographic parameters in the future.

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